Laminar analysis of the cerebellar cortex shows widespread damage in early MS patients: A pilot study at 7T MRI

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Abstract
Background: To date, little is known about the presence and extent of cerebellar cortical pathology in early stages of MS.
Objective: The aims of this study were to (i) investigate microstructural changes in the normal-appearing cerebellar cortex of early MS patients by using 7 T MRI and (ii) evaluate the influence of those changes on clinical performance.
Methods: Eighteen RRMS patients and nine healthy controls underwent quantitative T1 and T2* measurement at 7 T MRI using high-resolution MP2RAGE and multi-echo gradient-echo imaging. After subtracting lesion masks, average T1 and T2* maps were computed for three layers in the cerebellar cortex and compared between groups using mixed effects models.
Results: The volume of the cerebellar cortex and its layers did not differ between patients and controls. In MS patients, significantly longer T1 values were observed in all vermis cortical layers and in the middle and external cortical layer of the cerebellar hemispheres. No between-group differences in T2* values were found. T1 values correlated with EDSS, SDMT and PASAT.
Conclusions: We found MRI evidence of damage in the normal-appearing cerebellar cortex at early MS stages and before volumetric changes. This microstructural alteration appears to be related to EDSS and cognitive performance.

Keywords: Multiple sclerosis, MRI, ultra-high field MRI, cerebellum

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Introduction
Histopathological studies have shown that the cerebellar cortex of multiple sclerosis (MS) patients represents a predilection site for demyelination and that this region is particularly affected in late-stage MS patients, where about 40% of cerebellar cortical area is affected. Similarly to what reported in the forebrain cortex, focal demyelinated areas are also found in the cerebellar cortex of MS patients: they involve the cerebellar cortex and the adjacent white matter (leukocortical lesions), small intracortical perivenous areas (pure intracortical lesions) and (most frequently) they affect the cerebellar cortex in a band-like manner (subpial lesions). In most instances, the cerebellar cortex is affected independently from white matter (WM) lesions and areas of cortical demyelination in the cerebellum show relative preservation of neurons, axons and synapses.

Cerebellar damage in MS patients has been shown to be a major determinant of disability and to represent a predictor of poor outcome. Cerebellar abnormalities may affect both the coordination and control of movements as well as cognition and affective processes in patients suffering from MS. Besides, alterations in cerebellar–neocortical connectivity may...
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For all these reasons, sensitive measures of alterations in cerebellar cortical integrity might provide not only a new window into early changes in MS pathology but also potential means to screen patients requiring therapies that are more aggressive.

Ultra-high-field MRI may provide new insights into cerebellar pathology throughout early MS stages, due to the improved spatial-resolution, the lower partial-volume effects, and the higher contrast-to-noise-ratio (CNR) achievable in clinically compatible scan times. Magnetization-prepared 2 inversion-contrast rapid gradient-echo (MP2RAGE)\(^9\) at 7T enables the generation of quantitative T\(_1\) maps. T\(_1\), the longitudinal relaxation time in MRI, is influenced by the amount of macromolecules (eg. myelin) in the tissue and, to a lesser extent, by the presence of paramagnetic substances, like iron.\(^10\)

Hence, shorter T\(_1\) relaxation time (RT) indicates an increase of brain macromolecules or iron content while longer T\(_1\)-values suggest a loss of brain structural integrity or iron concentration. Multi-echo gradient-recalled echo (ME-GRE) at 7T MRI provides high-spatial-resolution quantitative T\(_2^*\) maps. The T\(_2^*\)-RT describes the loss of transverse magnetization due to T\(_2\) relaxation and to magnetic field inhomogeneities. Essentially, it reflects microscopic susceptibility variations which are induced by tissue components with different magnetic behavior.

An increase in T\(_2^*\) most often indicates a reduction of macromolecules (myelin, cell membranes, proteins), while a decrease suggests an augmented iron concentration or increased density of macromolecules.\(^11\)

By combining T\(_1\) and T\(_2^*\)-measurements at 7T, we have recently shown a decrease in mean T\(_1\)-RT from the outer to the inner layer of the cerebellar cortex in healthy subjects. This pattern was attributed to an increase in myelination in the deeper layers of the cerebellar cortex,\(^12\) similar to what has previously been reported for the forebrain.\(^13,14\)

By exploiting the above-mentioned methodology, in this work we aimed to explore (i) the presence of microstructural changes in the normal-appearing cerebellar cortex of early MS patients by using ultra-high field magnetic resonance imaging (MRI) and (ii) the potential relation of those changes with clinical disability, motor and cognitive performance.

Methods

In this pilot study, we recruited eighteen relapsing-remitting MS (RRMS) patients (14 females, mean age 34 years, less than five years disease duration, median Expanded Disability Status Scale (EDSS) 1.6 (range 1–2), 9/18 had very mild cerebellar deficits (as defined by SARA – Scale for assessment and rating of Ataxia – 1 ≤ 4) and nine age-matched healthy controls (7 females, mean age 32 years). All participants provided written informed consent and the studies were approved by the local ethics committee.

Imaging

MS patients and healthy controls underwent quantitative T\(_1\) and T\(_2^*\)-MR imaging using high-spatial resolution MP2RAGE\(^15\) (repetition time TR = 6000 ms, echo time TE = 2.84 ms, first inversion time T\(_{\text{I1}}\) = 750 ms, second inversion time T\(_{\text{I2}}\) = 2350 ms, first flip angle = 4°, second flip angle = 5°, matrix size 300 × 320 × 160, voxel size 0.75 × 0.75 × 0.9 mm\(^3\)) and a ME-GRE sequence (TR = 45 ms, TE1 = 4.59 ms, TE9 = 41.3 ms, with an echo spacing, ΔTE, of 4.59 ms, matrix size 300 × 320 × 160, voxel size 0.75 × 0.75 × 0.9 mm\(^3\)) acquired on a Siemens Magnetom 7T head-only scanner (Siemens Healthcare Sector, Germany) with a single-channel transmit and 32-channel receive volume coil (Nova Medical Inc, MA, USA). A map of the transmit B1 field was acquired by means of a SA2RAGE sequence (TR = 2400ms, TE = 0.72 ms, matrix 116 × 128 × 64, voxel size 2.3 × 2.3 × 4 mm\(^3\), same transmit voltage as MP2RAGE). The T\(_2^*\)-maps were generated from a 3D multi-gradient echo dataset with nine echo times.

Three dielectric pads were placed around the upper neck to improve the inversion efficiency over the cerebellum and whole brain B1 homogeneity as reported in.\(^12\) T\(_1\) and T\(_2^*\)-RT were mapped onto three different layers of the cerebellar cortex using the method recently presented by Boillat et al.\(^12\) In short: the cerebellar cortex was segmented into three layers of equal dimension using CBS Tool (www.cbs.mpg.de/institute/software/cbs-tools) as previously reported by our group.\(^16\)

Before extracting the T\(_1\) and T\(_2^*\)-values, the lesion masks were subtracted in order to study the normal-appearing cerebellar GM pathology in our patients’ cohort. Average T\(_1\) and T\(_2^*\)-maps were computed for each layer in the hemispheres and vermis.
Both hemispheres and the vermis were separated based on the CHROMA atlas (a group-wise average of a T1-map acquired at high resolution), which is part of the CBS Tools. (Fig. 1)

Cerebellar cortical and cerebellar layers volumes
Cerebellar cortical volume was assessed on MP2RAGE images by using the CBS Tool\(^{16}\) in both patients and controls.

Cerebellar cortical lesions
Lesions were identified and delineated manually on the so-called uniform MP2RAGE images, which are obtained through a complex ratio of images acquired at two different inversion times, as described by Marques et al.\(^{15}\) (see Fig. 2) The manual segmentations were performed by consensus between 1 radiologist (A.T., 6 years of experience) and 1 neurologist with expertise in MS and neuroimaging (C.G., 13 years of experience). Each lesion was labeled as either 1) leukocortical, extensions of WM lesions in the folia, affecting adjacent cortical tissue and 2) intracortical, lesions within the cerebellar cortex without contact to the WM. Mean T1 and T2* values were extracted from cortical cerebellar lesions.

Total cerebellar cortical lesion load provided by the lesion mask was subtracted from total cerebellar cortex volume, in order to segment the normal appearing cerebellar cortex. Mean T1 and T2* RT were then computed for the normal-appearing cortical tissue.

Clinical assessment
Clinical disability was measured using the Expanded Disability Status Scale (EDSS) before the MRI scan. Grade of cerebellar involvement was assessed by means of SARA – Scale for assessment and rating of Ataxia, composed of 8 items related to gait, stance, sitting, speech, finger-chase test, nose-finger test, fast alternating movements and heel-shin test. The motor performance was evaluated by means of the Timed 25-Foot Walking (T25-FW) and the 9-Hole Peg Test (9-HPT). Cognition was assessed using selective reminding tests (SRT) (SRT-Long Term Storage, SRT-Consistent Long-Term Retrieval and SRT-Delayed), Spatial Recall Test (SPART), Spatial Recall Test Delayed (SPART-D), Symbol Digit Modalities Test (SDMT) and Paced Auditory Serial Addition Test (PASAT).\(^{17,18}\)

Statistical analysis
Average T1- and T2* maps were computed for each layer in the hemispheres and vermis and then compared between patients and controls using mixed effects models. False discovery rate was applied to correct for multiple comparisons.

Between-group comparison in cerebellar layers volume and cerebellar volume was performed...
using a univariate t-test since the two groups did not differ for age and gender (Table 1).

As explorative analysis, a generalized linear model was performed using the mean T1 in the middle/external cerebellar cortical layers (vermis and hemispheres were considered as one region), the mean lesion volume in the cerebellum, and age as independent predictors and clinical scores (EDSS, 9-HPT, T25-FW, SRT-LTS, SRT-CLTR, SRT-D, SPART, SPART-D, SDMT and PASAT) as outcome. Backward-stepwise selection was performed to select the best prediction model for each clinical score. A leave-one-out cross-validation (LOOCV) was conducted to assess the prediction quality of each model.

### Results

Demographics and number of cortical cerebellar lesions are reported in Table 1, \( T_1 \) and \( T_2^* \) RT of cerebellar normal-appearing grey matter (NAGM) and cortical lesions in Table 2. Clinical scores in MS patients are summarized in Table 3.

**Cerebellar lesions**

Based on manual segmentation of MS lesions, our cohort of patients exhibited a total of 15 cerebellar cortical lesions (median, range: 0, 0–10) with an average lesion volume of 72.1 \( \mu \text{L} \). The total number/average volume per lesion of the subcategories of cortical lesions were 14/76.4 \( \mu \text{L} \) (leukocortical) and 1/11.6 \( \mu \text{L} \) (intracortical), respectively.

\( T_1 \) RT in the grey matter part of cortical lesions (1899.4 ± 198.6 ms) were longer than those in normal-appearing cortical tissue (1794.8 ± 85.6 ms, \( p < 0.05 \)), whereas \( T_2^* \) RT (33.2 ± 5.9 ms) did not differ (32.6 ± 2.1 ms).

**Volumetric and \( T_1/T_2^* \) relaxometry in the normal appearing tissue of three cerebellar cortical layers in MS patients and controls**

Cerebellar cortex volume did not statistically differ between MS patients and healthy controls, nor did the volume of the different layers.
In MS subjects, significantly longer T1 RT values were observed in all vermis layers (p for individual layers < 0.01 to < 0.02), and in the middle and external layer of the cerebellar hemispheres (p < 0.03, Fig. 3).

No between-group differences in T2* values were found (see Table 2).

### Table 2. T1 and T2* RT in cerebellar normal appearing grey matter (NAGM) of patients and cerebellar grey matter (GM) of healthy controls (HC) and in cerebellar cortical lesions.

| T1 RT (ms) | Inner layer | Middle layer | Outer layer |
|------------|-------------|--------------|-------------|
| NAGM MS patients | 1704 ± 46.1 | 1806.4 ± 44.9 | 1874 ± 56 |
| GM HC     | 1668.1 ± 5  | 1758.9 ± 50.5 | 1806.4 ± 54.2 |

| T2* RT (ms) | Inner layer | Middle layer | Outer layer |
|-------------|-------------|--------------|-------------|
| NAGM MS patients | 32.7 ± 2.1  | 33.0 ± 1.9   | 32.2 ± 2.6  |
| GM HC       | 32.8 ± 1.8  | 33.5 ± 2.2   | 32.3 ± 2.1  |

### Table 3. Clinical scores in MS patients.

| Tests               | Average ± STD | Function                  |
|---------------------|---------------|---------------------------|
| EDSS                | 1.6 ± 0.2     | Disability                |
| 9-HPT               | 18.7 ± 1.6    | Arm-motor function        |
| T25-FW              | 3.94 ± 0.78   | Leg-motor function        |
| SRT-LTS             | 62.7 ± 6.7    | Cognition                 |
| SRT-CLTR            | 59.2 ± 7.6    | Cognition                 |
| SRT-D               | 11.4 ± 1      | Cognition                 |
| SPART               | 24.4 ± 3.8    | Cognition                 |
| SPART-D             | 8.6 ± 2.1     | Cognition                 |
| SDMT                | 59.3 ± 7.5    | Cognition                 |
| PASAT               | 47.4 ± 8.8    | Cognition                 |

EDSS: Expanded Disability Status Scale; 9-HPT: 9-Hole Peg Test; T25-FW: Timed 25-Foot Walk Test; SRT-LTS: Selective Reminding Test - Long Term Storage; SRT-CLTR: Selective Reminding Test - Consistent Long - Term Retrieval; SPART: Spatial Recall Test; SPART-D: Spatial Recall Test Delayed; SRT-D: Selective Reminding Test-Delayed Recall; SDMT: Symbol Digit Modalities Test; PASAT = Paced Auditory Serial Addition Test.

In MS subjects, significantly longer T1 RT values were observed in all vermis layers (p for individual layers <0.01 to <0.02), and in the middle and external layer of the cerebellar hemispheres (p < 0.03, Fig. 3)

No between-group differences in T2* values were found (see Table 2).

Relationship between T1 relaxometry in the normal appearing tissue of three cerebellar cortical layers in MS patients and clinical tests

The T1 RT values of the mean outer layer of the cerebellar cortex correlated with EDSS (P < 0.01, adjusted R² = 0.4), see supplementary data. Besides, T1 RT values in the middle layer and mean outer layer were associated to SDMT (P < 0.05, adjusted R² = 0.5) and PASAT scores (P < 0.05, adjusted R² = 0.5), see supplementary data.

### Discussion

In this pilot study, we measured longer T1 RT in some layers of the normal-appearing cerebellar cortex in early MS patients compared to healthy subjects. These results suggest the presence of widespread microstructural damage in the cerebellar cortex at early stages of the disease, which appear to be independent from the presence of detectable focal lesions and of cerebellar cortical atrophy.

Since cerebellar involvement is predictive of development of greater disability and poor prognosis in MS patients, it is of the utmost importance to have...
it recognized as early as possible. The detection of early cerebellar involvement in MS could in fact permit to identify patients that may benefit of a more aggressive therapeutic approach than the one currently suggested.

To date, few studies addressed the presence of cerebellar abnormalities in early MS. Cortical cerebellar lesions and cortical atrophy in the cerebellum was reported in clinically isolated syndrome (CIS) patients or at early RRMS stages in patients with mild disability and on therapy.9,19–21 Previous work of our group using multicontrast connectometry revealed subtle local connectivity disruptions in a group of early MS patients and suggested loss of axonal integrity in local cortico-cortical cerebellar connections.22 Up to the present time, however, there are no studies attempting to measure microstructural damage in the normal-appearing cerebellar cortex in early stage MS. This is mainly due to technical challenges arising from the tight folding of this histological structure and the relatively poor contrast and resolution of conventional brain imaging.

Thanks to ultra-high-field MRI, it is now possible to investigate the brain tissue in-vivo at a sub-millimeter resolution, which is of crucial importance to study thin and convoluted brain structures like the cerebellar cortex.23,24 In this study, we performed a quantitative laminar analysis at 7 T MRI and compared the T1/T2* RT measured in different cerebellar cortical layers between early MS patients and healthy controls.

In MS patients, we showed an increase in T1 RT in all cortical layers in the vermis as well as in the middle and external cortical layer of the cerebellar hemispheres compared to healthy subjects. Interestingly, those abnormalities occurred outside areas of focal damage as identified with 7T MP2RAGE and independently from the presence of cerebellar cortical atrophy. The observed alterations in the most external layers may be due to demyelinating processes developing from the subpial region of the cerebellar cortex (i.e. molecular layer), as previously reported in postmortem1 and experimental studies.25,26 In alternative or in addition, it cannot be ruled out that the observed gradient of damage may be a consequence of more effective remyelinating activity in the inner cortical layers, such as observed in the forebrain cortex.27 However, the presence of partial volume effects might also contribute to the observed gradient. Therefore, we have tried to limit such effects by thresholding out values that are in the range of the T1 RT in the cerebrospinal fluid.

As to T2* RT, we have not observed any significant differences between patients and controls: this may be due to the lower sensitivity of T2* RT (compared to T1 RT) to occurring mechanisms of damage or to an inadequate statistical power. Besides, the high sensitivity of the T2* contrast to changes other than demyelination or axonal loss (e.g. iron accumulation) could also overshadow the sensitivity to the former.
The reported microstructural alterations in our cohort of RRMS patients correlated to clinical score (EDSS). Moreover, our exploratory analysis showed a relationship between the measured T1 RT in the outer and middle cerebellar layers of both vermis and hemispheres and auditory information processing speed and flexibility (measured by the PASAT) but also attention, visual scanning, and motor speed performances (measured by the SDMT). The modest association between T1 changes and cognitive performance might well reflect the limited sample size, therefore larger studies are required to verify the robustness of the reported relationship.

The major limitation of this study is the limited sample size and the fact that we have investigated a cohort of RRMS patients at early stage but with minimal functional impairment. Another important limitation is the lack of any analysis of the rest of the brain. Future work will aim to expand the current findings in larger cohorts of early MS patients and in longitudinal settings.

In conclusion, our pilot study showed that quantitative T1 imaging at ultra-high-field MRI is sensitive to alterations in the normal-appearing cortex of the cerebellum in early-MS patients, which are compatible with diffuse subpial demyelination and widespread microstructural damage. Indeed, subpial demyelination can be extensive, subtle, and not well delimited, and as such easily missed during lesion segmentation. Those alterations appeared to be independent from focal disease activity, intended as leukocortical or pure intracortical lesions, and volume loss in the cerebellar cortex and were related to clinical score and cognitive performance in our cohort of mildly impaired early-stage RRMS patients.

Data availability
The data that support the findings of this study are available from the corresponding author on request.

Conflict of Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Riccardo Galbusera reports no disclosures in relationship to this work Katrin Parmar reports no disclosures in relationship to this work Yohan Boillat reports no disclosures in relationship to this work Mario Joao Fartaria works for Siemens Switzerland Alexandra-Ramona Todea reports no disclosures in relationship to this work Kieran O’Brien works for Siemens Australia Anna Smolinski reports no disclosures in relationship to this work Ludwig Kappos reports no disclosures in relationship to this work Wietske van der Zwaag reports no disclosures in relationship to this work Cristina Granziera reports no disclosures in relationship to this work.

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Supplemental Material
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References
1. Kutzelnigg A, Faber-Rod JC, Bauer J, et al. Widespread demyelination in the cerebellar cortex in multiple sclerosis. Brain Pathol Zurich Pathol 2007; 17: 38–44.
2. Gilmore CP, Donaldson I, Bø L, et al. Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis: a comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. J Neurol Neurosurg Psychiatry 2009; 80: 182–187. DOI:10.1136/jnnp.2008.148767.
3. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain J Brain 2005; 128: 2705–2712.
4. Amato MP and Ponziani G. A prospective study on the prognosis of multiple sclerosis. Neurol Sci off Sci 2000; 21: S831–838.
5. Schmahmann JD. Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. J Neuropsychiatry Clin Neurosci 2004; 16: 367–378.
6. Saini S, DeStefano N, Smith S, et al. Altered cerebellar functional connectivity mediates potential adaptive
plasticity in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004; 75: 840–846.
7. Doyon J, Song AW, Karni A, et al. Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc Natl Acad Sci USA* 2002; 99: 1017–1022.
8. Ungerleider LG, Doyon J and Karni A. Imaging brain plasticity during motor skill learning. *Neurobiol Learn Mem* 2002; 78: 553–564.
9. Fartaria MJ, O’brien K, Søregå A, et al. An ultra-high field study of cerebellar pathology in early relapsing-remitting multiple sclerosis using MP2RAGE. *Invest Radiol* 2017; 52: 265–273.
10. Helms G. Tissue properties from quantitative MRI. *Brain Mapp Encycl Ref* 2015; 1: 287–294.
11. Ropele S, de Graaf W, Khalil M, et al. MRI assessment of iron deposition in multiple sclerosis. *J Magn Reson Imaging JMRI* 2011; 34: 13–21.
12. Boillat Y, Bazin P-L, O’Brien K, et al. Surface-based characteristics of the cerebellar cortex visualized with ultra-high field MRI. *NeuroImage* 2018; 172: 1–8. 15
13. Sereno MI, Lutti A, Weiskopf N, et al. Mapping the human cortical surface by combining quantitative T(1) with retinotopy. *Cereb Cortex N Cortex* 2013; 23: 2261–2268.
14. Waehnert MD, Dinse J, Schäfer A, et al. A subject-specific framework for in vivo myeloarchitectonic analysis using high resolution quantitative MRI. *NeuroImage* 2016; 125: 94–107.
15. Marques JP, Kober T, Krueger G, et al. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. *NeuroImage* 2010; 49: 1271–1281.
16. Bazin P-L, Weiss M, Dinse J, et al. A computational framework for ultra-high resolution cortical segmentation at 7Tesla. *NeuroImage* 2014; 93 Pt 2: 201–209.
17. Simioni S, Amari F, Bonnier G, et al. MP2RAGE provides new clinically-compatible correlates of mild cognitive deficits in relapsing-remitting multiple sclerosis. *J Neurol* 2014; 261: 1606–1613. DOI:10.1007/s00415-014-7398-4.
18. Bever CT, Grattan L, Panitch HS, et al. The brief repeatable battery of neuropsychological tests for multiple sclerosis: a preliminary serial study. *Mult Scler* 1995; 1: 165–169. DOI:10.1177/13524585950010306.
19. Calabrese M, Mattisi I, Rinaldi F, et al. Magnetic resonance evidence of cerebellar cortical pathology in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2010; 81: 401–404. DOI:10.1136/jnnp.2009.177733.
20. Favaretto A, Lazzarotto A, Poggiali D, et al. MRI-detectable cortical lesions in the cerebellum and their clinical relevance in multiple sclerosis. *Mult Scler* 2016; 22: 494–501. DOI:10.1177/1352458515594043.
21. Anderson VM, Fisniku LK, Altmann DR, et al. MRI measures show significant cerebellar gray matter volume loss in multiple sclerosis and are associated with cerebellar dysfunction. *Mult Scler Houndmills Scler* 2009; 15: 811–817.
22. Romascoano D, Meskaldji D-E, Bonnier G, et al. Multicontrast connectometry: a new tool to assess cerebellum alterations in early relapsing-remitting multiple sclerosis. *Hum Brain Mapp* 2015; 36: 1609–1619. DOI:10.1002/hbm.22698.
23. Andersen BB, Gundersen HJG and Pakkenberg B. Aging of the human cerebellum: a stereological study. *J Comp Neurol* 2003; 466: 356–365.
24. Marques JP, van der Zwaag W, Granziera C, et al. Cerebellar cortical layers: in vivo visualization with structural high-field-strength MR imaging. *Radiology* 2010; 254: 942–948.
25. MacKenzie-Graham A, Tiwari-Woodruff SK, Sharma G, et al. Purkinje cell loss in experimental autoimmune encephalomyelitis. *NeuroImage* 2009; 48: 637–651.
26. MacKenzie-Graham A, Rinek GA, Avedisian A, et al. Cortical atrophy in experimental autoimmune encephalomyelitis: in vivo imaging. *NeuroImage* 2012; 60: 95–104.
27. Strijbis EMM, Kooi E-J, van der Valk P, et al. Cortical remyelination is heterogeneous in multiple sclerosis. *J Neuropathol Exp Neurol* 2017 01; 76: 390–401.