Multiple sclerosis is an inflammatory, degenerative disease of the central nervous system. The most obvious pathological change in multiple sclerosis is multifocal demyelination of the white matter, but grey matter demyelination may be of equal or even greater importance for its clinical manifestations. In order to assess the pathogenetic role of lesions in the grey and white matter, and to explore the association between demyelinated and non-lesional brain tissue, tools are needed to depict each of these tissue components accurately in vivo. Due to its sensitivity in detecting white matter lesions, T2-weighted magnetic resonance imaging at 1.5 T is important in the diagnosis of multiple sclerosis. However, magnetic resonance imaging at 1.5 T largely fails to detect grey matter lesions. In this study, we used T2-weighted magnetic resonance imaging at 9.4 T to detect grey matter lesions in fixed post-mortem multiple sclerosis motor cortex. Furthermore, we produced T1, T2 and magnetization transfer ratio maps, and correlated these indices with quantitative histology [neuronal density, intensity of immunostaining for myelin basic protein (reflecting myelin content) and phosphorylated neurofilament (reflecting axonal area)] using t-tests and multivariate regression. In 21 tissue samples, 28 cortical grey matter lesions were visible on both T2-weighted magnetic resonance imaging and sections immunostained for myelin basic protein, 15/28 being mixed white and grey matter and 11/28 subpial cortical grey matter lesions; 2/28 cortical grey matter lesions involved all layers of the cortex. Compared with non-lesional cortex, cortical grey matter lesions showed reduction of neuronal density (98/mm², SD = 34/mm²; versus 129/mm², SD = 44; P < 0.01), phosphorylated neurofilament (1/transmittance = 1.16; SD = 0.09 versus 1.24; SD = 0.1; P < 0.01) and magnetization transfer ratio (31.1 pu; SD = 11.9 versus 37.5 pu; SD = 8.7; P = 0.01), and an increase of T2 (25.9: SD = 5 versus 22.6 ms; SD = 4.7; P < 0.01). Associations were detected between phosphorylated neurofilament and myelin basic protein (r = 0.58, P < 0.01), myelin basic protein and T2 (r = −0.59, P < 0.01), and neuronal density...
and $T_1$ ($r = -0.57$, $P < 0.01$). All indices correlated with duration of tissue fixation, however, including the latter in the analysis did not fundamentally affect the associations described. Our data show that $T_2$-weighted magnetic resonance imaging at 9.4 T enables detection of cortical grey matter lesion in post-mortem multiple sclerosis brain. The quantitative associations suggest that in cortical grey matter $T_1$ may be a predictor of neuronal density, and $T_2$ of myelin content (and—secondarily—axons). Successful translation of these results into in vivo studies using high field magnetic resonance imaging (e.g. 3 T and 7 T) will improve the assessment of cortical pathology and thereby have an impact on the diagnosis and natural history studies of patients with multiple sclerosis, as well as clinical trial designs for putative treatments to prevent cortical demyelination and neuronal loss.

**Keywords:** multiple sclerosis; magnetic resonance imaging; neuropathology; neuronal loss; cortical grey matter; grey matter lesions; post mortem brain

**Abbreviations:** IMBP = intensity of myelin basic protein staining; iNF = intensity of phosphorylated neurofilament staining; MBP = myelin basic protein; MTR = magnetization transfer ratio; SMI = Sternberger Monoclonal Incorporated

### Introduction

Multiple sclerosis is an inflammatory and degenerative disease of the central nervous system (CNS) (Compston and Coles, 2008). Until recently, multiple sclerosis has generally been thought of as a disease of the white matter. However, improvements in immunohistochemical and molecular techniques enabling detection of distinct changes in the so-called normal appearing white matter (Zeis et al., 2008) and in the grey matter (Peterson et al., 2001; Albert et al., 2007), as well as magnetic resonance imaging (MRI) modalities allowing quantification of global changes in specific tissue compartments (Fisniku et al., 2008b), have fostered a reappraisal of the disseminated nature of multiple sclerosis across the CNS, described for more than 100 years (Dawson, 1916; Brownell and Hughes, 1962).

Recent studies using post-mortem multiple sclerosis brain revealed the large extent of demyelination in the cerebral (Kutzelnigg et al., 2005) and cerebellar (Kutzelnigg et al., 2007) grey matter. Not only axonal damage (Peterson et al., 2001) and neuronal loss (Peterson et al., 2001; Wegner et al., 2006), but also remyelination (Albert et al., 2007) have all been detected in the cortical grey matter. Although such changes appear to be more extensive in progressive forms of multiple sclerosis (Geurts et al., 2009), cortical atrophy indicating grey matter loss can already be detected early in the disease course (Chard and Miller, 2009b).

Until recently, the detailed assessment of cortical grey matter pathology in multiple sclerosis using tools other than histology seemed impossible as visualization of grey matter lesions using MRI proved to be challenging (Geurts et al., 2005a). Even when using MRI on a high field 4.7 T system a post-mortem magnetic resonance-histology correlation study suggested that up to 80% of cortical grey matter lesions remain undetected (Geurts et al., 2008b).

In this study, we employed MRI at a field strength of 9.4 T and quantitative histology in order to (i) visualize cortical grey matter lesions; and (ii) explore the association between histological features (myelin content, neuronal density, axonal preservation) and quantitative magnetic resonance measures including $T_1$, $T_2$ and magnetization transfer ratio (MTR; an MRI measure of macromolecular protons in biological tissues) in multiple sclerosis brain samples of the motor cortex. The study aimed to identify MRI techniques that would be sensitive in detecting grey matter lesions and specific in measuring key pathological features including loss of neurons and myelin.

### Methods

#### Patients and samples

This study was approved by the Joint Ethics Committees of the University College London Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK. Post-mortem brain from 21 subjects with multiple sclerosis who had signed up to the tissue donation scheme of the UK Multiple Sclerosis Tissue Bank based at Imperial College London (Hammersmith Hospital Campus), London, UK was used for this study. The date and time of death were provided by the Multiple Sclerosis Tissue Bank; duration and course of multiple sclerosis in each subject were obtained from the case notes. The tissue samples were coronal brain slices (thickness ~1 cm) of one hemisphere, all of which included primary motor cortex. Following tissue retrieval from the Multiple Sclerosis Tissue Bank the unfixed brain slices were kept in plastic bags at ~2–8°C before being immersed in 10% formalin solution prior to MRI. The mean time between death and fixation of the brain tissue was 42 (SD = 32) h. The duration of fixation prior to MRI was 746 (SD = 582) days.

Using a magnifying glass, the brain slices were carefully inspected for the presence of lesions involving grey matter of the motor cortex. Tissue blocks (size ~25 × 20 × 5 mm) were dissected of areas fulfilling this criterion and placed in standard histology cassettes. Each cassette containing one tissue block was then inserted into a universal tube and immersed in perfluoropolyether (Perfluorosolv PFS-1; Solvay Solexis, Milan, Italy) to avoid susceptibility artefacts at the tissue air interface.

#### Magnetic resonance imaging

Universal tubes containing tissue blocks were then inserted into a quadrature $^1$H volume magnetic resonance coil (Oxford Research
Histology

After scanning, the tissue blocks—whilst still retained in their respective histology cassette—were taken out of the universal tube and re-immersed in 10% formalin solution before being processed for embedding in paraffin. Paraffin sections were obtained and stained for haematoxylin and eosin and cresyl-violet (neurons), and luxol fast blue. Immunohistochemistry was run on Bond-Max (Vision Biosystems, Hemel Hempstead, UK). The primary antibodies used (all from Covance, Cambridge Bioscience, Cambridge, UK) were anti-myelin basic protein (MBP; 1:1000, 60 min, room temperature) to reflect myelin content, glial fibrillary acidic protein (1:2500, anti-myelin basic protein (MBP; 1:1000, 60 min, room temperature) to visualize phosphorylated and non-phosphorylated neurofilaments of axons, respectively. Paraffin section thickness was 20 μm for cresyl-violet and 10 μm for luxol fast blue. For all other stains section thickness was 5 μm.

Histological image analysis was performed using Image Pro Plus, version 6.2 software (Media Cybernetics, Bethesda, MD, USA) installed on a PC, which received its signal through a KY-F550E colour video camera (JVC) mounted on a Zeiss Axioscope microscope (Carl Zeiss, Welwyn Garden City, UK). The microscope was equipped with a motorized stage control system for the Image Pro Plus stereology plug-in.

Cortical grey matter lesions were identified on sections stained for MBP as clearly distinct, sharply demarcated areas of myelin loss. For this study, we slightly modified an earlier classification system for cortical grey matter lesions (Peterson et al., 2001; Bo et al., 2003b), sub-dividing them into the following groups: (i) type I lesions, affecting subcortical white matter and some or all layers of the cortex; (ii) type II lesions, i.e. very small lesion located within the cortical grey matter; (iii) type III lesions, i.e. sub-pial, usually extensive lesions affecting superficial layers of the cortex; and (iv) type IV lesions, involving all layers of the cortical grey matter but no white matter.

Matching between magnetic resonance imaging and histology

Using the tiling function of the Image Pro Plus stereology toolkit, and a final magnification of ×312.5, tiled images were acquired of sections stained for MBP, and stored as JPEG files. On a separate workstation, these images were then displayed alongside the corresponding T2-weighted image for visual comparison. Once the MRI slice matching best with the histological section was identified, regions of interest were placed on T2-weighted scans in both cortical grey matter lesions and non-lesioned cortex using ImageJ. Hard-copies of these scans, including region of interest, were printed out to serve as a reference during quantitative histology (see below). Regions of interest on T2-weighted magnetic resonance images were registered to the quantitative MRI maps, and values of T1, T2 and MTR obtained.

Using the Image Pro Plus system, the in-plane size of histologically detected cortical grey matter lesions were measured at a final magnification of ×312.5. In type I cortical grey matter lesions, only the portion of the lesion affecting the cortical grey matter was included. Care was taken to match each cortical grey matter lesion area with an equal sized area of non-lesioned cortex for quantitative measurements (see below).

For quantification of myelin content and axonal area in the region of interest their transmittance (T) was calculated using a final magnification of ×250 according to

$$ T = \frac{\text{Transmitted light}}{\text{Incident light}} $$

on MBP and SMI31 stained sections (Schmierer et al., 2004). In order to display results more intuitively (higher values correspond to greater presence of the tissue feature concerned) measurements of T were inverted so that 1/T on sections stained for MBP = intensity of MBP staining (μMBP), and 1/T on sections stained for SMI31 = intensity of phosphorylated neurofilament staining (NI). The incident light intensity was kept constant between 105/256 and 108/256 throughout the measurements. Three measurements (area size: 360 × 280μm) were obtained in each region of interest, and then averaged.

Pyramidal cells were identified on cresyl-violet stained sections according to the following criteria: cresyl-violet stained cytoplasm, a single nucleus, generally larger in shape than surrounding cells, and a non-spherical outline (Fig. 1K) (Wegner et al., 2006). Unbiased sampling of pyramidal cells was performed by using the Image Pro Plus stereology plug-in, which drives the motorized stage controller. A final magnification of ×787.5 and a counting frame of 75 μm² were used to sample pyramidal cells within a region of interest. A section thickness of ×20μm allowed a centrally located 10μm thick virtual ‘brick’ (Howard et al., 1985) to be used in order to rule out overlay of cells in the z-dimension. In order to achieve a coefficient of error ≤0.05 on average ~100 neurons had to be counted on the same section within equal-sized and equal-located region of interest (i.e. taking into account the cortical layers affected by demyelination) of cortical grey matter lesions and non-lesioned cortex (Gundersen and Systems, Oxford, UK; diameter = 25 mm) of a 9.4 T Varian Inova magnetic resonance system (Varian Inc., Palo Alto, CA, USA). After manual shimming seven contiguous slices of 1 mm thickness, using a field of view of 30 × 30 mm were acquired of the following:

(i) 2D spin echo with repetition time = 3000 ms and echo time = 60 ms, matrix size = 256 × 256, 16 averages for high signal-to-noise ratio T1-weighted scans (∼117 μm in plane resolution). Scanning time was 136 min.

(ii) 2D spin echo with echo time = 16 ms and varying repetition time = 480/600/800/1000/2000/4000 ms, matrix size 256 × 192 to obtain T1 maps. Scanning time was 85 min.

(iii) 2D spin echo with repetition time = 2000 ms and varying echo time = 15/24/36/48/60 ms, matrix size 256 × 192 to obtain T2 maps. Scanning time was 85 min.

(iv) 2D gradient echo with application of a saturation radio frequency-pre-pulses at 100 kHz (non-saturated acquisition) and 6 kHz (saturated acquisition) offset from water resonance for MTR maps. Scanning time was 26 min.

Total scan time for the entire protocol was 5 h and 32 min.

T1 and T2 maps were produced using ImageJ (National Institutes of Health, Bethesda, MD, USA; http://rsbweb.nih.gov/ij/) by fitting signal intensities (SI) to the equations, SI = M0 [1−exp(−TR/T1)] and SI = M0 [1−exp(−TE/T2)], respectively, on a pixel-by-pixel basis (M0 ~ constant; TR = repetition time; TE = echo time). MTR maps were produced on a pixel by pixel basis according to the equation: 100(M0 − Mf)/M0, where Mf and M0 are signal intensities with application of the saturation pre-pulse at 6 and 100 kHz offset from water frequency, respectively. MTR values are reported in percent units (pu) as described earlier (Barker et al., 1996).
The density of microglial cells and astrocytes was assessed on tissue sections immuno-labelled for CD68 and glial fibrillary acidic protein, respectively, in cortical grey matter lesion and non-lesioned cortex (each cell type separately) using the following scoring system: 0 = for both CD68 and glial fibrillary acidic protein stained sections virtual absence of the above mentioned cell types; 1 = presence of an occasional CD68 positive microglial cells, or presence of occasional glial fibrillary acidic protein positive astrocytes was also scored; 2 = a moderate density of CD68 positive microglial cells, or an increased number of astrocytes with signs of proliferation (enlarged cell bodies). Fibrillary gliosis in cortical grey matter lesion and non-lesioned cortex was assessed as either 'present' or 'absent'.

Statistics

In order to assess reproducibility of the obtained magnetic resonance indices, five tissue blocks were scanned twice under identical conditions. To assess the reproducibility of histological measurements, repeat measurements were taken of neuronal density, iMBP, and iNF in one-third of the cortical grey matter lesion, and respective areas of non-lesioned cortex. Coefficients of variation were calculated as the square root of the mean variance, divided by the mean of the measurements. For comparison of T1, T2, MTR, iMBP, iNF and neuronal density between cortical grey matter lesion and non-lesioned cortex, Student’s paired t-test was used on patient means averaged over respective tissue compartments (cortical grey matter lesion or non-lesioned cortex). For associations between variables, linear regression was applied to patient means over respective tissue compartments, resulting in two data points (cortical grey matter lesion, non-lesioned cortex) per tissue block. To investigate confounding of the pair-wise associations by other variables, these were entered as covariates into the regression. Analysis was carried out using Statistical Package for the Social Sciences version 16 (SPSS, Chicago, IL, USA).

Results

The average age of the patients at death was 53 (SD = 11) years, and their disease duration 26 (SD = 14) years. The course of the disease was secondary progressive in 18 and primary progressive in three patients. The cause of death was given as breast cancer in one, septicaemia due to urinary tract infection in two, multiple sclerosis in three and pneumonia in the remaining 15 patients.

In 21 tissue blocks, 36 cortical grey matter lesions were detected on MBP stained sections. On average, 1.7 cortical grey matter lesions were detected per tissue block (SD = 1; range = 1–4). Sixteen/36 cortical grey matter lesions were classified as type I (Fig. 1), 18/36 as type III (Fig. 1), and 2/36 as type IV (Fig. 2). No type II cortical grey matter lesion was detected in our sample. The average size of cortical grey matter lesion was 13.2mm² (SD = 17.3 mm²).

The visibility of lesions on MRI was assessed retrospectively, i.e. with knowledge of the corresponding histological results. Of the 36 cortical grey matter lesions detected histologically, 28 were visible on T2-weighted MRI, whereas eight cortical grey matter lesions were not. Seven/36 cortical grey matter lesion (one type I, six type III) were not visible as they were not sufficiently covered by the magnetic resonance coil; 1/36 type III cortical grey matter lesion may not have been visible due to a combination of small size (1.6 mm²) and volume averaging by MRI (Moore, 2003). Excellent correspondence in terms of morphology was achieved between histology and T2-weighted MRI of the 28 cortical grey matter lesions visible on both modalities (Fig. 1 and 2).

The cortical grey matter lesions were generally chronic, hypocellular lesions and signs of microglial proliferation were rare. The presence of microglia was scored ‘0’ in 28, and ‘1’ in the remaining eight of the 36 cortical grey matter lesions. Slightly more—however still relatively few—microglial cells were detected in the non-lesioned cortex: nine areas of non-lesioned cortex were scored ‘0’, 24 as ‘1’, and three were scored ‘2’. Activated astrocytes in the cortical grey matter were in our sample—again—relatively sparse. Scores were as follows: thirteen cortical grey matter lesions were scored ‘0’, 15 were scored ‘1’ and eight were scored ‘2’. The respective figures in non-lesioned cortex were eight (score 0), 21 (score 1) and 7 (score 2). Only a few small patches of fibrillary gliosis were observed in both cortical grey matter lesions and non-lesioned cortex, whereas otherwise protoplasmic astrocytosis dominated (see scoring above).

Table 1 summarizes the means comparisons of T1, T2, MTR, iMBP, iNF and neuronal density in cortical grey matter lesion and non-lesioned cortex. Compared to non-lesioned cortex, cortical grey matter lesions exhibited a reduction of iMBP (by 13%), iNF (7%), pyramidal cells (24%) and MTR (17%), and an increase of T2 by 15%. A trend difference (statistically not significant) was detected for T1, which was 6% higher in cortical grey matter lesion. No significant difference was detected in T1, T2, MTR, iMBP, iNF and neuronal density between type I and type III cortical grey matter lesions (Table 2).

Figure 3 illustrates the most significant associations detected in this study between (i) iNF and iMBP (r = 0.58, P < 0.01); (ii) iMBP and T2 (r = –0.59, P < 0.01); and (iii) T1 and neuronal density (r = –0.57, P < 0.01). Correlation was also detected between (i) iNF and T2 (r = –0.55, P < 0.01); (ii) iMBP and MTR (r = 0.52, P = 0.02); and (iii) T2 and neuronal density (r = –0.51, P = 0.02).

In order to investigate primary and secondary associations in the above relationships, multivariate analysis was performed. Regression of T2 on both iMBP and iNF simultaneously (partial r of iMBP versus T2 = –0.41, P = 0.05; partial r of iNF versus T2 = –0.32, P = 0.11) suggested that the association between iNF and T2 is mainly due to the correlation between iNF and iMBP, and thus secondary to the association between iMBP and T2.

Regression of iMBP on both T2 and magnetization transfer ratio indicated that the association between iMBP and T2 (partial r = –0.47, P = 0.06) is more robust than the association between iMBP and magnetization transfer ratio (partial r = 0.23, P = 0.35).

Regression of neuronal density on T1 and T2 simultaneously revealed associations of similar strength (partial r of T1 versus neuronal density = –0.47, P = 0.02; partial r of T2 versus neuronal density = –0.42, P = 0.02), suggesting neither of these indices as the single best predictor of neuronal density. However, when T2 was regressed on both neuronal density and iMBP only the
Figure 1 Correlation of MRI and histology in post-mortem brain of a patient with multiple sclerosis. Following dissection (A) the tissue block was scanned at 9.4 T to produce high-resolution T2-weighted (T2w) images (B) and maps of T2 (D), T1 (E) and MTR (F). Post-MRI the tissue block was processed for embedding in paraffin and (immuno-)stained for myelin basic protein (MBP; C, G, J), cresyl-violet (Nissl) (H, K) and SMI31 (neurofilaments; I, L). Myelin content and axonal area were estimated at a final magnification of ×250 on sections stained for MBP (J) and SMI31 (L), respectively (see text for further details). Pyramidal cells were quantified at a final magnification of ×785.5 (K). For further details see ‘Methods’ section. The case shown here reveals two cortical grey matter lesion types, type III (blue arrows) and type I (red arrows) (B and C). The red line in (B and C) indicates the border between grey and white matter both being affected by the type I cortical grey matter lesion. Asterisks in (B and C) indicate a multiple sclerosis white matter lesion. Bar = 10 mm (G–I); 200 μm (J, L); 40 μm (K).
The association between iMBP and T2 remained significant (partial $r = 0.51$, $P = 0.01$), whereas the correlation between T2 and neuronal density vanished. This suggests that T2 rather depends on iMBP than on neuronal density, whereas T1 was associated with no tissue feature other than neuronal density.

All quantitative indices investigated in this study correlated with duration of fixation (T1: $r = -0.67$, $P < 0.01$; T2: $r = 0.41$, $P = 0.03$; MTR: $r = -0.75$, $P < 0.01$; iMBP: $r = -0.41$, $P = 0.02$; iNF: $r = -0.52$, $P < 0.01$; neuronal density: $r = 0.44$, $P = 0.02$). Duration of fixation was therefore included throughout the statistical analyses of correlations. Except for the association between T2 and neuronal density, which only became apparent after duration of fixation had been included in the analysis, none of the other correlations detected were fundamentally affected by duration of fixation. No confounding effects were detected between any of the detected correlations and the patient’s age at death, disease duration, course of the disease, or time between death and fixation of the tissue.

The coefficients of variation of quantitative histology indices were as follows: neuronal density: 9% (cortical grey matter lesion) and 8% (non-lesioned cortex); iMBP: 3.1%; (cortical grey matter lesion) and 6.6% (non-lesioned cortex); iNF: 9.1% (cortical grey matter lesion) and 9.7% (non-lesioned cortex). Coefficients of variation of magnetic resonance measures obtained in identical regions of non-lesioned cortex 21 days (SD = 1) apart were T1 = 1.9%; T2 = 2.1% and MTR = 2.6%.

### Discussion

This study revealed two main findings. First, we have shown that in post-mortem tissue of patients with multiple sclerosis, T2-weighted MRI acquired at 9.4 T substantially improves detection of histologically defined subtypes of cortical grey matter lesion. Second, we discovered quantitative associations between three magnetic resonance indices and histological features in post-mortem multiple sclerosis motor cortex: (i) T1 was a predictor of neuronal density; (ii) T2 was a predictor of iMBP and—secondarily—inF; and (iii) MTR also predicted iMBP. However, the association between MTR and iMBP was less robust than between T2 and iMBP.

Multiple sclerosis can affect every region and tissue compartment of the CNS (Chard and Miller, 2009a). T2-weighted MRI at a
magnetic field strength of 1.5 T readily enables detection of white matter lesions in multiple sclerosis, which is helpful in diagnosis (Polman et al., 2005) and disease monitoring (Fisniku et al., 2008a). However, evidence suggests that, in multiple sclerosis, grey matter changes are at least as important as white matter pathology for the accrual of disability (Chard and Miller, 2009b; Geurts et al., 2009). Significant correlation has been reported in patients with multiple sclerosis of disability with grey (but not white) matter atrophy (Fisniku et al., 2008b) and mean grey matter magnetization transfer ratio (Fisniku et al., 2009), which is not surprising given that cortical grey matter lesion can occupy large areas of the brain as detected by histology. For example, Kutzelnigg et al. (2005) described demyelination of up to 68% of the total forebrain, and up to 92% of the total cerebellar cortical area (Kutzelnigg et al., 2007). Sub-pial intracortical (type III) cortical grey matter lesions represent the largest proportion of cortical grey matter lesions, accounting for about 65% of the total cortical demyelination (Bo et al., 2003b; Albert et al., 2007). In our sample, 50% of all cortical grey matter lesions detected were type III lesions.

Until recently, the detailed assessment of cortical grey matter pathology in multiple sclerosis using tools other than histology seemed virtually impossible as visualization of grey matter lesions using MRI proved to be challenging (Geurts et al., 2006a). The reasons for this shortcoming include the location of cortical grey matter lesions, their size and intrinsic properties of lesional and non-lesional multiple sclerosis cortex. As most cortical grey matter lesions affect the outermost layers of the cortex, they are in close contact with the sub-arachnoid space, resulting in susceptibility artefacts at the interface between cortex and CSF. Though cortical grey matter lesions may occur as long ‘ribbons’ of demyelination, these ribbons are often only a few millimetres wide, thereby approaching the limits of magnetic resonance resolution (usually ~1 mm² in plane at 1.5 T). Intrinsic properties complicating cortical grey matter lesion detection include the significantly less pronounced inflammatory response compared to white matter.

![Figure 3](image_url) Association between (A) intensity of staining for myelin basic protein (‘myelin content’) and phosphorylated neurofilaments (‘axonal area’); (B) iMBP and T₂ and (C) neuronal density and T₁ in post-mortem cortical grey matter of patients with multiple sclerosis. Blue markers = cortical grey matter lesions; green markers = non-lesional cortex; T = transmittance.
lesion (Bo et al., 2003a), the lack of blood–brain barrier disruption in cortical grey matter lesion (van Horssen et al., 2007) and the smaller difference—compared to white matter lesion and normal appearing white matter—in relaxation times between cortical grey matter lesions and non-lesioned cortex (Bainbridge et al., 2004).

Some progress in cortical grey matter lesion detection has been achieved through the introduction of inversion recovery (Boggild et al., 1996), and particularly of double inversion recovery MRI, with the latter revealing up to five times more cortical lesions than conventional T2-weighted MRI at 1.5 T in one in vivo study (Geurts et al., 2005b). Nevertheless, at 1.5 T cortical grey matter lesion—particularly those solely located within the cortex (i.e. types II–IV)—are massively under-reported (Geurts et al., 2005b).

MRI at field strengths higher than 1.5 T has been variably successful in detecting cortical lesions. Geurts et al. (2008a) compared the sensitivity for cortical lesions of T2-weighted and inversion recovery MRI acquired at 1.5 T with proton density weighted MRI at 4.7 T using post-mortem brain slices obtained from 10 subjects with multiple sclerosis. Despite higher signal-to-noise ratio, reduced slice thickness and superior in plane resolution (380 × 380 μm²) provided by a combination of 4.7 T and a longer scan time compared to the 1.5 T imaging (500 × 500 μm²), the contrast between cortical grey matter lesions and non-lesioned cortex was not sufficient to identify cortical grey matter lesions reliably. This presumably reflects the relative low density of myelin in the upper cortical layers and the correspondingly smaller absolute change that occurs when cortical grey matter lesions develop, especially as lesions in these layers are largely non-inflammatory in nature. For cortical grey matter lesions that are larger than 1 mm, detectability might be improved by increasing signal-to-noise ratio, at the relative cost of lower resolution. However, our data suggest that for smaller lesions an improvement in both image characteristics is conducive for cortical grey matter lesion detection.

In a recent study, Bagnato et al. (2009) applied a multimodal protocol including T1-based magnetization prepared rapid acquisition gradient echo, proton density weighted and T2-weighted MRI acquired at 3 T to post-mortem brain slices of one patient with multiple sclerosis. Forty neocortical lesions were detected on MRI scans, of which 20% were visible in all MRI modalities, 7.5% only on the proton density weighted images, and 12.5% only on T2-weighted images, suggesting that a combination of inversion recovery and conventional acquisitions at 3 T may allow a more comprehensive estimate of the number and extent of cortical grey matter lesion (Bagnato et al., 2009).

Kangarlu et al. (2007) studied post-mortem brain of one subject with multiple sclerosis using magnetic resonance systems operating at 1.5 T and 8 T, and correlated their findings with histology. Magnetic resonance sequences employed included T1 and T2-weighted images at both 1.5 T and 8 T, and fluid attenuation inversion recovery at 1.5 T. The images included in their paper (MRI and histology) indicate that MRI at 8 T may allow detection of various histological subtypes of cortical grey matter lesion. However, no quantitative analysis of the data was undertaken.

Our study employing an even higher field strength of 9.4 T confirms that the gain in signal-to-noise ratio and resolution (here ~117 μm² in plane) achievable at ultra-high magnetic field strength allows reliable detection of multiple sclerosis cortical grey matter lesions on standard spin echo T2-weighted images. The basis for the excellent contrast in our sample between cortical grey matter lesion and non-lesioned cortex appeared to be the significant difference in T2 between these tissue compartments of, on average, 3.3 ms (~14%). In line with this observation, we detected a robust association between T2 and iMBP (Fig. 3B) suggesting that quantitative T2 may be a useful magnetic resonance index to predict changes in myelin content of multiple sclerosis cortex. Of note, in an earlier MRI/pathology study of formalin fixed brain using a 1.5 T magnetic resonance system, T2 was the strongest predictor of myelin content in multiple sclerosis white matter (Schmierer et al., 2008). In the same study—and similar to our findings in multiple sclerosis cortex—T2 was superior to MTR as a predictor of myelin in the white matter once the tissue had been fixed in formalin.

Our study revealed that T1 may provide a tool with which to estimate pyramidal cell loss in multiple sclerosis cortex (Fig. 3C). T1 is related to macromolecule concentration, water binding and water content (Gowland and Stevenson, 2003). Loss of neuronal cell bodies is likely to result in a net loss of macromolecules in affected regions of the cortex, thereby increasing the amount of free water, which subsequently leads to an increase of T1. Given a correlation coefficient in our study of r = −0.57 (P < 0.01) for the relationship between neuronal density and T1, the latter may be influenced by factors other than neuronal density; for example neuronal size, which should be explored in future studies. In our sample, the degree of microglial activation and astrocytosis in the cortical grey matter did not confound any of the relationships detected. This may be due to (i) the generally limited cellularity of cortical grey matter (the proportion of microglia and astrocytes among non-neuronal cells of the human cortex is approximately 6% and 19%, respectively) (Pelvig et al., 2008); (ii) the less pronounced inflammatory response in cortical grey matter lesions compared to white matter lesions (Bo et al., 2006); and (iii) the chronicity of multiple sclerosis in the brain samples used.

A reduction of neuronal density in cortical grey matter lesion of 10% has been described before in a study by Wegner et al. (2006). The reduction (compared to non-lesioned cortex) detected in our study was ~24%, i.e. over twice the figure reported by Wegner et al. although their study also focused on pyramidal neurons (Wegner et al., 2006). The discrepancy between studies may be due to the cortical layers used for cell counting; whereas Wegner et al. (2006) focused on layers V/VI, pyramidal cell densities in our study are based on counts throughout the cortical ribbon. More superficial layers (with respect to pyramidal cells of major interest: layers II/III) may be more susceptible to damage with reportedly up to >50% neuronal loss in layer III in some cases of multiple sclerosis (personal communication, Richard Reynolds, Imperial College London).

The lack of a difference in our sample for any quantitative index acquired between type I cortical grey matter lesions (affecting basal layers of the cortex and adjacent white matter) and subpial type III cortical grey matter lesions (Table 2) does not underpin the concept of variation in neuronal susceptibility. However, our study was not powered to address this question specifically, and therefore no final conclusion can be drawn.
Limitations of the study

There were some limitations to this study. First, control brain from patients with non-neurological conditions was not included, so we did not have any direct comparison with ‘normal’ cortex. It is noteworthy, however, that Wegner et al. (2006) found no difference of neuronal density between normal controls and non-lesioned cortex, suggesting that neuronal degeneration in multiple sclerosis may significantly depend on the presence of cortical grey matter lesions.

Second, immersing the tissue in a water-free liquid (perfluoropolyether) helped to avoid susceptibility artefacts that would have been produced at the air-surface interface. This is obviously not possible for experiments in vivo, where suppression of the signal caused by CSF currently has to be achieved by inversion recovery techniques (Nelson et al., 2008).

Third, although we did not formally test this hypothesis, formalin fixation has certainly affected tissue properties and—as a result—the quantitative MRI data obtained. The molecular mechanism of fixation is not well understood. It is thought that formaldehyde solutions react with macromolecules to form intra- and intermolecular cross-links. The cross-linking of macromolecules alters the characteristics of tissues and results in a gel that largely retains the cellular constituents in their topographic relationships to each other (Schmierer et al., 2008). Due to its association with MRI and histology indices, duration of fixation was a significant potential confounder. However, including duration of fixation in the analysis did not alter any of the detected relationships between variables. Nevertheless, using unfixed tissue could be advantageous as interpretation of data requires only one translational step (from post-mortem to in vivo or vice versa), whereas fixation introduces an additional source of uncertainty. An example of the latter is the association in post-mortem multiple sclerosis white matter at 1.5 T between MTR and myelin content, which—having been strong in unfixed condition ($r=0.83, P<0.01$)—significantly weakens once the tissue has been fixed (Schmierer et al., 2008).

Translation into the clinical arena

Our study clearly shows that MRI at 9.4 T allows detection of cortical grey matter lesions, and reasonable estimates of neuronal density as well as myelin content in multiple sclerosis cortex. Will it be possible to translate these results into the clinical arena? Preliminary studies on patients with multiple sclerosis at 7 T in vivo certainly suggest that reliable detection of cortical grey matter lesions may be feasible at this field strength (Kollia et al., 2009; Mainero et al., 2009). Moreover, novel strategies such as driven equilibrium single pulse observation of $T_1$ and $T_2$ have been developed to provide excellent resolution alongside significant reduction in scanning times for whole brain mapping of $T_1$ and $T_2$ (Deoni et al., 2005). These techniques are currently being developed further for use at field strengths of 3 T and higher suggesting that high resolution mapping of relaxation times in a clinical setting to glean quantitative information about specific cell types and other tissue features may become feasible in the not so distant future (Deoni, 2007). Successful translation of our results into in vivo studies will improve the assessment of cortical pathology with the likelihood of effects on diagnosis and natural history studies of patients with multiple sclerosis, and clinical trial designs for putative treatments to prevent cortical demyelination and neuronal loss.

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