
MR-based technology for in vivo detection, characterization, and quantification of pathology of relapsing-remitting multiple sclerosis

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Abstract—In relapsing-remitting (RR) multiple sclerosis (MS), conventional magnetic resonance (MR) imaging (MRI) has proved to be a valuable tool to assess the lesion burden and activity over time. However, conventional MRI cannot characterize and quantify the tissue damage within and outside such lesions and only can provide some gross measures reflecting the presence of irreversible tissue damage, such as the load of T1 “black holes” and the severity of brain or cord atrophy. Other MR-based techniques, including cell-specific imaging, magnetization transfer (MT) MRI (MT-MRI), diffusion-weighted (DW) MRI (DW-MRI), proton magnetic resonance spectroscopy (^1H -MRS), and functional MRI (fMRI), have the potential to overcome this limitation and, consequently, to provide additional information about the nature and the extent of MS tissue damage, which would inevitably remain undetected when only a conventional MRI is obtained. Cell-specific imaging should result in a better definition of the cellular mechanisms associated with MS inflammation. Metrics derived from MT- and DW-MRI can quantify the structural changes occurring within and outside lesions visible on conventional MRI scans. ^1H -MRS could add information on the biochemical nature of such changes. fMRI is a promising technique to assess the mechanisms of cortical reorganization, which may limit the consequences of an MS-related injury. The application of these MR techniques to the study of RRMS is likely to provide useful insights into the pathophysiology of this disease

and to improve our ability to assess the efficacy of experimental treatments.

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INTRODUCTION

In recent years, major advances in the use of magnetic resonance (MR) imaging (MRI) for assessing patients with multiple sclerosis (MS) have been achieved. Conventional MRI (i.e., dual-echo spin-echo and postcontrast T1-weighted spin-echo scans) (**Figure 1**) has become established as the most important paraclinical tool not only for diagnosing MS (1) but also for understanding its natural history and for monitoring the efficacy of experimental treatments (2). This is particularly true for relapsing-remitting (RR) MS (RRMS). In patients with RRMS, measures

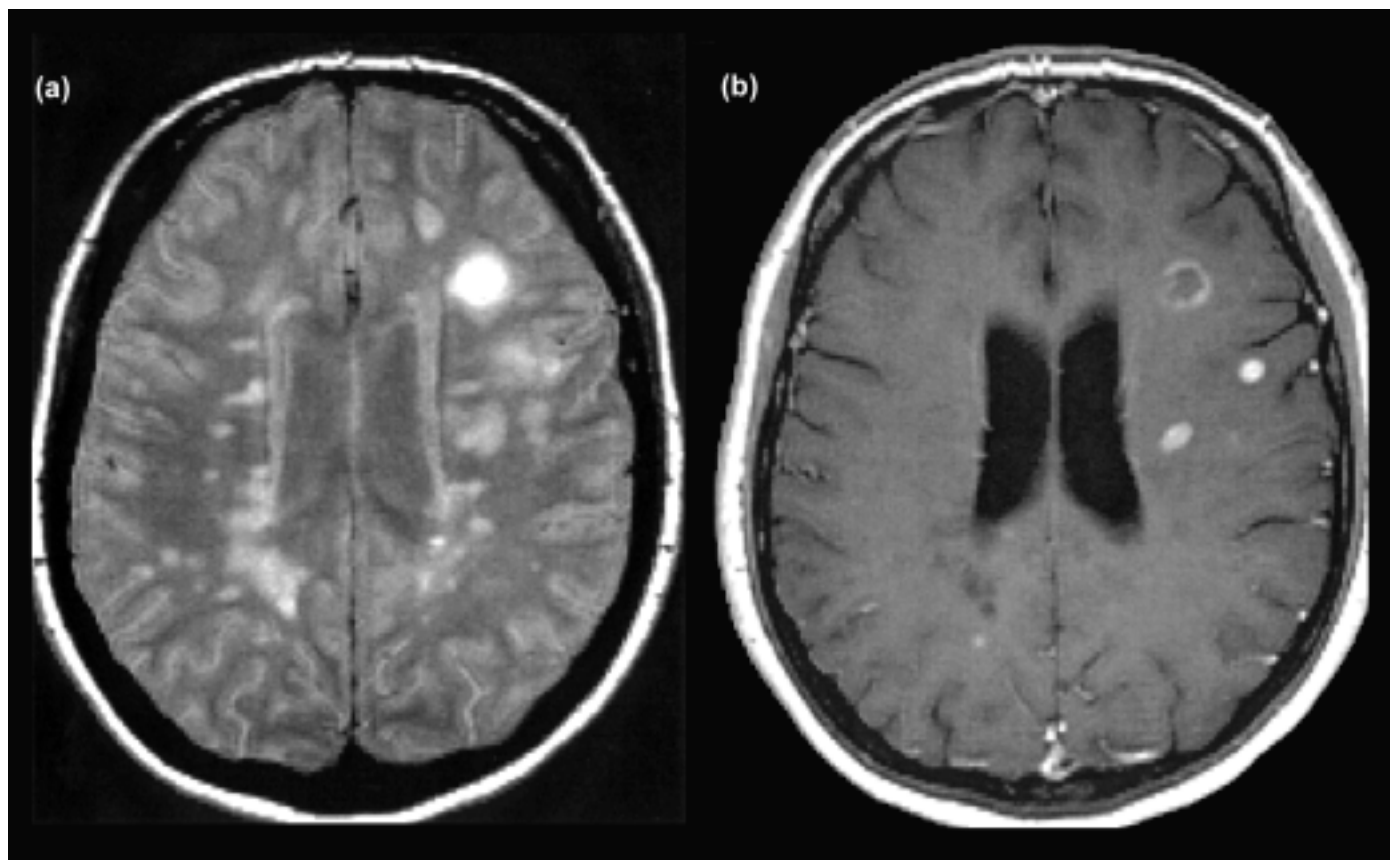


Figure 1.

(a) Axial proton density-weighted spin-echo: multiple hyperintense lesions are visible, suggestive of multifocal white matter pathology, with a predominant involvement of periventricular regions and (b) postcontrast (gadolinium DTPA, 0.1 mmol/kg) T1-weighted spin-echo MRI scans of brain of a patient with clinically definite MS: Some of these lesions are contrast-enhanced, indicating presence of a local blood-brain barrier disruption. This pattern highly suggests MS, which demonstrates a pathological process presence with spatial and temporal dissemination.

derived from conventional MRI scans are much more sensitive than clinical assessments for the detection of disease activity over time (3,4). The application of the measures in large-scale clinical trials has given a fundamental contribution to the approval of disease-modifying treatments for this MS phenotype (5). However, the correlation found between the burden and activity of lesions as seen on conventional MRI scans and the clinical manifestations of RRMS is, at best, moderate (6,7). In addition, conventional MRI findings have a modest predictive value for the subsequent evolution of RRMS patients into a secondary progressive (SP) disease course, which is characterized by the irreversible accumulation of neurological deficits (8).

The discrepancy between conventional MRI and clinical assessment in MS may arise from several factors, including the known limitations of the clinical scoring

scales in terms of reliability and responsiveness (9). However, such a discrepancy can be explained largely by the limited capability of a conventional MRI to quantify the extent and to characterize the nature of tissue damage in MS. Other quantitative MR techniques have the potential to overcome these limitations. Among these techniques, cell-specific imaging, magnetization transfer (MT) MRI (MT-MRI), diffusion-weighted (DW) MRI (DW-MRI), proton MR spectroscopy (^1H -MRS), and functional MRI (fMRI), are those which have been most extensively applied to the assessment of RRMS.

CONVENTIONAL MRI

Conventional MRI findings support the hypothesis that in RRMS, inflammatory processes play a relevant

role in driving the disease activity. This is indicated by the high frequency of contrast-enhancing lesions visible on T1-weighted scans obtained after the administration of gadolinium (Gd) diethylenetriamine pentaacetic acid (DTPA) in untreated patients (3,4). Hyperintense lesions seen on unenhanced T2-weighted MRI are characterized by a variety of underlying pathological substrates, ranging from edema to demyelination and axonal loss (10). However, studies in animals and in humans with MS have demonstrated that the presence of Gd enhancement is always consistent with histopathological findings of blood-brain barrier (BBB) breakdown (11–13). Perivascular inflammation appears to be a necessary precondition to the development of enhancement, since noninflammatory demyelination is unaccompanied by changes of BBB permeability (14,15). Studies in animals with experimental allergic encephalomyelitis (EAE) have shown that Gd enhancement correlates with the number of inflammatory cells within the lesions and mainly represents macrophage activation (16–18). Because chronic relapsing EAE and MS present similar morphological and functional changes, it is, therefore, likely that enhancement in MS lesions predominantly reflects active inflammation. Although an enhanced MRI with a standard dose (SD) of Gd is sensitive enough to detect numerous active lesions in RRMS, there is evidence that a relevant amount of brain inflammation still goes undetected with the use of this technique. With the use of a triple dose (TD) of Gd, 70 to 80 percent more enhancing lesions can be seen than with the use of an SD (19–22). These findings indicate that enhancing MS lesions form a heterogeneous population and those enhancing only after a TD of Gd are characterized by a milder and shorter opening of the BBB, which is probably most associated with less severe inflammatory changes.

Several studies found that the number of enhancing lesions increases shortly before and during clinical relapses and correlates with the MRI activity in the subsequent months (3,23–26). However, the correlation between enhancement frequency and long-term MS clinical evolution is only modest (6,27). It has been reported that when RRMS patients enter the SP phase of the disease, a decrease of MRI-detectable inflammatory activity can be observed (28,29). Because ring-enhancing lesions may reflect a more destructive pathology, their potential association with disease severity has been recently studied in a small cohort of RRMS patients (12,30). The per-

centage of ring-enhancing lesions was found to be correlated with patients' clinical disability, T2 lesion load, and duration of disease and to predict the occurrence of relapses during the baseline period of observation, as well as after a 3-year follow-up. This finding suggests that the pathological process reflected by the presence of these lesions may contribute to a more severe clinical evolution of RRMS.

Conventional MRI scans of the spinal cord can demonstrate the presence of hyperintense lesions in 80 to 90 percent of patients with RRMS and in 30 to 40 percent of patients at the onset of the disease (31–34). The latter figure is higher, however, with patients who present with neurological manifestations attributable to myelopathy (34). The demonstration of spinal cord lesions can help (1) in the differentiation between patients with MS and healthy subjects, in whom intrinsic spinal cord lesions are extremely rare and do not seem to occur as a result of aging per se (35), and (2) in the differential diagnosis between RRMS and other neurological conditions with a similar clinical course, such as of equivocal brain MRI findings (36). On the contrary, monitoring an RRMS course with serial cervical cord scans does not significantly increase the harvest of brain MRI-detectable disease activity, even though acute MS symptoms are caused more often by cord lesions than by brain lesions and cord abnormalities are well correlated with the degree of physical disability (31,37–39).

A conventional MRI also can provide measures with increased specificity to the most destructive aspects of MS pathology. These measures include the burden of T1-hypointense lesions and the assessment of brain or cord atrophy. Hypointense lesions on enhanced T1-weighted images (known as “black holes”) correspond to areas where chronic severe tissue disruption has occurred (40). Although later studies based on much larger samples of patients have not confirmed the initial strong correlation found between the T1-hypointense lesion volume and disability, a general tendency is to consider assessing the extent of black holes as a valid surrogate measure to monitor RRMS evolution (41–43). However, this approach is not without major limitations, including the arbitrary process underlying black hole identification and the inability to provide any information about the pathology of normal-appearing brain tissue.

The measurement of brain atrophy has also been applied to assess the extent of tissue loss in RRMS (44,45). However, the pathological basis of this process is

still unclear. Although it is intuitive that myelin and axonal loss might contribute to the development of atrophy, the role of other factors is largely unexplored. For instance, reactive gliosis can potentially mask considerable tissue loss. Measurements of brain atrophy are also likely to be biased by fluctuations of tissue water content related to important aspects of MS pathology or management, such as the vasogenic edema associated to active lesions or the administration of “anti-inflammatory” treatment. In addition, atrophy is an end-stage phenomenon. Although detection of atrophy is a hard end point, a series of events conceivably would precede MRI-detectable atrophy. Finally, atrophy is relatively insensitive to disease changes. On average, brain volume decreases by about 1 percent yearly in patients with RRMS and other MS phenotypes, despite evidence of highly variable disease activity and characteristics (45–49). Cross-sectional studies have demonstrated robust correlations between cervical cord atrophy and patients’ disability across the different MS subgroups (50–52). A significant increase in cord atrophy over 1 year has been seen in both RRMS and secondary progressive multiple sclerosis (SPMS), with mean reduction in cord area of 2 to 3 percent per annum (51,52). However, no or modest correlations were found between cord area decrease and patients’ disability changes (51,52). All these data indicate that MRI-measurable atrophy provides only a limited view on MS heterogeneity and that large patient samples and long follow-up periods might be needed to detect treatment effect on the rate of atrophy development.

The correlation between the occurrence of conventional MRI-measured brain inflammation and the development of permanent tissue damage in RRMS is still not completely elucidated. Longitudinal studies with monthly or weekly MRI scans indicate that only a minority of MS lesions appears without prior Gd enhancement (3,4,53,54). Molyneux et al. noted a correlation between the number of enhancing lesions and changes of T2 hyperintense lesion load in both RRMS and SPMS patients (25). Other longitudinal studies reported that the frequency and extent of enhancement only partially predict the accumulation of T1 hypointense lesions and are only poorly correlated with the rate of development of brain atrophy, which can proceed despite the capability of some treatments to suppress the inflammatory activity (55–57). Patients with RRMS and SPMS have higher MRI activity than patients with benign courses do (58–60). All these findings indicate

that brain inflammation does significantly, but not exclusively, contribute to the development of tissue damage in RRMS.

Despite the limitations of the conventional MRI, its sensitivity in revealing RRMS activity makes it a valuable tool to monitor the efficacy of treatments with the potential to modify the clinical course of the disease (5). Since an enhanced brain MRI is 5 to 10 times more sensitive than clinical evaluation, its application allows clinical trials to be performed with reduced sample sizes and follow-up durations (3,4,37,59,61). At present, most large-scale multicenter clinical trials in MS are using enhanced MRI as a primary (phase II) or secondary (phase III) outcome measure (5). Future MS trials, however, will most likely be conducted with the reference arm receiving one of the already available treatments instead of placebo. In a recent study, despite using a powerful measure of outcome, such as the count of new Gd-enhancing lesions, Sormani et al. found that the number of patients needed to detect a significant additional effect of a new treatment compared with those already achievable is relatively high (62). This finding suggests the use of outcome measures derived from quantitative MR techniques, based upon their improved sensitivity and specificity to tissue loss, might render comparative MS trials more easily feasible.

CELL-SPECIFIC IMAGING

Gd-enhanced MRI can depict active MS lesions, but it cannot identify the presence of activated inflammatory cells. New methods for cell-specific imaging use markers for tracking various cell components of the immune system (63). A superparamagnetic iron oxide contrast agent, also known as monocrystalline iron oxide nanoparticles (MION), can be used to label lymphocytes *in vitro* and *in vivo* for trafficking studies (63–65). This technique has been applied to study relapsing-remitting EAE in Lewis rats (63,64). A MION-enhanced MRI showed a higher sensitivity for the detection of EAE lesions than that of conventional T2-weighted and Gd-enhanced images. The histopathological analysis revealed the presence of macrophages at the sites where MION-enhanced abnormalities were seen (64). Another study has demonstrated that human mononuclear cells labeled with MION can be detected by MRI *in vitro*, thus suggesting the possibility that the technique could provide new *in vivo* information on lymphocyte and monocyte trafficking in MS lesions

(65). Preliminary data on patients with RRMS have shown that there is a relatively large group of “active” MS lesions that enhance only after either MION or Gd injection (V. Dousset, personal conversation, June 2001). Understanding this “active” MS lesion heterogeneity might add significantly to our understanding of the disease pathobiology.

MAGNETIZATION TRANSFER MRI

MT-MRI is based on interactions between two predominant pools of water hydrogen protons, bound to macromolecules or free. In the central nervous system (CNS), these two pools correspond to the protons in tissue water and in the macromolecules of myelin and other cell membranes. Off-resonance irradiation is applied, which saturates the magnetization of the less mobile protons, but this is transferred to the mobile protons, thus reducing the signal intensity from the observable magnetization. The degree of signal loss depends on the density of the macromolecules in a given tissue. Thus, low MT ratio (MTR) indicates a reduced capacity of the macromolecules in the CNS to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane. Several lines of evidence suggest that a marked reduction of MTR values in MS lesions indicates severe tissue damage (66). The most compelling one comes from a postmortem study showing a strong correlation of MTR values from MS lesions and normal-appearing white matter (NAWM) with the percentage of residual axons and the degree of demyelination (67).

Using MT-MRI and variable frequencies of scanning, several authors have investigated the structural changes of new enhancing MS lesions for time periods ranging from 3 to 36 months (66). The results of all these studies consistently showed that, on average, MTR drops dramatically when the lesions start to enhance and can show a partial or complete recovery in the subsequent 1 to 6 months. The relatively good preservation of axons, which is usual in acute MS lesions, and the rapid and marked increase of the MTR suggest demyelination and remyelination as the most likely pathological mechanisms underlying these short-term MTR changes. Nevertheless, edema and its subsequent resolution can also give rise to the observed pattern of MTR behavior, because of the diluting effect of extracellular water.

However, edema alone seems unlikely to be sufficient to explain these findings, since previous studies showed that edema in the absence of demyelination results in only modest MTR reductions (68,69). MT-MRI studies of individual-enhancing lesions also confirmed the perception that the pathological nature of such lesions and the severity of the associated changes in the inflamed tissue may vary considerably (70–72). These changes seem to be related to the severity and duration of the opening of the BBB (20,73).

These results suggest that the balance between damaging and reparative mechanisms is highly variable since the early phases of MS lesion formation. Consequently, different proportions of lesions with different degrees of structural changes might contribute to the evolution of the disease. At present, however, few data support this concept. A 3-year follow-up study showed that newly formed lesions from patients with SPMS have a more severe MTR deterioration than do those from patients with mildly disabling RRMS (74). Established MS lesions have a wide range of MTR values (70,75). Lower MTR has been reported in black holes than in lesions that are isointense to NAWM on T1-weighted scans, and MTR has been found to be inversely correlated with the degree of hypointensity (71,76). In a longitudinal study with monthly MT-MRI and T1-weighted scans, van Waesberghe et al. found that MS lesions that changed from T1-hypointense to T1-isointense when Gd enhancement ceased also had a significant MTR increase (76), whereas a markedly decreased MTR at the time of initial enhancement was predictive of a persistent T1-weighted hypointensity and lower MTR after 6 months. Decreased MTR has also been found for NAWM from RRMS even in the absence of T2-visible lesions (77–79). These changes are more pronounced in NAWM areas adjacent to focal T2-weighted MS lesions, and this is more evident in SPMS than in RRMS patients (77,78). MTR reductions can also be detected in the NAWM before lesion formation (80). Edema, marked astrocytic proliferation, perivascular inflammation, and demyelination may all account for an increased amount of unbound water in the NAWM and, as a consequence, determine MTR changes (81).

MT-MRI can also be used to assess global MS lesion burden by means of an MTR histogram analysis (82). This highly automated technique can provide several metrics reflecting both macroscopic and microscopic MS pathology in the whole of the brain or in selected regions.

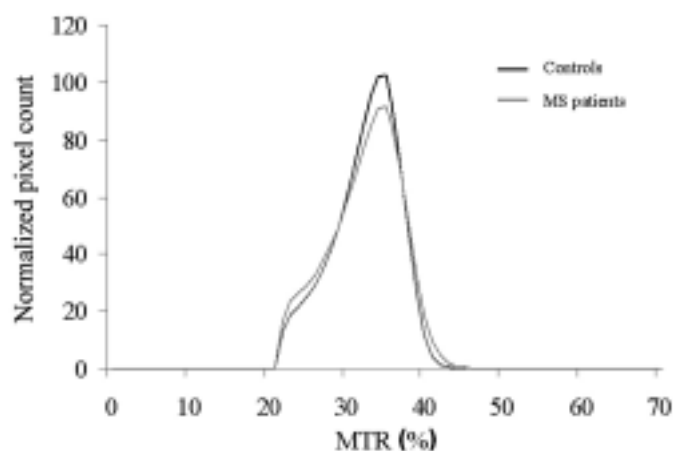


Figure 2.

Histograms of MTR values from whole of brain tissue of 60 patients with MS (gray line) and 40 age- and sex-matched healthy controls (black line). In MS patients, height of MTR histogram peak is reduced when compared to controls, and shape of histogram reflects a shift of pixels toward lower range of MTR values, indicating an increased amount of disrupted brain tissue.

In general, MS patients have lower average MTR, histogram peak height, and position than normal subjects (66,82,83) (**Figure 2**). MTR histogram parameters can be different in the various clinical forms of MS (83). RRMS patients have lower average MTR and peak height than benign MS, whose histograms are similar to those of healthy subjects, while patients with SPMS had the lowest MTR histogram metrics among these three disease subtypes.

Macroscopic lesions segmented on T2-weighted images can be superimposed onto the coregistered MTR maps, and the areas corresponding to the segmented lesions can be masked out, thus obtaining MTR maps of the normal-appearing brain tissue (NABT) in isolation. NABT-MTR histogram measures are different and evolve at a different pace in the major MS clinical phenotypes (49,84). SPMS patients have significantly lower NABT-MTR peak height and position than RRMS patients who, in turn, do not significantly differ from patients with benign MS (84). In addition, NABT changes are only partially correlated with the extent of macroscopic lesions and the severity of intrinsic lesion damage, thus suggesting that NABT pathology does not only reflect Wallerian degeneration of axons transversing large focal abnormalities (84). Reduced NABT-MTR has also been found in patients at presentation with clinically isolated syndromes (CIS) suggestive of MS (85). The

extent of NABT changes in these patients is an independent predictor of subsequent evolution to clinically definite RRMS (85). In patients with RRMS, both whole brain and NABT MTR histogram-derived measures were found to be more sensitive than conventional MRI lesion-load assessment in detecting disease-related changes over a 1-year period (49). Both T2 lesion load and NABT histogram changes were significantly less pronounced in RRMS than in SPMS patients but more evident than in benign cases. These findings indicate that lesion accumulation over time and tissue damage within and outside T2-visible lesions might all be important in determining the evolution from RRMS to SPMS. That the amount of truly normal brain tissue is critical in determining the subsequent evolution of RRMS is shown also by the progressive and significant decline of MTR histogram peak heights observed in these patients, since the peak height of the MTR histogram is considered to be a measure of the residual amount of truly normal tissue (82). However, one must be cautious before drawing firm conclusions, since this study was not longitudinal; as a consequence, patients with RRMS were not the same as those who had SPMS some years later (49). The feasibility of such a longitudinal study is debatable, however, considering the large sample of RRMS patients needed, the duration of the follow-up, and the MRI scanner changes and upgrades that would inevitably occur over such a long time period, which would bias the measurements (66).

Using automated techniques for brain tissue segmentation, MTR histograms can be obtained from the gray matter in isolation (86,87). Recent studies have shown that the average values of gray matter MTR from healthy subjects are similar to those of RRMS patients, which are lower than SPMS patients' values (86,87). Again, this suggests that increased tissue damage is a critical foot-step in determining the evolution from RRMS to SPMS.

Reliable MTR measurements can also be obtained from the cervical cord (88). Cervical cord MTR histogram-derived quantities are significantly lower in patients with SPMS than with RRMS and in patients with locomotor disability than in those without (89). Interestingly, in patients with MS, cord MTR is only partially correlated with brain MTR, suggesting that MS pathology in the cord is not a mere reflection of brain pathology (90). Consequently, measuring cord pathology might be a rewarding exercise in understanding MS pathophysiology.

Although MTR changes of T2-visible lesions and NAWM are not MS-specific, they may give important

diagnostic information. In patients with RRMS and no or few MRI-visible lesions, whole brain MTR histogram-derived metrics are similar to those from healthy controls, but a region-of-interest analysis revealed the presence of tissue damage in several white matter areas (79). The absence of MTR changes in NAWM from patients with migraine or systemic immune-mediate disorders and multiple T2 lesions reasonably excludes a diagnosis of MS (91,92). The absence of MTR changes in the NAWM of patients with optic neuropathy and myelopathy increases the confidence in diagnosing Devic's disease (93).

DIFFUSION-WEIGHTED MRI

Diffusion is the random translational motion of molecules in a fluid system. In CNS, diffusion is influenced by the microstructural components of tissue, including cell membranes and organelles. The diffusion coefficient of biological tissues (which can be measured in vivo by MRI) is, therefore, lower than the diffusion coefficient in free water and, for this reason, is named apparent diffusion coefficient (ADC) (94). Pathological processes that modify tissue integrity, thus resulting in a loss or increased permeability of "restricting" barriers, can determine an increase of the ADC. Since some cellular structures are aligned on the scale of an image pixel, the measurement of diffusion also depends on the direction in which diffusion is measured. Therefore, diffusion measurements can give information about the size, shape, and orientation of tissues (95). A measure of diffusion that is independent of the orientation of structures is provided by the mean diffusivity, \bar{D} , the average of the ADCs measured in three orthogonal directions. A full characterization of diffusion can be obtained in terms of a tensor, a 3×3 matrix that accounts for the correlation existing between molecular displacement along orthogonal directions (96). From the tensor, it is possible to derive \bar{D} , equal to one-third of its trace and some other dimensionless indexes of anisotropy. One of the most used is the fractional anisotropy (FA), which is a measure of deviation from isotropy and reflects the degree of alignment of cellular structures within fiber tracts, as well as their structural integrity. Tissue disruption, by removing structural barriers to water molecular motion, typically causes increased \bar{D} and decreased FA values (96,97).

The pathological elements of MS can alter the permeability or geometry of structural barriers to water diffusion in the brain (**Figure 3**). The application of DW-MRI to MS is, therefore, appealing, since it can provide quantitative estimates of the degree of tissue damage and, therefore, might improve the understanding of the mechanisms leading to irreversible disability. The first report of water diffusion in MS showed that MS lesions had increased ADC values compared to NAWM (98). A subsequent study with more stable diffusion measurements confirmed the preliminary results and demonstrated that NAWM of MS patients had higher ADC values than white matter from controls (99). However, these studies suffered from motion artifacts, limited brain coverage, and the application of diffusion gradients in a single direction.

A more recent study used a navigator echo strategy to correct for motion artifacts in a spin-echo diffusion sequence and to cover larger portions of the brain than previous studies (100). Again, previous results were confirmed, and in addition, a significantly increased \bar{D} was found in T1-hypointense compared to T1-isointense lesions and in nonenhancing compared to enhancing lesions (100). In the latter study, the \bar{D} patterns of lesions did not differ between patients with RRMS and those with other MS phenotypes (100).

Many of the problems with the studies just mentioned (98–101) can be addressed by the use of echo-planar imaging (EPI), which is less prone to motion and permits greater brain coverage, with more diffusion gradient directions, in a given time. Recent studies used such an approach and achieved the following results (102–106):

1. \bar{D} values of NAWM from MS patients are diffusely higher than the corresponding values of white matter from controls.
2. \bar{D} values increase in areas of NAWM subsequently involved by MS lesions (105,107).
3. Values continue to increase at the time of enhancement onset and then decrease rapidly in the next few weeks (105,107).
4. T2-visible lesions have higher \bar{D} values than NAWM.
5. T1-hypointense lesions have the highest \bar{D} values.

Conflicting results have been obtained when comparing enhancing versus nonenhancing lesions: one study confirmed that enhancing lesions have higher \bar{D} values, but others, which were based on larger samples of patients and lesions, did not find any significant differ-

ence between the two lesion groups (103,104,106,108). FA has also been found to be reduced within and outside T2-visible lesions (104,106). Among lesions, FA was found lower in enhancing versus nonenhancing lesions (106). \overline{D} and FA also vary significantly among the various types of enhancing lesions (106–110). All these data suggest a diffuse loss of structural barriers to water molecular motion in NAWM from MS patients. As expected, the loss of structural barriers is even greater in macroscopic lesions, and its magnitude seems to be correlated with the intrinsic tissue damage. Since “inflammatory” changes and gliosis could potentially restrict water molecular motion, myelin and axonal loss are the most likely contributors to the increased \overline{D} and decreased FA in MS NAWM and lesions. The correlation between the average \overline{D} values in the lesions and NAWM has been investigated in one study, and it was found not significant (102). This again suggests that subtle NAWM changes are not merely the result of Wallerian degeneration of axons transversing larger lesions. Recent work has also detected \overline{D} changes in the gray matter of MS patients (87), which are likely to be secondary to cortical damage, since \overline{D} and MTR of basal ganglia from patients with MS do not differ from the corresponding quantities from normal controls (111).

As for MT-MRI, the analysis of diffusion changes can also be performed more globally with the use of \overline{D} histograms (102,112–114). RRMS patients have a significantly higher average \overline{D} and lower histogram peak height than normals. Histogram broadening and the consequent decrease of peak height show that fewer pixels in patients’ brain have normal \overline{D} values. FA histogram-derived metrics were also significantly different from those of normals (112). Conflicting results have been obtained when comparing \overline{D} histogram-derived quantities between MS subtypes, since a preliminary report of higher average \overline{D} values in SPMS than in RRMS patients has not been confirmed by recent studies conducted with larger patient samples (112,114). The magnitude of the correlation between MTR and \overline{D} changes has also been investigated (102). In MS lesions, a strong inverse correlation between average MTR and \overline{D} was found (102). However, this correlation was not found when considering NAWM and the whole of the brain tissue (102). The lack of correlation between MTR and \overline{D} in the brain tissue might be the result of the complex relationship between destructive and reparative mechanisms occur-

ring in the NAWM and their variable effects on MTR and \overline{D} values.

Significant correlations between DW-MRI findings and MS clinical manifestations or disability were not found in some of the earliest studies, perhaps because of the relatively small samples studied, the limited brain coverage, or the narrow range of disabilities that was considered (100–103). With improved DW-MRI technology and increased numbers of patients being studied, correlations between DW-MRI findings and MS clinical manifestations or disability are now emerging (104,112,114,115). Average lesion \overline{D} , but not average lesion FA, was found to be significantly correlated, albeit moderately, with clinical disability in a study of 78 patients with MS, including a subgroup with RRMS (104). The lack of correlation between disability and FA indicates that the loss of overall impediment to diffusional motion is more important than the loss of tissue anisotropy in determining patients’ clinical status. Interestingly, a significant correlation between disability and T2-lesion volume was found in patients with RRMS and not in those with SPMS, where, in turn, there was a correlation between average lesion \overline{D} or FA and disability. These findings suggest that mechanisms leading to disability are likely to be different in patients with RRMS and SPMS. Although caution must be exercised, one might speculate that new lesion formation is a relevant pathological aspect in RRMS, whereas tissue loss in pre-existing lesions is one of the pathological hallmarks of SPMS. Consistently with these observations, water diffusivity in T2-visible lesions has been shown to significantly increase in patients with SPMS when compared to those with RRMS (115). The same authors also found strong correlations between average lesion diffusivity, disability, and disease duration.

PROTON MAGNETIC RESONANCE SPECTROSCOPY

^1H -MRS can complement conventional MRI in the assessment of patients with RRMS by defining simultaneously several chemical correlates of the pathological changes occurring within and outside T2-visible lesions. Water-suppressed proton MR spectra of the normal human brain at long echo times reveal four major resonances: one at 3.2 ppm from tetramethylamines (mainly from choline-containing phospholipids (Cho),

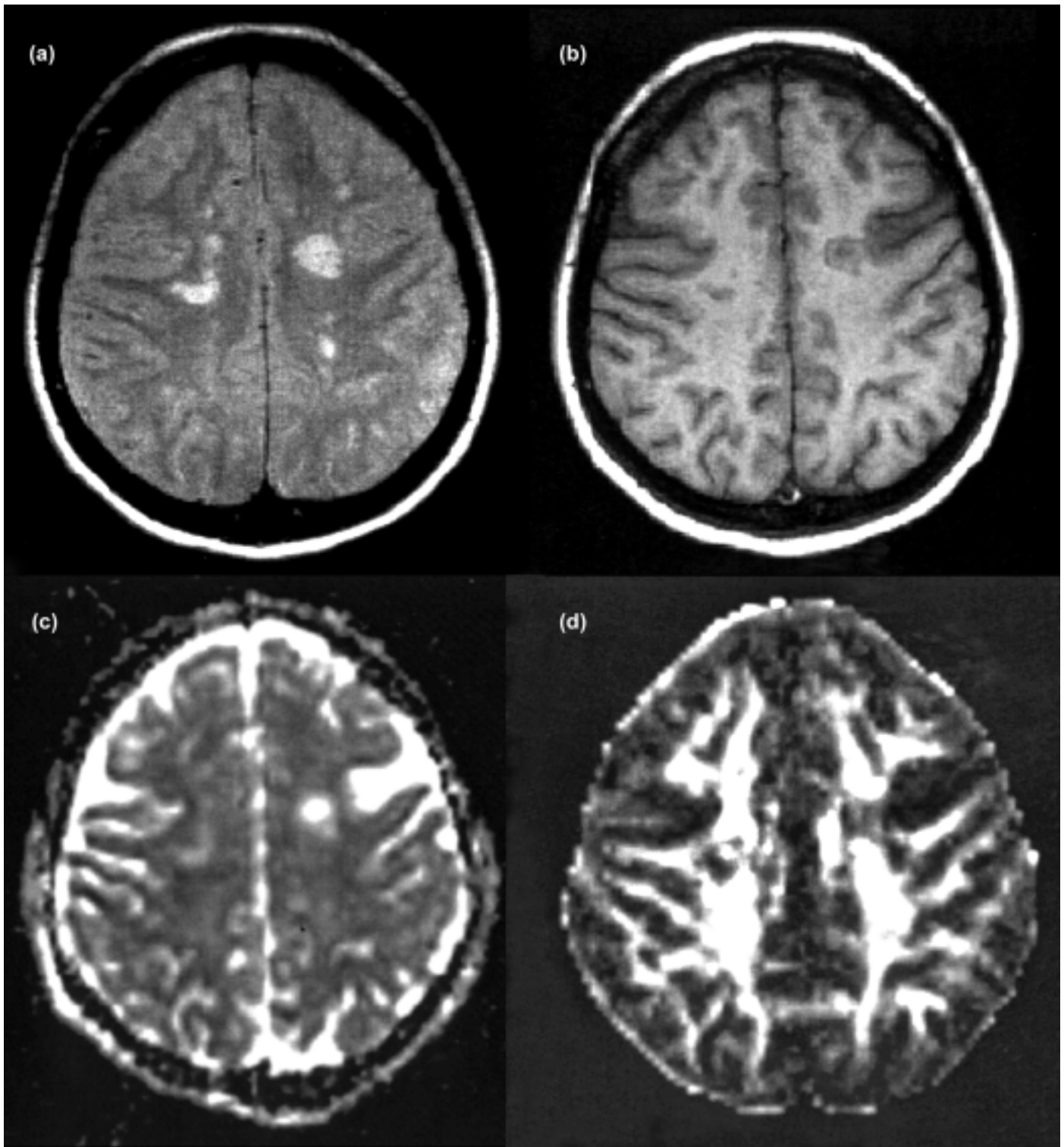


Figure 3.

(a) Axial proton density-weighted spin-echo image: At roof level of lateral ventricles, multiple hyperintense lesions are visible; (b) T1-weighted spin-echo image: Some of these lesions appear T1-hypointense, indicating presence of severe white matter disruption; (c) mean diffusivity (\bar{D}) map: Diffusivity is increased within MS lesions, which appear hyperintense on \bar{D} maps; and (d) fractional anisotropy (FA) map from brain of a patient with MS: Conversely, some of these lesions are visible as areas of decreased signal on FA maps, indicating a local decrease of anisotropic diffusion. Both \bar{D} increase and FA decrease are more pronounced for T1-hypointense MS lesions.

one at 3.0 ppm from creatine and phospho-creatine (Cr)), one at 2.0 ppm from N-acetyl groups (mainly N-acetyl-aspartate (NAA), and one at 1.3 ppm from the methyl resonance of lactate (Lac). Although more technically demanding, additional metabolites (including lipids and myoinositol (mI) can be detected with the use of short-echo time measurements.

^1H -MRS of acute MS lesions at both short and long echo times reveals increases in Cho and Lac resonance intensities since the early phases of the pathological process (116,117). Changes in the resonance intensity of Cho result mainly from increases in the steady-state levels of phosphocholine and glycerol-phosphocholine, both membrane phospholipids that are released during active myelin breakdown. Increases in Lac are likely to reflect the metabolism of inflammatory cells. In large, acute demyelinating lesion decreases of Cr can also be seen (117). Short echo time spectra can detect transient increases of visible lipids, released during myelin breakdown and mI (116,118). All of these changes are usually followed by a decrease in NAA. Since NAA is a metabolite detected almost exclusively in neurons and their processes of the normal adult brain, the decrease in NAA is considered secondary to axonal dysfunction. After the acute phase and over a period of days to weeks, there is a progressive reduction of raised Lac resonance intensities to normal levels. Resonance intensities of Cr also return to normal within a few days. Cho, lipid, and mI resonance intensities return to normal over months. The signal intensity of NAA may remain decreased or show partial recovery, starting soon after the acute phase and lasting for several months (115,119).

Recovery of NAA may be related to resolution of edema, increases in the diameter of previously shrunk axons secondary to remyelination and clearance of inflammatory factors, and reversible metabolic changes in neurons. Although similar decreases in NAA are found in acute enhancing lesions of patients with benign and SPMS, chronic lesions from patients with benign MS have much higher NAA levels than do chronic lesions from SPMS patients, suggesting a greater recovery of NAA in acute lesions from less disabled MS patients (120). Since in acute MS lesions, Gd enhancement is usually ceased by 2 months, the metabolic changes shown by ^1H -MRS can reveal on-going pathology, which would otherwise go undetected. Interestingly, a recent study detected elevated lipid peaks also in NAWM regions. In

some of these regions, such ^1H -MRS abnormality preceded new MS lesion formation (118).

Since changes of axonal viability may be important determinants of functional impairment in MS, one of the major contributions of ^1H -MRS to the understanding of MS is likely to be the quantification of axonal pathology, by measuring NAA levels of lesions and NAWM. The importance of axonal damage in determining clinical deficits in MS has been shown by several authors (117,119–121). The most elegant study is by Davie et al. (122), who found reduced cerebellar NAA levels in patients with MS and cerebellar ataxia similar to that present in those with autosomal dominant spinocerebellar degeneration, whereas the levels of cerebellar NAA were normal in nonataxic MS patients. One can estimate the magnitude of the decrease in brain NAA from large portions of the central brain (119,121–125) or measure it directly using a recently developed technology that enables us to obtain NAA measurements from the whole brain. Decreased NAA levels are found in patients with established MS since the early phases of the disease (120,125). Although the extent of the decrease in NAA/unit lesion volume has been found to be greater in SPMS than in RRMS, the rate of NAA changes with time is faster in RRMS than in SPMS (124). Strong inverse correlations between NAA and disability levels have been found in patients with RRMS (123).

Decreases in NAA are not restricted to T2-visible lesions but also occur in the NAWM adjacent to or distant from them (81). This is consistent with postmortem studies showing axonal loss in the NAWM of MS patients (81). Anterograde and retrograde degeneration of axons transversing large lesions appears to be the most likely pathological substrate, at least in patients with high lesion loads. The role of this factor in determining ^1H -MRS changes in NAWM is supported by the recent finding of dramatic but reversible changes of NAA in the NAWM of the hemisphere contralateral to solitary acute MS lesions (127). However, small focal abnormalities independent of larger T2-visible lesions can also contribute to NAA decreases in NAWM. This seems to be the case for patients with primary progressive MS, who have markedly reduced NAA levels in the NAWM despite the paucity of T2 abnormalities (128). Recently, it has been shown that NAWM from SPMS patients has on average 8.2 percent lower NAA levels than NAWM from RRMS (129). However, in RRMS patients, a progressive reduction of NAWM NAA is detectable over time, and this de-

crease correlates strongly with accumulation of disability (129).

FUNCTIONAL MRI

fMRI measures changes of MRI signal that occur during brain activation as a consequence of the changes in the concentration of deoxygenated hemoglobin. Preliminary studies have suggested that fMRI can be used to monitor the recovery after an MS clinical relapse or to study the reorganization of neural pathways in the brain of patients with established MS (130–132) (**Figure 4**). In patients affected by arm paralysis, a correlation was found between fMRI findings and the severity of the functional deficit (131). A case report study has shown that during recovery after MS relapse, dynamic changes in the patterns of cortical activation with hand movements can be detected with fMRI (130). The observed pathologically decreased lateralization of cortical motor activation becomes less marked with progressive clinical recovery and precedes the normalization of NAA levels in the affected area. This suggests that cortical adaptive responses can compensate for MS-related brain injury to maintain normal motor functions despite lesion damage. On the contrary, the cortical activation was found to be reduced after visual stimulation of the affected eye in MS patients with optic neuritis (132).

More recently, the correlations between fMRI findings and other MRI-derived measures of MS disease burden were assessed to define whether and to what extent fMRI changes are adaptive to the underlying MS pathology. In a sample of clinically stable MS patients with varying degrees of upper-limb motor deficit, Lee et al. found that the patterns of cortical activation during a hand motor task were significantly different from those of healthy controls (133). In MS patients, the increase of ipsilateral cortex activation was significantly correlated with increasing T2 hyperintense lesion load in the contralateral hemisphere. In patients with RRMS and no residual motor disability, fMRI reveals an abnormal pattern of recruitment of elements of the cortical motor network, which is correlated with brain T2 lesion load (134). Using MRS imaging, Reddy et al. found that a similar fMRI finding (i.e., an increased activation of the ipsilateral sensorimotor cortex during finger movement) was strongly correlated with decreases in brain NAA (135). Filippi et al. (136) have found significantly different pat-

terns of movement-related cortical/subcortical activations in RRMS patients with and without fatigue. The results of these studies indicate that fMRI can provide estimates of the cortical adaptive changes that follow MS-related tissue damage. The presence of compensatory mechanisms may help to explain why MRI measures of MS lesion burden are only modestly correlated with clinical measures of disability. In addition, fMRI findings suggest that therapies promoting cortical plasticity might be useful to enhance MS recovery.

CONCLUSIONS

Conventional MRI has markedly increased our ability to detect the macroscopic abnormalities associated with RRMS. New quantitative MR approaches with increased sensitivity to subtle NAWM and gray matter changes and increased specificity to the heterogeneous pathological substrates of MS lesions may give complementary information to conventional MRI. Cell-specific imaging should result in a better definition of the cellular mechanisms associated with MS inflammation. MT-MRI and DW-MRI have the potential to provide relevant information on the structural changes occurring inside and outside T2-visible lesions. ¹H-MRS could add information on the biochemical nature of such changes. fMRI is a promising technique to assess the mechanisms of cortical reorganization, which may follow MS-related injury. The extensive application of all these MR-based techniques should improve the understanding of the mechanisms leading to a later accumulation of irreversible disability in patients with an RR disease course.

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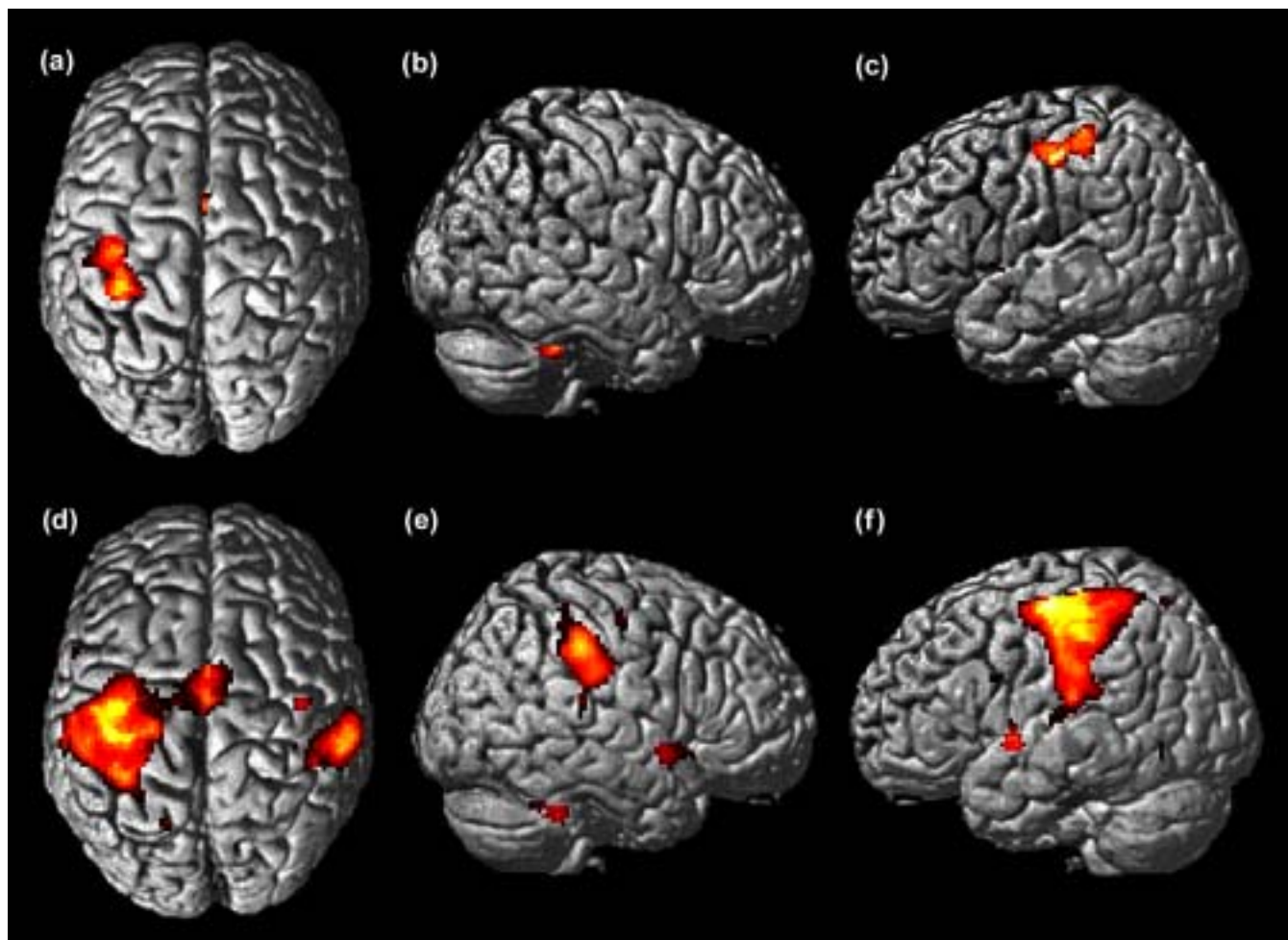


Figure 4.

(a), (b), and (c) are patterns of cortical activations on a rendered brain in 15 right-handed healthy subjects, and (d), (e), and (f) are patterns in 15 right-handed patients with SPMS during performance of a simple motor task with their clinically unimpaired and fully normal-functioning upper-right hands. Compared to controls, a larger and more significant activation of contralateral and ipsilateral primary sensorimotor cortex can be detected in SPMS patients.

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