Neuropathologic Correlates of Magnetic Resonance Imaging in Multiple Sclerosis

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INTRODUCTION
The application of magnetic resonance imaging (MRI) has revolutionized concepts of the pathogenesis of multiple sclerosis (MS) and has significantly impacted the diagnosis and management of the disorder. Magnetic resonance imaging has shown that MS is a highly dynamic disease process and has revealed abnormalities far afield from the classic plaques that had heretofore defined its pathogenesis, clinical expression, and neuropathology. Clearly, the histopathologic elucidation of the basis of these focal and diffuse imaging findings would contribute significantly to understanding MS and how it affects the function of the CNS of individuals whom it afflicts.

Magnetic resonance originates from magnetic dipoles manifesting in atoms (1). One of the most abundant elements in biologic systems is hydrogen, a major constituent of water. Hydrogen atoms each contain a single proton, the magnetic dipoles of which can be quantified and translated into signal intensity, and expressed as a 2- or 3-dimensional image by the MRI instrument (1). Because of the abundance of water protons in the body, the MR image is essentially an image of the distribution and quantity of free water and water associated with other molecular species in tissue (1). From a neuropathologist’s point of view, MRI may be considered to be a battery of “special stains” for water and its tissue associates. Thus, classic histopathologic techniques, which purposely remove water to allow paraffin embedding of tissue to stain proteins, nucleic acids, and, to some extent, lipids may be supplemented with MRI before dehydration to visualize (at least at the macroscopic level) the distribution of free water and water associated with other constituents in tissue. In fact, recently developed MRI technology has permitted the resolution of tissue detail equivalent to that of a low-to-intermediate magnification microscope objective. Moreover, the magnetic dipoles from atoms other than hydrogen, such as sodium and phosphorous, can also be used in other MRI techniques such as spectroscopy (1).

The quest for the determination of a specific MRI abnormality to correlate with a specific pathologic change in CNS tissue dominated the early literature on MRI-pathologic correlation in MS. As will become apparent in this review, we have subsequently come to realize that such a correlative specificity is often not possible nor should have been expected to be so, given the fact that MRI is detecting water and its anatomic relationship to other molecular species in tissue. Instead of revealing, with several important exceptions, specific imaging abnormalities that can be translated into specific histologic features, the contribution of MRI to understanding the pathogenesis of MS has come to rest on its ability to delineate the evolution of the disease process over time for correlative pathology studies. It has also become apparent that certain patterns in the 2- or 3-dimensional MRI sphere can correlate well with similar patterns of histopathology, thereby leading to insights into MS pathogenesis. A multimodality approach can lead to additional insights into the nature of MS lesions.

There are a number of recent reviews available on the neuropathology of MS (2–4). Here, we outline the past and
current status of our ability to correlate changes on MRI in MS with neuropathology.

INTEGRATION OF MRI AND HISTOPATHOLOGIC DATA

Although the correlation of the gray-scale images of the MRI scan with an equivalent slice of CNS tissue would at first glance seem relatively straightforward, there are a number of issues that soon become apparent (5).

The first challenge is the actual MRI acquisition from the tissue specimen to undergo the correlative study. If the specimen is a slice of tissue, the general anatomic orientation for correlation is dictated by the slice itself. On the other hand, if an entire brain is imaged, the acquisition of a slice of tissue

![Fig 1](https://example.com/fig1.png)

**FIGURE 1.** Examples of different in vivo magnetic resonance imaging (MRI) modalities on the same axial slice of a brain of a patient with relapsing-remitting multiple sclerosis. (A, B) Note that the periventricular frontal (white arrows) and occipital (blue arrows) plaques appear as hyperintense foci on the proton density (A) and T2-weighted (B) images. (C-F) The enhancement of both plaques on T1-weighted imaging after gadolinium (gad) (C) indicates blood-brain barrier breakdown. Both lesions show a reduction of magnetization transfer ratio (MTR) (D). Demyelination is evident in both on the myelin water map (E), the plot of the myelin water fraction derived from the short-T2 component. Both show a long-T2 component (F), the histopathologic basis of which is unknown at present but has been postulated to originate from extracellular fluid. There is a narrow rim of diffusely abnormal white matter (DAWM) adjacent to both plaques that is characterized by an intermediate grade of intensity on the proton density and T2-weighted images. The remaining white matter not involved in plaque or DAWM is referred to as normal-appearing white matter. (A) Proton density. (B) T2-weighted MRI. (C) T1-weighted image after gad infusion. (D) Magnetization transfer ratio. (E) Myelin water map. (F) Long-T2 component.
for correlation with a specific plane on the scan requires the identification of specific preexisting anatomic landmarks or artificial landmarks to allow proper registration of the pathology slice and the MRI slice. In some instances, it is useful to embed the formalin-fixed cerebral hemisphere in a gel in which MRI-opaque plastic fiducials are positioned to allow the correct registration of the MRI scanning planes. Efficient protocols for imaging both the entire brain in situ and unfixed brain slices with a short postmortem interval have been developed (6). The imaging of the brain slice allows for precise registration such that the histopathologic correlates of very small lesions visible on MRI can be determined (7). In fact, the MRI has been used to locate lesions that would otherwise have not come to the attention of the pathologist (8). Protocols for imaging of the brain in situ followed by registration of the brain slices by cutting the brain in a customized slicing box have also been used (9). Stereotaxis has also been adapted for the anatomic correlation of MS pathology (10).

It is important to appreciate that there is a significant discrepancy between the thickness of the tissue slice scanned by the MRI instrument and the thickness of the histologic section used for the corresponding correlation. Depending on the MRI scanner and the clinical or research question to be asked, the MRI slice may be 1 to 5 mm thick. Most clinical scanners currently use 3-mm-thick slices in MS patients, whereas the histopathologic tissue section is in the order of 3 to 10 μm thick. This slice thickness difference is particularly important with respect to volume averaging (11). Volume averaging refers to the fact that the image generated by the MRI scanner for a given slice incorporates imaging information through the entire slice thickness for each pixel on the image. Thus, it is possible that a very small diameter lesion may be averaged with an immediately underlying or overlying area of normal-appearing parenchyma, and it is purely a matter of chance as to which area will be represented in the histologic section. To minimize these issues, we routinely cut a series of histologic sections at 5 levels through the tissue block corresponding to the MRI slice. To determine that a given point on the MR image is not averaging 2 or more histopathologically different areas, that point can be examined on each of the 5 levels to ensure that it is not the case before it is used for MRI-histopathologic correlation. More recently, our use of 1-mm-thick slices for high-field strength studies allows us to cut one hundred 10-μm-thick sections through the entire MRI slice, thereby capturing all of the tissue that was imaged.

To obtain the full face of a paraffin block, it is necessary to trim the face of the block, and some tissue that was averaged in the MRI slice will thereby be lost. To avoid this in cases where the cerebral hemisphere is scanned, we cut the brain at 1 MR plane above and 1 MRI plane below the slice of interest and trim into the block until the slice of interest is reached.

As discussed above, many of the MRI-pathology correlation studies in MS have used autopsy tissue. Although this allows for complete sampling of the CNS, it would be expected that postmortem autolysis would degrade the MRI data and image. Surprisingly, however, it has been shown experimentally that $T_1$ and $T_2$ relaxation times are relatively stable for at least 24 hours postmortem (12). Autolysis is usually not an issue in biopsied tissue, but a biopsy generally represents only a small portion of MS lesions, which may be quite heterogeneous in topography of histopathologic features. Nevertheless, biopsy studies, particularly in conjunction with concomitant or follow-up clinical or imaging studies, can provide important correlations (13).

Formalin, which is the standard fixative used for most human histopathology studies, significantly shortens both $T_1$ and $T_2$ relaxation times (14–17) and reduces the magnetization transfer ratio (MTR) (18). However, it seems that the relative differences in signal intensities between pathologic processes and within normal CNS tissue remain similar to the unfixed state. Thus, the study of formalin-fixed MS tissue is relevant to the in vivo MRI state (17).

### Routine $T_1$-Weighted and $T_2$-Weighted MRI of MS Plaques

Relaxation MRI techniques are routinely used in the clinical setting to aid in the diagnosis and follow-up of MS patients (19). These relaxation techniques include $T_1$-weighted, $T_2$-weighted, and proton density imaging (1). Although highly sensitive, neither are they specific nor diagnostic for MS (20). Their use has been incorporated into internationally recognized criteria to render a diagnosis of MS (21); the criteria continue to evolve and recently have undergone major revisions (22).

The most characteristic pathologic feature MS is the plaque, the biology and imaging of which have dominated MS imaging and neuropathology research (23). These large areas of inflammatory demyelination with variable degrees of axonal loss are characteristically located in CNS white matter and are thought to have an immune-mediated pathogenesis that probably is autoimmune in origin (2). They appear as hyperintense foci on $T_2$-weighted, proton density, and fluid-attenuated inversion recovery (FLAIR) imaging and as areas of reduced signal on $T_1$-weighted imaging (Figs. 1 and 2) (19). Early MRI-pathology studies established the sensitivity of MRI in the detection of MS plaques and how the MR image could

![FIGURE 2](https://example.com/figure2.png)

**FIGURE 2.** Typical in-vivo clinical magnetic resonance imaging scans from a patient with relapsing-remitting multiple sclerosis. (A, B) The fluid-attenuated inversion recovery (FLAIR) (A) demonstrates many periventricular plaques, whereas only a subset is visible on the $T_1$-weighted image (B). The yellow arrow highlights a lesion visible on the FLAIR that is also identifiable as a $T_1$ black hole; the orange arrow demonstrates a lesion only visible on the FLAIR.
reproduce the topography, area, and shape of these focal lesions in exquisite detail (24, 25) (Fig. 3). Subsequent studies attempted to dissect the histopathologic basis for MRI changes (5). These studies showed that T2 changes could be attributed to demyelination (26), macrophages (27), vascular permeability (28), edema (29), expansion of the extracellular space secondary to tissue loss (30), or gliosis (31), and it became evident that a specific histopathologic correlate for any given T2 imaging abnormality was not possible. In retrospect, this is not surprising because routine MRI techniques essentially detect water protons instead of complex tissue molecules per se (1). This is also true for T1-weighted imaging, with the important exception of permanent “black holes,” which are areas of decreased signal that represent regions of axonal loss and parenchymal destruction (32) that would be expected to contribute to permanent neurologic deficits (33). Black holes that are transient (“acute black holes”) are not areas of irreversible damage, and their evanescent nature has been attributed to remyelination or to resolution of edema associated with inflammation (13). The use of a multimodality approach using relaxation techniques to predict the pathologic features in MS lesions is reinforced by the fact that the probability of demyelination in a lesion is high if it is evident both on T1-weighted and T2-weighted imaging and shows an abnormality on magnetic transfer imaging but low if seen only on the T2-weighted image (34).

Edema in MS lesions is generally attributed to the presence of inflammatory infiltrates disrupting the blood-brain barrier. Enhancement on the T1-weighted image after intravenous administration of gadolinium-diethylenetriamine penta-acetic acid (Fig. 1C) has been shown to correlate with the presence of chronic inflammatory infiltrates in the form of macrophages (27) or perivascular lymphocytic infiltrates (35). Enhancement may be also related to the formation of new blood vessels at the periphery of plaques (36).

Patterns Within Lesions on MRI

The patterns within a lesion on MRI may correlate with histopathology and provide information on the evolution of the lesion. The formation of an “open ring” of gadolinium enhancement is said to be highly characteristic of a demyelinating lesion (37), and ring enhancement has been reported to be characteristic of a specific pathologic type of MS lesion (38) with complement and immunoglobulin deposition (39). A rim of abnormal signal at the periphery of a lesion may indicate the presence of macrophages at that site (27, 40) and, by inference, ongoing demyelination. The characteristic ring patterns of Baló concentric sclerosis, a variant of MS that may represent an intermediary stage in the evolution of an acute plaque to a chronic active one (2, 41), may be seen by MRI (42, 43) (Fig. 4A). Baló concentric sclerosis diagnosed by MRI has been confirmed by histopathology (44).

Less Conventional MRI Techniques

Magnetic Resonance Spectroscopy

The use of proton magnetic resonance spectroscopy (MRS) would seem to be a logical choice for a technique that could determine the chemical constituents of a lesion. If a specific compound so analyzed was a specific marker for a given cell type, the relative increase or decrease of that marker would presumably be a reflection of the fate of that cell type in the lesion. Although this is intuitively the case, pathologic correlations of MRS findings to confirm this are extremely difficult to accomplish because the excised tissue is no longer biochemically the same as in vivo. An important MRS marker is N-acetyl aspartate (NAA), which has been shown to be a specific marker for axons (45). Magnetic resonance spectroscopic studies (Fig. 5) have found NAA to be reduced in MS plaques (46); this is supported by histologic studies demonstrating axonal damage and loss in MS plaques (47, 48) (Fig. 3). Magnetic resonance spectroscopy has also detected abnormal peaks of lipid in MS plaques, which have been attributed to myelin breakdown products (49). Elevation of choline in the plaque detected by MRS is thought to originate from increased numbers of cell membranes because of the hypercellularity of inflammatory and/or demyelinating lesions (49). An MRS-histopathology correlative study, however, found choline to be increased in lesions with fibrillary gliosis and suggested that astrocytes may be a source of choline (50). Increases in myoinositol (51) (Fig. 5) and creatine (52) are also thought to be related to gliosis. Increased lactate in MS lesions is thought to emanate from macrophages (51). Magnetic resonance spectroscopy for phosphorous shows a reduced peak in the plaque that is believed to be caused by a reduction in phospholipids (53).

T2 Relaxation: The Short-T2 Component (Myelin Water Fraction)

One of the newer techniques that offers promise for specific translation of MRI technology to histology is myelin water imaging based on the short-T2 component. This capitalizes on the observation that T2 decay occurs at different rates in different CNS structures and the overall decay curve does not fit a straight line, but instead seems to be a composite of individual water pools, each of which has its own linear T2 decay pattern (54). Thus, the T2 relaxation decay curve for healthy CNS tissue can typically be separated into 3 components by a non-negative least-squares algorithm (55). These components comprise the longest component of greater than 1-second duration from cerebrospinal fluid, an intermediate component of 80-100-millisecond duration from intracellular and extracellular fluid, and the short-T2 component of 10-50-millisecond duration thought to emanate from water trapped between myelin lamellae (55). The ratio of area under the short-T2 component peak to the area under the entire T2 distribution is referred to as the myelin water fraction (MWF), which can be used to generate a myelin water map (56) (Fig. 1E). The anatomic distribution of the short-T2 component corresponds to the distribution of myelin (57) and is absent in areas that are completely demyelinated (Fig. 4), yet still demonstrate axons (57), indicating that the short-T2 component is associated with the myelin sheath and not the axons they have myelinated. Furthermore, the MWF correlates with the intensity of Luxol fast blue (LFB) staining of myelin (58, 59). Thus, it seems the short-T2 component/MWF is detecting myelin, and we believe that this is related to the periodicity of the myelin sheath with trapping of water in the intraperiod line of the myelin sheath, which represents the apposition of the extracellular faces of adjacent oligodendroglial processes in compact myelin. Thus,
this technique represents a means for following demyelination and remyelination during the course of MS and may, thereby, have a significant role in clinical monitoring, management, and therapeutic trials. Indeed, there is in vivo evidence indicating that the short-T2 component can detect remyelination of previously demyelinated lesions (60), and this technique is to be used in some future clinical trials in MS (61). The short-T2 component is one of several techniques competing for the position of favored MRI marker for myelin (62), although the field is currently controversial. Several other techniques have been touted as the ideal marker for myelin, but it seems, at least from current data, that these techniques also detect other tissue components, most notably axons.

**T₂ Relaxation: The Intermediate and Long Components**

Virtually nothing is known of the histopathologic basis of the intermediate component of the T₂ relaxation distribution, although it is thought to originate from intracellular and extracellular water. An additional long-T₂ component has been described in some MS lesions in vivo, a phenomenon observed more frequently in patients with a longer disease duration (63) (Fig. 1F). It is unclear what the origin of the additional long-T₂ component is, but extracellular fluid has been postulated (63). However, there is a subset of lesions that has a long T₂ and specific changes on diffusion MRI that indicate that elongated processes are present; this has been speculated to be caused by isomorphic fibrillar gliosis (64).

**Magnetization Transfer Imaging**

Magnetization transfer imaging results from the interaction of mobile aqueous associated protons with protons in macromolecules (65), thereby providing an indirect assessment of tissue structural integrity. It is usually measured as an MTR but has also been modeled to extract tissue parameters, for example, F, the fraction of protons on nonaqueous tissue (66–68). Most magnetization transfer models assume a 2-pool system (mobile aqueous protons and nonaqueous protons), although white matter is known to be more complex. The magnetization transfer effect depends not only on the presence of 2 (or more) proton pools but also on the magnetization exchange rate between the pools, which itself may vary with pH, temperature, and pathology (69). The large literature on magnetization transfer imaging makes it clear that MTR is a very robust and sensitive measure of tissue damage. Although it has been associated with myelin (70), particularly in the unfixed state (71), its specificity for myelin has been controversial (72) because it has also been associated with axonal integrity (9, 33), inflammation (73), changes in water content (72), and, very recently, with axonal neurofilament proteins (74). Magnetization transfer ratio abnormality is also highly correlated with axonal swelling, a precursor of axonal degeneration and loss in MS (9). In any event, regardless of its origin, there is a reduction in MTR in MS plaques (75) (Fig. 1D), and the change in MTR is dependent on the age of the lesion (76).

**Diffusion-Tensor Imaging**

Diffusion-tensor imaging measures the degree of random motion of protons. This is expressed as the apparent diffusion coefficient and mean diffusivity (77). Mean diffusivity is increased, whereas fractional anisotropy, which is a measure of the directionality of proton motion, is reduced in MS plaques (77, 78). There is a reduction of both these parameters in postmortem brain compared with the in vivo state that is likely caused by postmortem autolysis, temperature changes, humidity, and other physical phenomena associated with autopsy tissue (79). Despite this, however, the relative differences between lesions and normal-appearing white matter (NAWM) postmortem are comparable to the living state (79), indicating that it is appropriate to use this methodology in MRI-pathology correlation studies. Reductions of mean diffusivity and fractional anisotropy are affected by myelin content and, to a lesser degree, axons (79), and an increase in radial diffusivity correlates with demyelination, although this parameter may also be a result of axonal damage (80). Thus, this technique can be used to track changes in the MS plaque over time and may have translational capacity to the clinical setting. Recent work demonstrated that elevated radial diffusivity during gadolinium enhancement was associated with an increased risk for the development of a persistent black hole, a surrogate of severe demyelination and axonal injury (81). The use of fractional anisotropy to generate tractography maps, which has been elegantly illustrated in vivo (82, 83), has proven difficult to reproduce in formalin-fixed postmortem tissue until recently (84). This will open up a new field of MRI-pathologic correlation to examine the effect of focal and diffuse abnormalities on tract degeneration in MS.

**Susceptibility-Weighted Imaging**

Susceptibility-weighted imaging, which is influenced by iron deposition, offers potential in the delineation of lesions and in the characterization of iron deposition in the deep gray matter

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**FIGURE 3.** Representative postmortem magnetic resonance imaging (MRI) and histopathology of an axial formalin-fixed slice of a cerebral hemisphere from a patient with multiple sclerosis. (A, B) Periventricular plaques (black arrows) and plaques unassociated with the ventricles (white arrows) are readily evident on proton density (A) and T₂-weighted (B) MRI. (C–F) In addition to the obvious myelin loss shown as absence of staining with Luxol fast blue (LFB) (C), LFB-Bielschowsky (E), and myelin basic protein (MBP) immunohistochemical (F) stains, there is marked axonal loss in these plaques seen in the Bielschowsky preparation (D). A small “leukocortical” plaque (red arrows), involving subcortical U fibers and (to a lesser degree) the overlying cortex, is not evident on the proton density scan (A), is barely perceptible on the T₂-weighted scan (B), and is best appreciated with the MBP immunostain (F). Diffusely abnormal white matter (DAWM), indicated by asterisks, is seen adjacent to the periventricular plaques and is more prominent posteriorly (large astersisks) than anteriorly (small astersisks). Note the normal MBP staining (F) despite a reduction in myelin lipids demonstrated with the LFB stain (C) and the axonal loss (D) in DAWM. The degree of axonal involvement in DAWM is variable; in this case, it is severe. Normal-appearing white matter (not labeled) is the remaining white matter not involved in plaques or DAWM. (A) Proton density MRI. (B) T₂-weighted MRI. (C) LFB. (D) Bielschowsky. (E) LFB-Bielschowsky. (F) MBP immunostain.
A  Baló’s Concentric Sclerosis Lesion in an MS patient at 7 Tesla

B  Diffusely Abnormal White Matter at 7 Tesla
in MS (85). Correlative studies of tissue magnetic susceptibility-derived measures in controls (86) and MS (87) are emerging. Recent studies suggest that susceptibility-weighted imaging may also be imaging tissue structure rather than exclusively iron distribution (88, 89).

Perfusion MRI

Perfusion MRI refers to the measurement of brain perfusion by tracking a contrast dye (bolus tracking) or blood (arterial spin labeling) (89). This methodology, which does not lend itself to direct pathologic correlation, has shown reductions in perfusion in MS that have been correlated with disability and neuropsychological abnormalities (89).

NONLESIONAL WHITE MATTER ABNORMALITIES

Normal-Appearing White Matter

It had generally been believed until recently that the white matter that was not involved with focal MS plaques, the so-called NAWM, was morphologically and physiologically normal. Although a few neuropathology studies had pointed out that abnormalities may be detected in NAWM, it had been generally accepted that the biochemical and histochemical abnormalities in assayed NAWM were caused by the inadvertent inclusion of small plaques in the material assayed (90). The morphologic findings documented in MS NAWM in neuropathology studies include diffuse gliosis, perivascular inflammation, perivascular lipofuscin, occasional demyelination (91, 92), diffuse inflammation (93), microglial activation (93, 94), and microglial cells with upregulation of transcription factors important in major histocompatibility complex expression (95). Lysosomes (96-98) and lysosomal enzymes (92) have also been detected in NAWM, particularly in astrocytes. Perivascular inflammatory cells in NAWM express matrix metalloproteinases (99), which are involved in the disintegration of the extracellular matrix and other functions. Abnormalities in extracellular matrix proteoglycans have been documented in MS NAWM (100). Furthermore, there is immunohistochemical evidence of breakdown of the blood-brain barrier in NAWM, characterized by abnormalities in endothelial tight junctions (101, 102), leading to leakage of high-molecular-weight serum compounds, which are normally impervious to the blood-brain barrier, into the CNS perivascular parenchyma (102). The latter

FIGURE 4. Multiple sclerosis (MS) magnetic resonance imaging–pathology correlations at 7 Tesla. (A) 7 Tesla (TE = 20.1 milliseconds [ms]) image, myelin water map, and corresponding Luxol fast blue histologic images of the temporal lobe of an MS patient. Arrows in upper panels indicate the alveus of the hippocampus. Faint rings of preserved myelin (arrows in lower panels) alternate with rings of myelin loss in the Baló concentric sclerosis lesion. Lower panels are higher magnifications of the area indicated by the dashed box in the upper panels. (B) Diffusely abnormal white matter (DAWM) at 7 Tesla. The DAWM has ill-defined edges (arrows) and is composed of an area with an intensity intermediate between that of the plaques (asterisks) and normal-appearing white matter (N). Within this is an area of reduced staining on the Luxol fast blue (phospholipid) stain and the axonal Bielschowsky preparation; this area is relatively normal in the immunohistochemical stains for the myelin proteins, myelin basic protein (MBP) and 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNP). (A) is reprinted from and (B) is modified from Laule C, Kozlowski P, Leung E, Li DKB, MacKay AL, Moore GRW. Myelin water imaging of multiple sclerosis at 7T: Correlations with histopathology. Neuroimage 2008;40:1575–80. Copyright 2008. Used with permission from Elsevier.

FIGURE 5. Magnetic resonance spectroscopy spectra from control white matter and a multiple sclerosis (MS) lesion. Various metabolites are evident, including N-acetyl-aspartate (NAA), creatine (Cre), choline (Cho), and myo-inositol (ml). The MS lesion shows a reduction in NAA (a marker of neuronal integrity) and an increase in ml (indicative of gliosis) versus the healthy white matter.
is likely the correlate of the mild increase in gadolinium enhancement appreciated in NAWM on quantitative assessment of contrast MRI scans (103). An important recent finding of relevance to our understanding of the clinical progression and MRI abnormalities in MS is the loss of axons within cerebral hemispheric NAWM that has been attributed to Wallerian degeneration secondary to axonal destruction within plaques (104, 105). Low levels of inflammation in the NAWM probably also contribute to the loss of axons (23, 93). Axonal loss has also been found in optic nerves and spinal cord and seems to involve mainly small-diameter axons (106, 107).

Which of these pathologic changes specifically contributes singly or in part to the MRI abnormalities in the NAWM has not been determined. The NAWM abnormalities have been firmly established by nonconventional MRI. Axonal loss, as shown by a reduction of NAA on MRS, has been documented in NAWM (108) and is, to a limited extent, reversible (109). Magnetic resonance spectroscopy has also shown reduction of phospholipid (53), lipid peaks (110), increased myoinositol (111, 112), increased creatine (112, 113), and increased choline (112, 113) in MS NAWM. Additional evidence of a widespread abnormality in NAWM is evident by reduced MTR (114), increased apparent diffusion coefficient (115), reduced fractional anisotropy (116), prolongation of T1 (117), reduction in the short- T2 component/MWF (118), and an increase in total water content (118). These abnormalities in NAWM are clinically important because (in contrast to plaque burden) they correlate with disability, cognitive impairment, and atrophy (119, 120). The MRI changes, as also noted above in the pathology studies on axonal loss, are not just confined to the cerebral hemispheres but are widespread and have also been documented in the spinal cord (121, 122) and optic nerve (123) and occur early in the disease process (120).

**Diffusely Abnormal White Matter**

Diffusely abnormal white matter (DAWM), also referred to as “dirty-appearing white matter,” is a relatively recently recognized region of pathology in MS nonlesional white matter, initially described by Zhao et al (124). It is appreciated on routine T2-weighted and proton density imaging as a region of intermediate intensity of signal between that of plaque and NAWM, that is, a signal intensity similar to that of gray matter (124) (Figs. 1, 3 and 4B). It is characteristically seen adjacent to periventricular plaques and in the occipital white matter, although it may also be unassociated with a plaque. It shows a reduced MWF and MTR (125, 126) and an increase in water content and T1 (66, 126). Inasmuch as DAWM was recognized only relatively recently, it is possible that some of the previous studies on NAWM may have included DAWM in their assessments. The DAWM is relatively common in MS but is not universally present and may be seen in both younger and older MS patients (127). It shows different findings on T1-weighted MRI and magnetization transfer imaging in secondary progressive MS, as compared with primary progressive MS (128). The DAWM seems to show a selective loss or perturbation of myelin phospholipid, as detected by LFB staining (126, 129). Curiously, in some studies, this is not accompanied by equivalent immunohistochemical changes in the protein constituents of myelin (126, 129) (Figs. 3 and 4), but other studies have not noted this disassociation (130). More recent preliminary data using other stains, including Weil myelin stain, which also stains phospholipids, have again demonstrated the selective reduction in phospholipids (131), suggesting that the findings are not related to the technical issues inherent in LFB staining. There is variable axonal loss in DAWM, but this is often not necessarily as widespread or severe as the phospholipid abnormality (126, 129). These changes are associated with a reduction of the short-T2 component, which is a very sensitive indicator of DAWM (126, 129). The clinical significance of this intriguing abnormality is uncertain, but in view of the prominent lipid abnormality of the myelin sheath, it is possible that there may be significant physiologic abnormalities in DAWM. The pathogenesis of DAWM, with the coexistence of a myelin lipid abnormality and axonal degeneration in the face of relative preservation of myelin proteins, is uncertain. These findings are consistent with an early lipotoxic effect on myelin, possibly extending from the adjacent plaque in the early phases of its expansion into the adjacent white matter, with subsequent secondary changes in the axon. The relationship of DAWM to abnormalities seen in NAWM is also unclear (132), but one interpretation is that DAWM is an intermediary stage between plaque and NAWM. There are also some indications that new plaques themselves may arise within the DAWM (133), suggesting that it may be the substrate for new lesion formation, possibly in response to unmasking the myelin lipid or axonal abnormality. Given the axonal changes, DAWM may well be an important contributor to neurodegeneration in MS. Furthermore, it is also unknown whether white matter hyperintensities, or leukoaraiosis (134, 135), which resemble the MRI appearance of MS and are seen in aging, subcortical dementia (136), and Alzheimer disease (137) have a similar pathogenesis to DAWM.

Magnetic resonance imaging and histopathologic evidence of axonal loss in NAWM and DAWM have highlighted the neurodegenerative component of MS and its clinical progression (23). Although some studies have shown a relationship between inflammation and neurodegeneration (138), the prominence of neurodegeneration in the disease has raised the question as to whether MS is a primary neurodegenerative process and the inflammatory demyelination, which has defined the disorder in the past, is a secondary phenomenon (139). No doubt, in the future, there will be considerable interest in this concept and the unfolding arguments and scientific evidence for the resolution of this fundamental question.

**THE INITIAL MS LESION**

Multiple sclerosis plaques that have been examined histopathologically (even in acute MS) have been clinically established for 24 hours (140) to weeks (141). In the past, this early lesion has been considered to be predominantly a manifestation of inflammatory demyelination, but more recent studies suggest that this may be a noninflammatory event characterized by oligodendroglial apoptosis (140) that may even be antedated by damage to perivascular astrocytic end-feet (142). Nonconventional MRI changes are evident in NAWM at the site of what will subsequently become an MS plaque weeks to
months before this is evident by routine imaging, when it appears as an enhancing lesion. The pathologic correlates of these very early focal abnormalities are unknown but have been speculated to be edema, gliosis, demyelination, remyelination, or an early immune-mediated attack on myelin (143). These early changes detected at sites of future lesions include a reduction in MTR (143, 144), an increase in choline (145), increases in blood volume and blood flow (146), and an increase in apparent diffusion coefficient on diffusion-tensor imaging (147). It is unknown whether these changes are one and the same or a precursor to the (pre)active lesions (8), which comprise foci of microglial activation detected on conventional postmortem MRI.

GRAY MATTER

Cerebral Cortex

It is now widely recognized that there are significant numbers of demyelinated plaques in the cortex in MS (23, 148, 149), although their presence was described in earlier studies (150). Cortical lesions are rare in pediatric patients with MS (151) but are clinically significant in adults (152–154). They are poorly visualized by the LFb stain but are easily seen using immunohistochemistry for myelin proteins (155). Using the latter technique, extensive cortical demyelination in MS has been demonstrated; in fact, the area of cortex demyelinated tends to be even greater than that in the white matter (155). The cingulate gyrus is particularly affected (155). Demyelinated plaques have also been shown in the hippocampus (156), which also shows neuronal loss (157) and a reduction of the acetylcholine synthesizing enzyme choline acetyltransferase but normal levels of its degradation enzyme acetylcholinesterase, indicating an imbalance in cholinergic projections to this structure (158). Such findings may be important correlates of the memory deficits in MS patients. In contrast to white matter lesions, cortical plaques usually show little inflammation (159). In view of a recent study that found cortical plaques with inflammation in patients with relatively earlier disease, this may be attributed to sampling the disease in its later stages (160). On the other hand, if cortical inflammation was a relatively frequent phenomenon, it would likely have been detected previously in active cases that show inflammatory white matter lesions in various stages of evolution. Cortical plaques are rarely detected by routine MRI (Fig. 3), but this situation has improved somewhat by the use of contrast agents, FLAIR imaging (148, 149), phase-sensitive inversion recovery (152), magnetization-prepared rapid acquisition with gradient echo (161), particularly when used as part of a multimodality approach (162), and double inversion recovery techniques (163). Double inversion recovery is the most commonly used MRI technique for which consensus scoring criteria are now available that will facilitate its use in multiple centers (164). Cortical lesions are readily visualized by high-field strength MRI (165–167), as demonstrated by recent histopathologic studies (168–170). Recent histologic evidence suggests that double inversion recovery is more sensitive to cortical plaques than FLAIR in MS (170); however, because other techniques are also sensitive to cortical lesion pathology (152), future MRI-histopathologic correlations should determine which of these techniques or which combination thereof is most sensitive and specific for cortical plaques. In addition to cerebral cortical lesions, it is now recognized that there is also extensive cerebellar cortical demyelination in MS (171).

There are also diffuse MRI abnormalities observed in cortical gray matter. These “normal-appearing gray matter” findings include abnormalities on magnetization transfer imaging and diffusion-tensor imaging, as well as reductions in NAA on MRS (120, 172). The pathologic substrate for normal-appearing gray matter abnormalities is unknown, but it has been speculated that they are caused by cortical plaques, diffuse degeneration of gray matter unrelated to cortical plaques, gray matter degeneration secondary to cortical and/or white matter plaques, or various combinations of these factors (120). In this regard, there is some histopathologic evidence of neuronal and synaptic loss in the cortical plaques of MS (173), and it is not clear at this time how much this contributes to the pathophysiology of MS. However, focal neuronal changes in cortical plaques cannot account for the widespread cortical thinning noted in MS (173). There is also recent MRS evidence suggesting that cortical neuronal/axonal loss may not be secondary to white matter plaques in relapsing-remitting MS but a part of a widespread neurodegenerative process in secondary progressive MS (172).

Of considerable interest is the presence of cortical functional plasticity evident on functional MRI in MS. There is a remarkable capacity for the cerebral cortex to take over the function of areas involved with MS lesions, which may explain, to some degree, functional recovery after MS relapses (174–176) (Fig. 6). Functional MRI studies may, therefore, become part of the clinical evaluation of MS patients in the future (175). Functional MRI is a measure of cerebral oxygen extraction. A focal region of increased neuronal activity is accompanied by increased blood flow and oxygen consumption, the former being larger than the latter. This increase in the ratio of oxygenated and deoxygenated hemoglobin gives an enhanced MR signal (174). In addition to the motor system, this technique is being applied to the visual system, higher cortical function and spinal cord (174). Not surprisingly, functional MRI–histopathologic correlations have not been carried out, but in view of the neovascularization present in MS plaques (36) and the increasing histopathologic evidence for mitochondrial failure in MS plaques and NAWM (177), these features may lend themselves to such studies.

Deep Gray Matter

There are very few studies addressing the pathology of deep gray matter plaques in MS (178) or of their MRI correlates. A study on the extent and pattern of gray matter demyelination in the motor cortex, cingulate gyrus, cerebellum, and thalamus found substantial variation in the extent of demyelination between the different gray matter regions, with the cerebellum exhibiting the most myelin loss and only a modest degree of demyelination in the thalamus (178). Examination of regions within the deep gray matter found demyelinating lesions to be common; they were most often in the thalamus and caudate, but they were also seen in the putamen, pallidum, claustrum, amygdala, hypothalamus, and substantia nigra. Neuronal loss was also observed, and most deep gray matter lesions involved both gray matter and white matter in
these structures (179). A study of plaques in the hypothalamus has demonstrated that they contain activated microglial and macrophages engaged in demyelination (180), but it seems that the degree of inflammation is intermediate between that in the cortex and in the white matter (179). Diffuse neuronal loss in the thalamus associated with atrophy and MRS abnormalities has been documented (181). The T2 hypointensity noted in deep gray matter structures is thought to be caused by iron deposition (182). Deep gray matter MTR abnormalities and volume loss are present even in the earliest stage of the disease (183, 184) and are related to cognitive deficits in MS patients (185). Increased plaque burden is associated with deep gray matter volume loss (186), and, in particular, thalamic and hippocampal atrophy is correlated with T1 lesion load even in the early stages (187).

**ATROPHY**

Cerebral atrophy in MS has been well documented by a variety of parameters on MRI. It correlates with physical and cognitive impairment, it can be evident early in the disease course (188), and it is progressive (189). Important in the assessment of progressive atrophy in MS is the caveat that this may be more apparent than real, particularly in the early stages of the disease because of resolution of edema either spontaneously or through treatment with corticosteroids, and may result in a reduction of cerebral volume in the absence of parenchymal tissue loss (188). In view of the fact that most of the brain mass is composed of axons and their myelin sheaths, it is generally assumed that true atrophy of the parenchyma is caused by the loss of these structures, but other histopathologic features may also play a role (188).

Atrophy is also evident in the MS spinal cord and has also been associated with disability (190). Axonal loss has been shown both in spinal cord plaques and in nonlesional white matter and likely contributes significantly to atrophy (107). By MRI, spinal cord atrophy seems to be independent of plaque load (188), suggesting that it might be a process that is independent of Wallerian degeneration.

**FIGURE 6.** Functional MRI (fMRI) for a motor activity for normal controls and a multiple sclerosis (MS) patient. (a-f) Left and right sides of the head are indicated by L and R, respectively, in (a). The proportions of normal controls exhibiting activation in the motor cortex are shown during right (a)- or left (e)-hand movements; the pixel brightness increases with frequency of activation at that location. (b-d) Scans show areas activated in the MS patient during movements of the impaired right hand at the first (b), second (c), and third (d) fMRI examinations, respectively. (f) Areas activated by the patient with movement of the unimpaired (left) hand, which did not change significantly during the study. Note that the first scan (b) shows activation of primary sensorimotor (red) and supplementary motor cortices (yellow) bilaterally and other areas that are not normally associated with motor function (blue). In the later scans (c, d), the areas recruited are less extensive and tend to return to the normal pattern as the patient gradually recovers function in the right hand. Modified from Reddy H, Narayanan S, Matthews PM, et al. Relating axonal injury to functional recovery in MS. Neurology 2000;54(1):236–9. Reproduced with permission.
Progressive atrophy of the optic nerve also occurs in and also likely is caused by axonal loss (106, 191). Optic nerve involvement is reflected in the reduced thickness of the nerve fiber layer in the retina that can be visualized by optical coherence tomography (192–194) (Fig. 7). Optical coherence tomography, although not an MRI technique, is mentioned here because it is an emerging technology of considerable interest in MS patient neuroimaging. This technique measures the magnitude and time delay of echoes in backscattered light from the retina (195) and has been shown to have an excellent correlation with retinal thickness, as determined by histology (196). Although there are some issues with optical coherence tomography, such as the normalization of data and protocols between different optical coherence tomographic instruments (195), this technique may serve as an indicator of the degree of CNS axonal loss in MS patients, in whom there is thinning of the retinal nerve fiber layer (Fig. 7), even without a previous episode of optic neuritis (192–194).

SPINAL CORD
Pathologic correlation studies have shown that routine MRI underestimates the degree of involvement of the spinal cord (197). Multiple sclerosis plaques can be visualized as focal abnormalities by routine relaxation imaging (198). Postmortem analysis has demonstrated that areas of less intense signal change correspond to regions of relative axonal preservation with mild reduction of LFB staining thought to be regions of partial demyelination (198). Furthermore, a recent ex vivo study found that increased radial diffusivity distinguished worsening severities of demyelination in MS spinal cord (80).

FUTURE DIRECTIONS
The recent use of high-field strength magnets provides high-resolution images that have heretofore not been possible. Recent pathologic correlative studies have shown that this technique can detect (in addition to the cortical plaques noted above [168, 169]) gray matter spinal cord plaques (199), small myelinated fiber bundles, and patterns within MS lesions that correlate well with histology (59) (Fig. 4), including remyelination (200). High-field strength MRI has also refined the quality of the short-T2/MWF image dramatically (59) (Fig. 4). Future MRI-pathology correlative studies using high-field strength techniques should shed further light on the pathogenesis not only of the white matter and gray matter plaques of MS but also the widespread abnormalities involving CNS parenchyma not involved by focal lesions. Such studies may help determine whether the focal inflammatory plaque or the diffuse neurodegenerative abnormalities that are present early in the disease are the fundamental initial abnormality in the pathogenesis of MS.

To date, MRI has provided significant insights into the dynamic and progressive evolution of disease processes in MS. This has been particularly so where there has been direct correlation with changes in the tissue. MRI-pathology correlation studies will be applied to newer MRI techniques as they become available and continue to unravel the complex web

FIGURE 7. Optical coherence tomography. (A, B) Upper panels demonstrate plots of retinal nerve fiber layer (RNFL) thickness versus the retinal topography. Normative data are shown as colored regions; the patient’s data are shown as a black line in the case of a normal retina (A) and as an interrupted line in the multiple sclerosis (MS)/optic neuritis retina (B). Note the thinning of the RNFL in most regions of the retina in the MS/optic neuritis patient compared with the normal retina. Lower panels demonstrate tomograms of the retina showing the retinal layers, with a decrease in the thickness of the RNFL of the MS/optic neuritis patient (B) versus the normal (A). Inf, inferior; Nas, nasal; Sup, superior; Temp, temporal. Modified from Pula JH, Reder AT. Multiple sclerosis. Part 1: Neuro-ophthalmic manifestations. Curr Opin Ophthal 2009;20(6):467–75. Reproduced with permission.
of interacting factors that produce the tissue and chemical changes responsible for the relapsing-remitting and progressive clinical manifestations of MS. Such studies will eventually be translated into therapeutic approaches that will hopefully ameliorate or abolish this disabling disease.

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REFERENCES


196. Blumenthal EZ, Parikh RS, Pe’er J, et al. Retinal nerve fibre layer imaging compared with histological measurements in a human eye. Eye (Lond) 2009;23:171–75