A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 T imaging

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We used a surface-based analysis of T2* relaxation rates at 7 T magnetic resonance imaging, which allows sampling quantitative T2* throughout the cortical width, to map in vivo the spatial distribution of intracortical pathology in multiple sclerosis. Ultra-high resolution quantitative T2* maps were obtained in 10 subjects with clinically isolated syndrome/early multiple sclerosis (≤3 years disease duration), 18 subjects with relapsing-remitting multiple sclerosis (≥4 years disease duration), 13 subjects with secondary progressive multiple sclerosis, and in 17 age-matched healthy controls. Quantitative T2* maps were registered to anatomical cortical surfaces for sampling T2* at 25%, 50% and 75% depth from the pial surface. Differences in laminar quantitative T2* between each patient group and controls were assessed using general linear model (P < 0.05 corrected for multiple comparisons). In all 41 multiple sclerosis cases, we tested for associations between laminar quantitative T2*, neurological disability, Multiple Sclerosis Severity Score, cortical thickness, and white matter lesions. In patients, we measured T2* in intracortical lesions and in the intracortical portion of leukocortical lesions visually detected on 7 T scans. Cortical lesional T2* was compared with patients’ normal-appearing cortical grey matter T2* (paired t-test) and with mean cortical T2* in controls (linear regression using age as nuisance factor). Subjects with multiple sclerosis exhibited relative to controls, independent from cortical thickness, significantly increased T2*, consistent with cortical myelin and iron loss. In early disease, T2* changes were focal and mainly confined at 25% depth, and in cortical sulci. In later disease stages T2* changes involved deeper cortical laminae, multiple cortical areas and gyri. In patients, T2* in intracortical and leukocortical lesions was increased compared with normal-appearing cortical grey matter (P < 10−10 and P < 10−7), and mean cortical T2* in controls (P < 10−5 and P < 10−4). In secondary progressive multiple sclerosis, T2* in normal-appearing cortical grey matter was significantly increased relative to controls (P < 0.001). Laminar T2* changes may, thus, result from cortical pathology within and outside focal cortical lesions. Neurological disability and Multiple Sclerosis Severity Score correlated each with the degree of laminar quantitative T2* changes, independently from white matter lesions, the greatest association being at 25% depth, while they did not correlate with cortical thickness and volume. These findings demonstrate a gradient in the expression of cortical pathology throughout stages of multiple sclerosis, which was associated with worse disability and provides in vivo evidence for the existence of a cortical pathological process driven from the pial surface.
Introduction

Multiple sclerosis is an inflammatory demyelinating and neurodegenerative disorder of the CNS, and the leading cause of non-traumatic disability in young adults in Western countries. Histopathological examinations of multiple sclerosis brains indicate that subpial demyelinating lesions, which extend intracortically from the pia mater without reaching the white matter, are potential biomarkers of multiple sclerosis progression (Peterson et al., 2001; Magliozzi et al., 2010; Reynolds et al., 2011). As cortical lesions appeared topographically related to focal meningeal inflammation in some pathological studies of chronic progressive multiple sclerosis (Magliozzi et al., 2007, 2010; Howell et al., 2011), it has been hypothesized that cortical demyelination in multiple sclerosis may be driven by organized meningeal inflammation, accompanied by a decreasing gradient of demyelination away from the pial surface. Histopathological evidence that the cortex can be the site of inflammatory demyelinating lesions near the time of multiple sclerosis onset (Lucchinetti et al., 2011) further supports the existence of an early pathological process that primarily targets the cortex, independently from white matter.

Although largely undetected on conventional MRI scans, cortical lesions, including the subpial type, have been imaged in vivo with improved sensitivity and spatial specificity at ultra-high field 7 T MRI (Filippi et al., 2014). We previously showed that a surface-based analysis of cortical $T_2^*$-weighted signal from 7 T $T_2^*$ gradient-echo images combined with a multichannel radiofrequency coil can disclose subpial $T_2^*$ signal abnormalities in subjects with multiple sclerosis relative to healthy subjects (Cohen-Adad et al., 2011), providing in vivo evidence for the existence of diffuse subpial pathology in multiple sclerosis, previously reported only post-mortem.

Surface-based estimation of cortical $T_2^*$ relaxation rates provides a quantitative estimate of the biophysical changes underlying tissue integrity (Cohen-Adad et al., 2012). This measure is less dependent of the technical limitations that affect measurements of $T_2^*$-weighted signal at ultra-high field MRI, and of potential biases that arise from cortical lesion detection based on visual inspection of scans. In the healthy brain, $T_2^*$ relaxation time ($1/R_2^*$) inversely correlates with myelin and iron content (Langkammer et al., 2010; Li et al., 2011). In both white matter and cortical multiple sclerosis lesions, histopathological–magnetic resonance correlations showed that demyelination and iron loss induce an increase in $T_2^*$ relaxation time (Yao et al., 2012, 2014), whereas iron accumulation relates to shorter $T_2^*$ (Bagnato et al., 2011).

We demonstrated that surface-based mapping of quantitative $T_2^*$ as a function of cortical depth (laminar analysis) from ultra-high resolution gradient echo 7 T MRI images is highly reproducible (Govindarajan et al., 2015) and could prove useful for studying the myelo-architecture of the cortex in vivo (Cohen-Adad et al., 2012), and for characterizing conditions associated with changes in cortical myelin and/or iron concentration.

In this study, we used a surface-based laminar analysis of 7 T quantitative $T_2^*$ to test the following hypotheses: (i) cortical pathology in multiple sclerosis is associated with changes in quantitative $T_2^*$, which differ across disease stages: quantitative $T_2^*$ abnormalities mainly involve the outer cortical layers and sulci in early disease; while they can also be detected in deeper cortical layers and gyri in later stages and progressive multiple sclerosis; and (ii) neurological disability and multiple sclerosis severity correlate with the degree of laminar quantitative $T_2^*$ abnormalities. A further aim was to investigate the contribution of cortical laminar pathology, as measured by quantitative $T_2^*$, to cortical thinning in multiple sclerosis. Finally, we measured quantitative $T_2^*$ in multiple sclerosis cortical lesions, detected visually on 7 T scans, as well as in normal-appearing cortical grey matter (NACGM), to better understand their role in determining laminar quantitative $T_2^*$ changes in multiple sclerosis.

Materials and methods

Subjects

Forty-one patients [29 females; mean age $= 43.2 \pm 8.8$ standard deviation (SD) years] were prospectively included in the study. Eligibility criteria were: age between 18 and 60 years, a diagnosis of clinically isolated syndrome or multiple sclerosis (McDonald et al., 2001). Patients were enrolled according to three disease phenotypic categories: (i) clinically isolated syndrome - early multiple sclerosis ($n = 10$); $\leq$ 3 years disease duration; (ii) relapsing remitting multiple sclerosis (RRMS, $n = 18$) with $\geq$ 4 years disease duration; and (iii) secondary progressive multiple sclerosis (SPMS, $n = 13$) (Lublin and Reingold, 1996).
Exclusion criteria for multiple sclerosis subjects included a clinical relapse within 3 months of enrolment, corticosteroid therapy within 1 month of scanning, and other neurologic and/or significant psychiatric disease. Neurological disability in patients was assessed using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). The Multiple Sclerosis Severity Score (MSSS) was calculated on each patient using their EDSS and duration from onset of multiple sclerosis symptoms, as previously detailed (Roxburgh et al., 2005). Thirty-three of 41 patients were on treatment with disease-modifying agents for at least 6 months, while the remaining eight subjects were not receiving any therapy for multiple sclerosis.

Seventeen age-matched healthy volunteers (nine females; mean age = 39.3 ± 8.8 SD years) served as controls. General exclusion criteria included significant psychiatric and/or neurological disease (other than multiple sclerosis for patients), major medical comorbidity, pregnancy, and contraindications for MRI.

Subjects gave their written informed consent to participate in the study and the local Ethics Committee of our Institution approved the study procedures.

MRI data acquisition

All subjects underwent, on a 7 T Siemens whole-body scanner using a custom-built 32-channel phased array coil, acquisition of: (i) multi-echo 2D FLASH T$_2^*$-weighted spoiled gradient-echo imaging, repetition time = 2210 ms, echo time = 6.44 + 3.32 n [n = 0, ..., 11] ms, flip angle = 55°, two slabs of 40 slices each to cover the supratentorial brain, field of view = 192 × 168 mm$^2$, resolution = 0.33 × 0.33 × 1 mm$^3$ (25% gap), bandwidth = 335 Hz/pixel, acquisition time for each slab = ~10 min; (ii) a T$_1$-weighted 3D magnetization-prepared rapid acquisition gradient echo sequence (MPRAGE, repetition time/inversion time/echo time = 2600/1100/3.26 ms, flip angle = 9°, field of view = 174 × 192 mm$^2$, resolution = 0.60 × 0.60 × 1.5 mm$^3$, bandwidth = 200 Hz/pixel, acquisition time = 5.5 min) for co-registration of 7 T gradient-echo data with cortical surfaces; and (iii) a single-echo 2D FLASH T$_2^*$-weighted spoiled gradient-echo pulse sequence (repetition time/echo time = 1700/21.8 ms, the other parameters being identical to the multi-echo 2D FLASH T$_2^*$ sequence).

In addition to the 7 T session, all subjects were scanned once on a 3 T Siemens scanner (Tim Trio) using the Siemens 32-channel coil to acquire a structural scan with a 3D magnetization-prepared rapid acquisition with multiple gradient echoes (MEMPR) [repetition time/inversion time = 2530/1200 ms, echo time = 1.7, 3.6, 5.4, 7.3 ms, flip angle = 7°, field of view = 230 × 230 mm$^2$, resolution = 0.9 × 0.9 × 0.9 mm$^3$, bandwidth = 651 Hz/pixel, acquisition time = ~6.5 min] for cortical surface reconstruction, co-registration with 7 T data, and cortical thickness and cortical volume estimation.

MRI data processing

White matter lesion volume

White matter lesion volume (mm$^3$) was assessed from white matter lesions segmented on magnitude images from 7 T single-echo FLASH T$_2^*$ scans using a semi-automated method implemented in 3D Slicer version 4.2.0 (http://www.slicer.org).

Cortical surface reconstruction, cortical thickness and volume estimation

Pial and white matter surfaces, cortical thickness maps, and cortical volumes were obtained using the software FreeSurfer, version 5.3.0 (http://surfer.nmr.mgh.harvard.edu/), according to a multi-step procedure that calculates the grey matter/white matter border and the CSF/grey matter (pial) border in the 3D MEMPR volume (Dale et al., 1999). Topological defects in cortical surfaces due to white matter and leukocortical lesions were corrected using a semi-automated procedure with lesions filling.

Mean cortical thickness was measured in each subject as the distance (mm) between the grey matter/white matter boundary and the pial surface (Fischl and Dale, 2000). For each subject, we also estimated the mean normalized cortical volume defined as the ratio between mean cortical volume and total intracranial volume as assessed in FreeSurfer.

For vertex-by-vertex surface-based cortical thickness analyses across subjects’ groups, each individual subject’s surface was smoothed using a 10 mm full-width at half-maximum Gaussian kernel, and subsequently registered to a surface template ’fsaverage’ using FreeSurfer.

Quantitative T$_2^*$ mapping along the cortex

T$_2^*$ signal was corrected at each voxel for susceptibility-induced through-slice dephasing as described previously (Cohen-Adad et al., 2012). A Levenberg–Marquardt nonlinear regression model was then used to fit voxel-wise the corrected T$_2^*$ signal versus echo time; R$^2$ goodness of fit was measured, and voxels with poor fit (R$^2$ < 0.8) were excluded from further analyses. Poor fits were typically present in lower brain regions including the temporal pole, and in regions at the tissue/air interface (close to the sinuses).

Each individual T$_2^*$ map was registered to the corresponding 3 T cortical surface using a boundary-based registration algorithm (Greve and Fischl, 2009), as previously detailed (Cohen-Adad et al., 2012). The registered T$_2^*$ data were concatenated into a whole brain volume using FreeSurfer tools and resampled at 0.33 mm$^3$ isotropic resolution. T$_2^*$ was sampled along the entire cortex in right and left hemispheres at 25%, 50% and 75% depth from the pial surface (0% depth) towards grey matter/white matter boundary (100% depth) over the surface of each individual subject, and smoothed along the cortical surface using a 5 mm full-width at half-maximum Gaussian kernel. Given that cortical thickness varies throughout the cortex, depth was not defined as an absolute distance between the pial and the grey matter/white matter boundary, but rather as a relative distance between the pial and white matter surface. We used an equidistant model for sampling quantitative T$_2^*$ within the cortex as it has shown excellent reproducibility (Govindarajan et al., 2015), and has been found comparable to equivolume modeling when investigating in vivo data with spatial resolution similar to those used in our study (Waehnert et al., 2014).

Because it is unclear whether leukocortical lesions, which extend across cortex and white matter but do not reach the pial surface, originate from cortex or white matter, such lesions were not masked in quantitative T$_2^*$ maps.
For group analyses, all subjects’ surfaces were then registered to the surface template ‘fsaverage’ using FreeSurfer.

**Quantitative T$_{2}^{*}$ in focal cortical lesions**

Using Slicer, two raters, blinded to patients’ demographic and clinical data, segmented by consensus, on magnitude images from 7 T single-echo FLASH T$_{2}^{*}$, focal cortical multiple sclerosis lesions that appeared as focal cortical hyperintensities extending for at least three voxels and across two consecutive slices. In subjects with multiple sclerosis, focal cortical lesions were characterized as (i) intracortical lesions including lesions originating from the pial surface and extending at different depths throughout the cortical width (type III-IV lesions), and type II lesions (Peterson et al., 2001; Bo et al., 2003b); and (ii) leukocortical lesions extending through grey matter/white matter without reaching the pial surface. Areas of NACGM were also identified in each multiple sclerosis subject throughout the cortex.

Subsequently, in each subject, intracortical lesions, leukocortical lesions and NACGM masks were created and coregistered, using a boundary-based registration method (Greve and Fischl, 2009), to the corresponding cortical quantitative T$_{2}^{*}$ maps for estimating mean quantitative T$_{2}^{*}$ (ms) in each mask. Given that the focus of the study was the cortex, for leukocortical lesions quantitative T$_{2}^{*}$ was measured only in the intracortical portion of the mask.

**Statistical methods**

A general linear model (GLM) was run on a vertex-by-vertex basis across the whole cortex, using Freesurfer tools, to assess: (i) differences in quantitative T$_{2}^{*}$ at each depth from the pial surface (25%, 50%, 75%) between controls and the three subgroups of patients (early multiple sclerosis, RRMS, SPMS); (ii) the relationship, in all patients, between quantitative T$_{2}^{*}$ at 25%, 50% and 75% depth and EDSS and MSSS; (iii) cortical thickness differences between patients and controls; and (iv) the relationship between quantitative T$_{2}^{*}$ at each depth and cortical thickness across the whole cortex using the per-vertex regressor option in Freesurfer, which allows testing at the vertex level for possible correlations between two imaging modalities. Age was used as a nuisance factor (covariate of no interest) in all GLM analyses.

For all surface-based group analyses we performed a cluster-wise correction for multiple comparisons using a Monte-Carlo simulation with 10 000 iterations (Hagler et al., 2006). Localization of significant clusters across the cortex was performed using the Desikan atlas in Freesurfer.

For comparisons of conventional MRI metrics (whole mean cortical thickness, normalized cortical volume, and white matter lesion volume) across patients’ groups relative to controls and correlations with clinical variables, analysis of covariance (ANCOVA) controlled for age, and Spearman rank correlation coefficient were performed using the software R (version 2.13.1). In patients, differences between mean quantitative T$_{2}^{*}$ in intracortical lesions, and leukocortical lesions relative to mean quantitative T$_{2}^{*}$ in NACGM were assessed using paired t-test; differences between mean quantitative T$_{2}^{*}$ in intracortical lesions, leukocortical lesions, NACGM in patients and mean cortical quantitative T$_{2}^{*}$ in healthy subjects were assessed using linear regression using age as covariate of no interest (R software, version 2.13.1). For all analyses, statistically significant threshold was set at P-value < 0.05.

**Sulci and gyri analysis**

Using the Freesurfer masks of gyri and sulci, we assessed whether quantitative T$_{2}^{*}$ changes in patients preferentially involved cortical sulci or cortical gyri. The masks of gyri and sulci were applied to all clusters that exhibited significant differences in quantitative T$_{2}^{*}$ at the GLM (corrected P < 0.05) in each patient subgroup (early multiple sclerosis, RRMS, SPMS) relative to controls, at each depth (25%, 50%, 75%). In each mask the surface area (mm$^2$) of vertices exhibiting significant quantitative T$_{2}^{*}$ changes was computed. For comparison, given that the surface area of sulci is ~14% smaller than the surface area of gyri, the surface areas (mm$^2$) of significant quantitative T$_{2}^{*}$ changes in sulci and gyri were normalized by the total cortical surface area.

**Results**

Participants’ demographics, including EDSS and MSSS in patients, and conventional MRI metrics are reported in Table 1.

**Laminar quantitative T$_{2}^{*}$ in multiple sclerosis across the cortex**

Figure 1 and Supplementary Table 1 illustrate the results of the GLM laminar analysis comparing 7 T quantitative T$_{2}^{*}$ at 25%, 50% and 75% depth from the pial surface in the earliest disease stages, RRMS and SPMS relative to healthy subjects, and the overlap of significant clusters across the three patients’ groups. In all subgroups of patients we observed, relative to controls and in both hemispheres, a significant increase in T$_{2}^{*}$ relaxation time (clusterwise P < 0.05, corrected for multiple comparisons), consistent with myelin and iron loss. Scattered small clusters of shorter quantitative T$_{2}^{*}$, suggestive of increased susceptibility effects and/or increased iron content, were seen in each disease group mainly in frontal and temporal areas (Supplementary Table 1).

In early multiple sclerosis, significant clusters of longer T$_{2}^{*}$ were mainly located in the outer cortical layers, at 25% depth from the pial surface, in the rostral anterior cingulate and parietal regions, as well as in the precentral and postcentral cortex. Fewer clusters of longer T$_{2}^{*}$ were present in deeper cortical laminae, specifically in the postcentral, and occipital cortex at 50% depth, whereas there was only one cluster of increased quantitative T$_{2}^{*}$ in the calcarine cortex at 75% depth (Supplementary Table 1). Subjects with RRMS and longer disease duration showed a discrete involvement of all cortical layers across frontal, parietal, occipital regions, as well as in the isthmus cingulate and temporal cortex (Supplementary Table 1). Subjects with SPMS showed diffuse cortical involvement at all depths throughout the cortical mantle (Supplementary Table 1).
In patients, the surface area of increased quantitative T2* relative to controls (normalized by the total cortical surface area) was greater in cortical sulci than in gyri, across all cortical depths in early multiple sclerosis and SPMS, and at 25% depth in RRMS (Fig. 2). The greater involvement of cortical sulci relative to gyri was prominent in early disease.

Quantitative T2* in focal cortical lesions and normal-appearing cortical grey matter

Cortical lesions counts in all multiple sclerosis subjects and in each multiple sclerosis subgroup are shown in Table 1.
In all patients, mean quantitative $T_2^*$ in intracortical lesions (40.2 ± 5.1 ms) and leukocortical lesions (40.1 ± 4.7 ms) was significantly higher than mean NACGM quantitative $T_2^*$ (33.5 ± 2.5 ms) ($P < 10^{-10}$ and $P < 10^{-7}$ by paired $t$-test), and than mean cortical quantitative $T_2^*$ (33.03 ± 1.6 ms) in controls ($P < 10^{-5}$ and $P < 10^{-6}$ by linear regression). This comparison remained significant in each multiple sclerosis subgroup (Supplementary Table 2).

Although there were no significant differences between NACGM quantitative $T_2^*$ in all patients and mean cortical quantitative $T_2^*$ in controls, NACGM quantitative $T_2^*$ in SPMS cases was significantly increased relative to cortical quantitative $T_2^*$ from healthy subjects (Supplementary Table 2).

**Cortical thickness and laminar quantitative $T_2^*$**

Overall, patients ($n = 41$) showed significantly decreased mean cortical thickness and normalized cortical volume (ie. entire cortex) relative to healthy subjects ($P < 0.02$ and $P < 0.005$ by ANCOVA, Table 1). Significant cortical thinning was also observed in SPMS subjects ($P < 0.02$ by ANCOVA, Table 1), and there was a trend towards significance for decreased normalized cortical volume in the RRMS and SPMS subgroups ($P < 0.08$ and $P < 0.07$ by ANCOVA, Table 1). The observed decrease in mean global cortical thickness and normalized cortical volume in the entire multiple sclerosis group ($n = 41$) relative to controls was not exclusively driven by SPMS subjects, as we also observed significant cortical thinning and cortical volume loss in early multiple sclerosis and RRMS when grouped together ($P < 0.02$ and $P < 0.05$ by ANCOVA, data not shown).

The GLM analysis between patients and controls did not disclose significant regional changes in cortical thickness after correction for multiple comparisons. We found that in patients, in several regions of both hemispheres, thinning of the cortex corresponded to longer $T_2^*$ ($P < 0.05$ corrected) at all three depths from the pial surface (Fig. 3 and Table 2). Because of the negative correlation in subjects with multiple sclerosis between laminar quantitative $T_2^*$ and cortical thickness across several cortical areas, we assessed differences in laminar quantitative $T_2^*$ (25%, 50% and 75% depth) between each patients’ subgroup and controls using a GLM with per-vertex regression of cortical thickness as a covariate of no interest (nuisance factor), along with age. Increase in quantitative $T_2^*$ across disease stages and depths remained significant ($P < 0.05$ corrected) and substantially unchanged after regressing out cortical thickness (Fig. 4).

**Correlation with clinical measures**

We did not find any relation between either regional or global cortical thickness and normalized cortical volume and either EDSS or MSSS, whereas white matter lesion volume was positively associated with EDSS ($P < 0.008$ by Spearman test) but not with MSSS.

Figure 5 shows the results of the GLM analysis investigating the relationship between both EDSS and MSSS in all patients and laminar quantitative $T_2^*$ at all three depths (25%, 50%, 75%) from the pial surface. There was a positive correlation ($P < 0.05$ corrected) between quantitative $T_2^*$ and both EDSS and MSSS at all depths, the greatest association being observed at 25% depth from the pial surface for both EDSS and MSSS (Table 3). Across all cortical depths, clusters showing a significant correlation between quantitative $T_2^*$ and EDSS and MSSS were mostly...
located in the sensorimotor cortex, though other significant clusters were observed in the insula, cingulate, prefrontal, parietal, and temporal cortex, and this was mainly observed at 25% depth. The significant association between EDSS and laminar quantitative T2* remained significant after including in the GLM analysis, in addition to age, white matter lesion volume as a nuisance variable (covariate of no interest) (Fig. 5 and Table 3).

**Discussion**

Subpial lesions are thought to constitute a major pathological substrate for disease progression in multiple sclerosis. Ultra-high field MRI enables improved detection and classification of focal cortical lesions in multiple sclerosis relative to lower field (<3 T) MRI (Filippi et al., 2014), but in vivo quantification of disseminated subpial lesions is limited. The current study extended previous reports by adding laminar T2* measurements of cortical thickness, which is a potentially more sensitive measure of disease progression than T2* relaxation time of cortical lesions alone.
Figure 4  Quantitative T$_2^*$ differences between multiple sclerosis patients and controls independent from cortical thickness. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) on the average pial surface showing in early multiple sclerosis (MS), RRMS and SPMS, clusters of increased T$_2^*$ relaxation time relative to healthy controls at 25%, 50% and 75% depth from the pial surface, after including in the GLM cortical thickness at the vertex level as a covariate of no interest, along with age. WM = white matter.

Figure 5  Relationship between quantitative T$_2^*$, disability and disease severity. Overlay of the GLM significance maps ($P < 0.05$ corrected for multiple comparisons) showing in 41 subjects with multiple sclerosis clusters exhibiting a positive correlation between T$_2^*$ relaxation time at 25%, 50% and 75% depth from the pial surface and neurological disability, as measured by EDSS, and disease severity, as measured by the MSSS. Overlaid are also significant clusters overlapping across the three depths. The positive correlation between EDSS and quantitative T$_2^*$ remained significant even after including white matter (WM) lesion load as a covariate of no interest (nuisance variable) in the GLM (bottom).
Table 3 Clusters of significant positive correlation between quantitative $T_2^*$ at 25%, 50% and 75% depth from the pial surface and EDSS and MSSS in 41 subjects with multiple sclerosis

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Clusters in bold represent clusters that remained significant after including in the GLM white matter lesions as a nuisance factor (covariate of no interest). S-area = surface area; ant = anterior.
demyelination remains challenging. In this study, using a surface-based technique on ultra-high resolution 7 T quantitative T2* maps (~0.13 mm³ voxel size), we demonstrated in vivo the presence of pathological changes that involve predominantly the outer layers of the cerebral cortex and cortical sulci in the earliest stages of multiple sclerosis, and extend to deeper cortical laminae and gyri in later disease, becoming diffuse across the whole cortical width and mantle in SPMS.

Comparative post-mortem MRI and histopathology data suggested that conventional imaging methods are not able to reliably detect cortical disease cases in which pathology is confined to the juxtameningeal cortex, based on the observation that visibility of multiple sclerosis cortical lesions at lower strength field MRI depended uniquely on lesions size and involvement of several cortical layers (Seewann et al., 2011). The surface-based technique that allows quantitative T2* at 7 T to be estimated vertex-wise across the whole cortical mantle and width could, thus, prove to be a powerful alternative imaging tool for assessing in vivo juxtameningeal cortical pathology in such multiple sclerosis cases.

Histopathological studies suggested that all brain lobes can be the site of cortical lesions, although some pathological findings reported a preferential distribution of cortical demyelination in the insula, cingulate and the temporobasal cortex (Bo et al., 2003b; Kutzelnigg and Lassmann, 2006). In our multiple sclerosis cohort, changes in cortical quantitative T2*, particularly in SPMS, were distributed across the cortical mantle. Frontal, sensorimotor and parietal areas seemed to be preferred sites of pathological changes at all disease stages.

**Quantitative T2* changes in cortical sulci and gyri**

There is evidence that the histopathological and immunological characteristics of subpial demyelination differ significantly from those in white matter lesions, which suggests a location-dependent expression of the multiple sclerosis immunopathological process (Peterson et al., 2001; Bo et al., 2003a, 2007). Autopsy studies of progressive multiple sclerosis showed that more aggressive subpial pathology was associated with the presence of ectopic meningeal B cell follicular-like structures that were located along and in the depth of the cerebral sulci (Magliozzi et al., 2007, 2010; Howell et al., 2011), and which are thought to trigger cortical demyelination through the activation of microglia (Lassmann and Lucchinetti, 2008). The role of ectopic meningeal B cell follicles in early multiple sclerosis has not been elucidated yet, and, in general, evidence of meningeal inflammation in the early stage of multiple sclerosis is limited. Biopsies on atypical multiple sclerosis cases at disease onset described meningeal inflammation by means of perivascular cells infiltration, in association with cortical demyelinating lesions (Lucchinetti et al., 2011). In vivo studies assessing at lower field strength MRI magnetization transfer imaging (MTR) in the cortex of patients with multiple sclerosis failed to observe a disease effect on cerebral sulci (Samson et al., 2013), possibly due to partial volume effects between the cortex and adjacent white matter and CSF (1 mm³ voxel size). The authors, however, were able to detect global changes in MTR in the outer portion of the cortex in RRMS and SPMS patients relative to healthy subjects (Samson et al., 2014). Other findings using a surface-based analysis on MTR images at 1.5 T found decreased MTR in the mid-cortical surface (50% depth from the pial surface) in a small cohort of multiple sclerosis subjects relative to healthy controls, however, a quantitative assessment of MTR differences across cortical layers and in sulci versus gyri was not performed (Derakhshan et al., 2014).

We found that, in early disease and in SPMS across all cortical depths and in RRMS at 25% depth, quantitative T2* changes preferentially involved cortical sulci rather than cortical gyri, corroborating the hypothesis that subpial demyelination is a process likely facilitated by the adjacent meningeal inflammatory milieu. The preferential localization of subpial demyelination in cortical sulci could be related to the tendency of meningeal inflammatory cells and soluble mediators to collect and concentrate in sulci, while being diluted at the outer gyral brain surface due to physiological flow variations of CSF. As disease progresses, persistence of inflammatory changes, and lack of effective repair mechanisms can induce further demyelination that spreads to cortical gyri leading to the phenomenon termed ‘general subpial demyelination’ (Bo et al., 2003b), described in late stage multiple sclerosis. Interestingly, in our study, involvement of cerebral sulci was prominent in early multiple sclerosis, whereas involvement of cortical gyri increased in later disease stages.

**Cortical quantitative T2* and clinical measures**

Post-mortem studies in chronic progressive multiple sclerosis demonstrated an association between a decreasing gradient of intracortical demyelination and neuronal loss away from the pial surface and disease severity as measured by age at which patients became wheelchair-dependent (Magliozzi et al., 2007, 2010).

We previously reported in different multiple sclerosis cohorts an association between neurologological disability and the number of focal subpial lesions on 7 T T2*-weighted images, which also included type IV cortical lesions that extend from the pial surface through the entire cortical width without reaching the subcortical white matter (Mainiero et al., 2009; Nielsen et al., 2013). Here, the laminar analysis of quantitative T2* revealed a significant positive relation between T2* relaxation time at all three cortical depths (25%, 50%, 75%) and both EDSS and MSSS, the greatest effect being in the outer cortical layers.
(25% depth). This association was independent of underlying white matter lesions, indicating that subpial pathology has a significant and unique contribution to disability and disease severity in multiple sclerosis.

The greatest association in our multiple sclerosis cohort between EDSS and MSSS and quantitative T2* at 25% depth is in line with neuropathological examinations that observed that multiple sclerosis brains with a decreasing gradient of demyelination and neuronal loss away from pial surface (likely linked to meningeal inflammation) could be distinguished from other multiple sclerosis cases that lacked a gradient in the expression of cortical pathology, despite the presence of neuronal loss and transmainer cortical demyelination (Magliozi et al., 2010). Clinically, these patients exhibited a milder disease course compared to the former group suggesting that a pathogenic mechanism for cortical demyelination driven from the cortical surface, and possibly associated with meningeal inflammation, is related with worse clinical outcome.

In our early multiple sclerosis cohort, cortical quantitative T2* changes were mainly found in the outer portion of the cortex. Interestingly, early patients showed levels of neurological disability similar to subjects with late RRMS. This, given the short disease duration, translated into higher MSSS, thus implying the presence of a clinically aggressive early disease course. It is also possible that some patients with a relatively benign disease course have been included in the late RRMS group. The term ‘benign’, however, is still controversial as the course of multiple sclerosis can worsen at any time, even after many years of apparent stability (Lublin, 2014), and frequently does not take into account cognitive deficits that may occur in multiple sclerosis in the presence of mild physical disability. Indeed our data demonstrate the presence of a clinically aggressive early disease course. It is also possible that some patients with a relatively benign disease course have been included in the late RRMS group. The term ‘benign’, however, is still controversial as the course of multiple sclerosis can worsen at any time, even after many years of apparent stability (Lublin, 2014), and frequently does not take into account cognitive deficits that may occur in multiple sclerosis in the presence of mild physical disability. Indeed our data demonstrate the presence of a clinically aggressive early disease course. It is also possible that some patients with a relatively benign disease course have been included in the late RRMS group.

We did not find any correlation between cortical thickness or normalized cortical volume and either EDSS or MSSS; rather, quantitative T2* proved to be a marker of neurological disability more sensitive than cortical tissue loss. This suggests that taking into account the spatial variation of tissue integrity measures in the cortex can greatly improve the ability to find multiple sclerosis-related pathological changes. Laminar quantitative T2* and cortical thickness may also reflect distinctive aspects of a degenerative process that targets the cortex, and as such different measures of clinical disability. Pathological data indeed suggested that neuronal and axonal loss seem to contribute more than demyelination to cortical atrophy in multiple sclerosis (Klaver et al., 2013). Longitudinal studies could help to elucidate the spatiotemporal events leading to cortical tissue loss in multiple sclerosis, as well as the contribution of white matter lesions.

**Origin of cortical quantitative T2* changes in multiple sclerosis**

In this study we detected an overall increase in quantitative T2* in patients, at all disease stages, relative to controls. Cortical T2* contrast has been associated predominantly with non-heme iron (Haacke et al., 2005; Fukunaga et al., 2010), which is stored in ferritin particles and provides a substrate for oligodendrocytes for producing and sustaining myelin sheaths surrounding axons. In healthy cortical laminae non-heme iron has been shown to colocalize with myelin on cellular and molecular levels (Connor and Menzies, 1990). In multiple sclerosis, in both white matter and cortical multiple sclerosis lesions longer T2* (or shorter R2*) has been pathologically related to iron and myelin loss (Yao et al., 2012, 2014). Other histopathological–magnetic resonance correlations of ex vivo multiple sclerosis brains using gradient echo T2*-weighted imaging at 7T found increased T2* signal in demyelinating lesions, and decreased T2* in focal areas characterized by the presence of iron rich microglia and/or macrophages (Pitt et al., 2010). Findings from our study,
thus, likely reflect the prevailing effects of a pathological process that underlies a decrease in iron and/or myelin content rather than changes due to iron accumulation in the cortex.

In our multiple sclerosis cohort, quantitative $\text{T}_2^*$ measured in focal cortical lesions was significantly increased (longer $\text{T}_2^*$) compared with NACGM quantitative $\text{T}_2^*$ and mean cortical quantitative $\text{T}_2^*$ in healthy controls, suggesting that focal cortical lesions may contribute, at least in part, to the observed changes in laminar quantitative $\text{T}_2^*$ in multiple sclerosis. Pathological–magnetic resonance correlations on gradient echo images at 7 T highlighted, however, that a consistent subset of cortical lesions can be missed at prospective visual inspection of magnetic resonance scans as cortical lesion counts greatly improved with retrospective scoring (i.e. after comparison of histological sections) (Pitt et al., 2010; Yao et al., 2014). Indeed in our study, in SPMS cases, quantitative $\text{T}_2^*$ changes relative to healthy controls also involved the NACGM. We previously reported in another multiple sclerosis cohort that in some patients, FLASH-$\text{T}_2^*$ magnitude images were characterized, in addition to focal subpial lesions, by the presence of diffuse band-like areas of subtle hyperintensity mainly involving the outer cortical laminae and extending over an entire gyrus or multiple gyri (Mainiero et al., 2009). These observations suggest that even at ultra-high field MRI visual characterization of focal cortical lesions does not account for the full spectrum of cortical pathology in multiple sclerosis, and that quantitative methods able to assess cortical damage voxel-wise could better depict the extent and pattern of cortical pathological changes in the disease.

Heme-bound iron, which may underlie normal vascularization, can also affect $\text{T}_2^*$ contrast. The contribution of intracortical vascular changes to cortical demyelination in multiple sclerosis, however, is still uncertain. Autopsy studies of late stage multiple sclerosis showed that cortical lesions lack the blood–brain barrier breakdown that characterizes active white matter lesions (Peterson et al., 2001; Bo et al., 2003a). Other histopathological examinations found blood–brain barrier breakdown and perivascular inflammation in the cortex of multiple sclerosis brains at disease onset (Lucchinetti et al., 2011). In vivo assessments described a significant reduction in cerebral blood flow and cerebral blood volume in cortical lesions compared with the normal-appearing grey matter, suggesting reduced metabolism due to loss of cortical neurons. A subset of cortical lesions showing an increased cerebral blood flow and/or cerebral blood volume, however, was also detected, possibly implying that perfusion could evolve during inflammation (Peruzzo et al., 2013). Other studies found, in early RRMS, decreased grey matter perfusion in the absence of volume loss, consistent with neuronal metabolic dysfunction (Debernard et al., 2014). Preliminary findings using gadolinium-enhanced $\text{T}_2$-fluid-attenuated-inversion-recovery (FLAIR) MRI reported, in a subset of multiple sclerosis cases, leptomeningeal contrast enhancement that was unrelated to contrast-enhancement in white matter lesions (Reich et al., 2014). Taken together, these observations lead to speculation that the time course of cortical blood–brain barrier breakdown could differ from that in white matter lesions and across disease stages. Future studies aimed at assessing intracortical vascular changes in multiple sclerosis could help to clarify its role in cortical pathology. In addition, quantitative $\text{T}_2^*$ measurements can be combined with other techniques specific to myelin such as $\text{T}_1$ mapping, quantitative magnetization transfer imaging (Dortch et al., 2013), and to iron such as quantitative susceptibility mapping (Deistung et al., 2013) to better characterize the contribution of myelin and iron to cortical multiple sclerosis pathology.

**Conclusion**

This study demonstrates that a surface-based analysis of ultra-high resolution quantitative $\text{T}_2^*$ MRI acquisition at 7 T with a highly parallelized radiofrequency coil can facilitate the in vivo characterization of cortical pathology at distinct depths from the pial surface in multiple sclerosis. We were able to detect in vivo a gradient in the expression of intracortical multiple sclerosis pathology across disease stages, which supports the hypothesis that cortical pathology in multiple sclerosis may be, at least in part, the consequence of a pathogenic process driven from the pial surface. Because we cannot exclude that changes in deeper cortical laminae (75% depth) could also be driven by white matter lesions extending into the cortex, longitudinal evaluations are needed to confirm our preliminary observations. Nevertheless, the significant association between laminar quantitative $\text{T}_2^*$, neurological disability and disease severity, prominent in the outer cortical layers and independent from white matter lesions, provides in vivo evidence that this pattern of cortical disease can be the pathological basis for disease progression in many multiple sclerosis cases.

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**Supplementary material**

Supplementary material is available at Brain online.
References


