Trans-Synaptic Axonal Degeneration in the Visual Pathway in Multiple Sclerosis

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Objective: To evaluate the association between the damage to the anterior and posterior visual pathway as evidence of the presence of retrograde and anterograde trans-synaptic degeneration in multiple sclerosis (MS).
Methods: We performed a longitudinal evaluation on a cohort of 100 patients with MS, acquiring retinal optical coherence tomography to measure anterior visual pathway damage (peripapillary retinal nerve fiber layer [RNFL] thickness and macular volume) and 3T brain magnetic resonance imaging (MRI) for posterior visual pathway damage (volumetry and spectroscopy of visual cortex, lesion volume within optic radiations) at inclusion and after 1 year. Freesurfer and SPM8 software was used for MRI analysis. We evaluated the relationships between the damage in the anterior and posterior visual pathway by voxel-based morphometry (VBM), multiple linear regressions, and general linear models.
Results: VBM analysis showed that RNFL thinning was specifically associated with atrophy of the visual cortex and with lesions in optic radiations at study inclusion ($p < 0.05$). Visual cortex volume ($\beta = +0.601$, 95% confidence interval [CI] = +0.04 to +1.16), N-acetyl aspartate in visual cortex ($\beta = +1.075$, 95% CI = +0.190 to +1.961), and lesion volume within optic radiations ($\beta = -2.551$, 95% CI = −3.910 to −1.192) significantly influenced average RNFL thinning at study inclusion independently of other confounders, especially optic neuritis (ON). The model indicates that a decrease of 1cm^3 in visual cortex volume predicts a reduction of 0.6μm in RNFL thickness. This association was also observed after 1 year of follow-up. Patients with severe prior ON (adjusted difference = −3.01, 95% CI = −5.08 to −0.95) and mild prior ON (adjusted difference = −1.03, 95% CI = −3.02 to +0.95) had a lower adjusted mean visual cortex volume than patients without ON.
Interpretation: Our results suggest the presence of trans-synaptic degeneration as a contributor to chronic axon damage in MS.

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The visual pathway is a suitable model to study the dynamics of neuronal degeneration in multiple sclerosis (MS), as it has a well-defined and eloquent structure[1] (Fig 1). The anterior visual pathway is composed of the axons from retinal ganglion cells, which form the optic nerve, optic chiasm, and optic tract, and synapse in the lateral geniculate nucleus (LGN). The posterior visual pathway is composed of axons from the LGN that comprise the optic radiations (OR) and project into the visual cortex (V1). Damage spreading from the posterior to anterior visual pathway or vice versa requires its transmission through the synapses in the LGN, a process called

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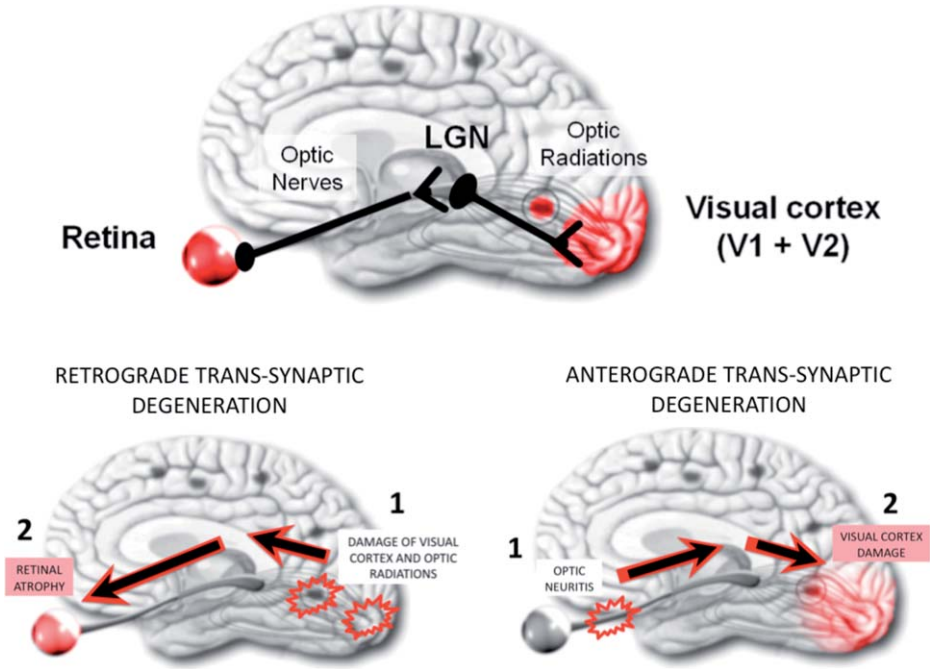

FIGURE 1: The visual pathway as a model to study trans-synaptic neuronal degeneration in multiple sclerosis. The afferent visual pathway has a distinct anatomy that can be simplified into 2 neuronal pathways: the anterior pathway, composed of the retina, optic nerves, chiasm, and optic tracts; and the posterior pathway, composed of the optic radiations and visual cortex. Both pathways are connected by a single synaptic relay, the lateral geniculate nuclei (LGN). At the bottom left, the model for retrograde trans-synaptic degeneration is represented as the effect of the damage to the posterior visual pathway (visual cortex [V1 + V2] atrophy and lesions within the optic radiations) on retinal atrophy. At the bottom right, the model for anterograde trans-synaptic degeneration is represented as the effect of optic neuritis on the posterior visual pathway (visual cortex [V1 + V2] atrophy).

trans-synaptic degeneration. This mechanism has been described in a number of central nervous system diseases after focal damage in different brain circuits, including the visual pathway,[2–5] but its role in MS remains to be clarified.

Long-term disability in MS is mainly due to axon damage,[6] and thus understanding the mechanisms behind this process could favor the development of prognostic biomarkers and neuroprotective therapies for MS. Damage to the anterior visual pathway can now be quantified by optical coherence tomography (OCT).[7–9] The retroorbital portion of the anterior visual pathway and the retrogeniculate portion of the visual pathway (made up of the OR and visual cortex) can be evaluated by magnetic resonance imaging (MRI).[10–14] The combination of both technologies can be used for quantifying the damage to the visual pathway induced by MS.

The aim of this study was to evaluate the association between anterior and posterior visual pathway damage as an indication of the presence of trans-synaptic (retrograde and anterograde) damage in patients with MS. In our model, retrograde trans-synaptic neuronal damage is represented by the effect of damage to posterior visual pathway (OR and visual cortex) on retinal atrophy, and anterograde trans-synaptic neuronal damage by the effect of optic neuritis (ON) on visual cortex atrophy (see Fig 1).

Patients and Methods

Study Population

The MS-VisualPath cohort is an ongoing cohort recruited at the center of Neuroimmunology of the Hospital Clinic of Barcelona. This cohort includes MS patients according to McDonald's 2005 criteria[15] with or without a past history of ON. The recruitment started in December 2010 and remains open. In the present study, we evaluated this cohort at study inclusion and after 1 year of follow-up. The Research Ethics Committee approved the study, and all participants gave their written informed consent before they were admitted to the study. All study subjects were 18 to 55 years old, and they were evaluated by clinical interview, as well as through a neurological and ophthalmological examination to discard any systemic, ophthalmological, or drug-related causes of potential retinal nerve fiber layer (RNFL) impairment other than MS (see Supplementary File 1). Our study was designed to identify the relationship between visible white matter (WM) damage and gray matter (GM) volume irrespective of the disease subtype, and thus we included patients with all MS subtypes. The use of immunomodulatory drugs was allowed. Patients were excluded if they had suffered acute ON in the 6 months prior to inclusion or during the follow-up period, or any other acute relapse or

systemic steroid treatment in the 30 days prior to enrollment or at 1 year of follow-up.

Clinical Assessment

At baseline and yearly thereafter, patients undergo a complete neurological and ophthalmological examination with OCT and MRI performance within a time interval <1 month. We collected a set of relevant clinical variables at baseline, including age at study inclusion, sex, disease subtype, disease duration, and the Expanded Disability Status Scale (EDSS) score. We excluded 4 eyes from 100 patients that did not fulfill the ophthalmological inclusion criteria. We considered only the other eye's value for those patients. The presence of prior ON was assessed in the subject's medical history (n = 38), as described in the Optic Neuritis Treatment Trial[16] and confirmed by careful ophthalmological assessment, MRI of the optic nerve (T2 sequence), and/or by identifying significant asymmetry in the RNFL by OCT and in the mean deviation of the visual field. A mean + 1 standard deviation of the interocular asymmetry of registered ON cases was considered as a cutoff point for abnormality. Diagnosis of neuromyelitis optica was ruled out and anti–aquaporin-4 antibody status was tested in cases with bilateral ON or functionally severe unilateral ON. Visual field was obtained by Humphrey Field Analyzer (Humphrey Instruments, San Leandro, CA) for each eye in every patient using the Swedish Interactive Thresholding Algorithm standard central 30-2 protocol.

Retinal Imaging

Spectral Domain retinal OCT (SPECTRALIS; Heidelberg Engineering, Carlsbad, CA; Heidelberg Eye Explorer version 1.7.0.0) was performed for each eye as described previously.[8] For statistical analysis, the mean values of the OCT measurements of both eyes were used unless 1 eye was excluded. In such cases, we used the other eye's measurement (n = 4). All OCT examinations fulfilled OSCAR-B criteria for OCT image acquisition.[17]

Brain MRI Acquisition and Preprocessing

Brain images were acquired using 3T MRI. The following sequences were obtained: (1) 3-dimensional structural T1-weighted magnetization-prepared rapid gradient echo (T1-MPRAGE); (2) coronal oblique T2 fat-saturated spin echo acquisitions for each optic nerve; and (3) single-voxel proton magnetic resonance spectroscopy of the visual cortex, calculating the absolute N-acetyl aspartate (NAA) levels. WM lesions were manually segmented from high spatial resolution T1-MPRAGE images. Freesurfer software[18] was used to automatically obtain the volume of the visual cortex and precentral cortex from T1 (bilateral visual cortex volume includes V1 and V2, both considered as a single region). The Jülich probabilistic MRI atlas[19] was used to obtain binary masks of the OR. To confirm the plausibility of their relation, we evaluated the association of retinal atrophy with regional GM atrophy and with the distribution of WM lesions at study inclusion by voxel-based morphometry (VBM) using SPM8, as described previously.[20]

Statistical Analysis

Results from VBM at study inclusion were assessed statistically by applying a multiple regression analysis. RNFL thickness and macular volume (MV) were used as the main explanatory variables for VBM of the GM (additional covariates in the model: age at acquisition of the scan, sex, EDSS, a head size scaling factor named VSCALING, and WM lesion volume) and WM lesions (additional covariates in the model: age at acquisition of the scan, EDSS, and VSCALING). The influence of prior ON was controlled by performing separate VBM analyses for the overall cohort (n = 100), for cases without prior ON (n = 62), and for cases with prior ON (n = 38). In addition, we evaluated the presence of anterograde and retrograde trans-synaptic degeneration in the visual pathway at study inclusion and after 1 year of follow-up. Retrograde trans-synaptic degeneration was defined as the association of posterior visual pathway damage (visual cortex or OR) with retinal atrophy. Visual cortex (V1 and V2) damage was measured by atrophy or decay in the NAA, and OR damage by quantifying the volume of the lesions within this tract. Retinal atrophy was measured by RNFL thickness and MV. This association was evaluated by multiple linear regression analysis adjusting for sex and age at study inclusion (parameters that influence anatomical measurements), EDSS (to control for disease severity), and prior ON (to control for the effect of ON on retinal atrophy). The models of visual cortex damage were also adjusted for lesion volume within the OR, and that of OR damage for visual cortex volume. Anterograde trans-synaptic degeneration was defined by the influence of prior ON on visual cortex atrophy. ON severity was determined using as a cutoff the median RNFL thickness and the median mean deviation of visual field of the affected eye in our cohort (78μm and -3.18dB, respectively). Accordingly, we considered 3 groups of patients based on ON history: no prior ON (control group, n = 62), mild prior ON (RNFL thickness $\geq$ 78μm and mean deviation ≥ -3.18dB, n = 20), and severe prior ON (RNFL thickness $<$ 78μm and mean deviation < -3.18dB, n = 18). The multivariate-adjusted mean of the visual cortex volume was estimated for each group, and general linear models were used for group comparisons, adjusting for sex, age at study inclusion, EDSS, disease duration, and lesion volume inside the OR. These analyses were performed independently for both time points (cross-sectionally), and then multiple linear regression analysis was performed with changes in visual cortex volume and retinal measures to evaluate longitudinally the presence of retrograde trans-synaptic degeneration of the retina. In all these longitudinal analyses, exclusively volumetric MRI measures were considered (and not NAA levels), because they are based on more robust and consistent image coregistration methods. Analyses were done using PASW Statistics 18 software (SPSS/IBM, Chicago, IL).

See Supplementary File 1 for additional information on methods.

Results

The MS-VisualPath cohort (n = 100) included 14 clinically isolated syndrome patients, 76 relapsing–remitting

TABLE 1. Demographic, Clinical, Magnetic Resonance Imaging, and Optical Coherence Tomography Characteristics of the MS-VisualPath cohort at Study Inclusion

Characteristic	All Cases, n = 100	NON, n = 62	ON, n = 38
Female, No.(%)	69 (69)	43 (69)	26 (68)
Age, yr	41.35 ± 9.54	42.51 ± 9.71	39.47 ± 9.07
Disease duration,yr	9.03 ± 7.89	8.48 ± 7.83	9.93 ± 8.00
Use of MS therapy, No. (%)	77 (77)	44 (71)	33 (87)
EDSS	1.87 ± 1.17	1.95 ± 1.28	1.74 ± 0.94
T1 lesion volume, cm^3	10.58 ± 11.32	10.54 ± 11.30	10.66 ± 11.50
NAWM volume, cm^3	730.82 ± 53.17	735.32 ± 51.68	723.59 ± 55.41
NAGM volume,cm^3	801.51 ± 56.58	799.52 ± 53.95	804.76 ± 61.23
Brain volume, cm^3	1,531.07 ± 97.6	1,532.73 ± 92.78	1,528.35 ± 106.21
Average RNFL thickness, μm	89.81 ± 13.72	94.50 ± 12.7	82.16 ± 11.86
Macular volume, mm^3	8.44 ± 0.39	8.52 ± 0.38	8.33 ± 0.39
Volume of visual cortex, cm^3	42.63 ± 4.27	43.10 ± 4.32	41.87 ± 4.14
NAA in visual cortex, ppm	20.74 ± 3.51	20.75 ± 3.54	20.72 ± 3.51
Lesion volume optic radiations,cm^3	1.45 ± 1.73	1.51 ± 1.88	1.35 ± 1.47

Means and standard deviations are given unless otherwise indicated.
EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; NAA = N-acetyl aspartate; NAGM = normal-appearing gray matter; NAWM = normal-appearing white matter;NON = no prior optic neuritis; ON = prior optic neuritis; RNFL = retinal nerve fiber layer.

MS (RRMS) patients, 4 secondary progressive MS patients, and 6 primary progressive cases. The baseline characteristics of these MS patients with the respective (MRI and OCT) measures of visual pathway damage are shown in Table 1. At the moment when the analysis of the present study was performed, 5 subjects (all RRMS) were pending to complete 1 year of follow-up. In addition, 1 subject (RRMS) suffered acute ON during follow-up and was excluded for year 1 analyses. Thus, 94 subjects of 100 were included for the follow-up evaluations.

VBM at Study Inclusion Identifies a Specific Association between Retinal Atrophy and Posterior Visual Pathway Damage

We aimed to identify the regions of the GM or WM in which lesions were associated with RNFL thickness, thereby assessing the association between the damage of the posterior and anterior visual pathways. When we performed a VBM analysis of the GM, we found that RNFL thinning was specifically associated with posterior visual pathway GM atrophy, defined by a decrease in visual cortex volume and thalamus (containing the LGN; $p < 0.05$). Table 2 and Figure 2 indicate all clusters and brain regions significantly associated with RNFL thinning. In our VBM analysis, we did not control for the balance of brain atrophy between both hemispheres, and thus the lateral distribution of atrophy may not be balanced; nor did we control for the tendency of these GM regions to become atrophied in MS, as shown by previous studies applying GM VBM.[21,22] Significantly, VBM analysis of WM lesions also confirmed that lesions inside the OR were associated with RNFL and MV atrophy (see Table 2 and Fig 2B). Of the clusters identified in the overall cohort, half matched with the OR, similarly to patients without prior ON.

Association between Injury of Posterior Visual Pathway and Retinal Damage as Indication of Retrograde Trans-Synaptic Degeneration in MS

We evaluated the influence of the damage to the posterior visual pathway on retinal atrophy by linear regression analysis performed with the data acquired at study inclusion. Retinal atrophy was used as the dependent variable, and measures of posterior visual pathway damage and other relevant clinical measures (sex, age at study inclusion, and EDSS score) were used as independent variables. Three different analyses were performed, evaluating the respective influence of visual cortex volume, NAA decay in the visual cortex, and lesion burden in

TABLE 2. Brain Clusters Associating Regional GM Atrophy and the Distribution of WM Lesions with Average Retinal Nerve Fiber Layer Thickness by Voxel-Based Morphometry at Study Inclusion

	All Cases, n = 100	MS-NON, n = 62	MS-ON, n = 38
GM volume			
GM cluster 1, size in voxels	811	476	NS
ROI for V1 and V2[a,b]	54.2 (6.7%)	30.9 (6.5%)	—
ROI for other visual cortex[b,c]	516.6(63.7 %)	332 (9.7%)	—
GM cluster 2, size in voxels	2,039	1,091	NS
ROI for thalamus[b]	1,316 (64.5%)	573 (52.5%)	—
WM lesions			
WM lesions,cluster size in voxels	4,254	582	864
ROI for optic radiations[b]	510.5 (12%)	81 (13.9%)	—

[a]Calcarine sulcus (V1) and lingual gyrus (V2).
[b]Represents the voxels and the percentage of the ROI inside the cluster.
[c]Occipitalmiddle gyrus (MT+/V3A), occipital inferior gyrus (V3A/VP), temporal middle gyrus (V5), fusiform gyrus (V4).
GM = gray matter; MS = multiple sclerosis; MS-NON = MS cases without prior optic neuritis; MS-ON = MS cases with prior optic neuritis; NS = nonsignificant; ROI = region of interest; WM = white matter.

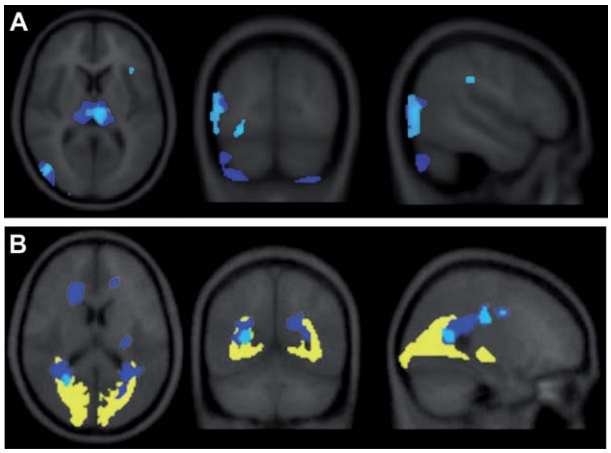

FIGURE 2: Topography of regional gray matter (GM) atrophy and white matter (WM) lesion distribution in the brain associated with retinal nerve fiber layer (RNFL) thinning. (A) Correlation analysis between GM volume and retina thickness by voxel-based morphometry (VBM). GM voxels correlated with average RNFL thickness in the overall cohort (dark blue) and for the patients without prior optic neuritis (ON; light blue). No significant clusters where found in cases with ON history. WM total lesion load was used as an additional covariate in this model. (B) Correlation analysis between WM lesion distribution and retina thickness by VBM. WM lesions correlated with average RNFL thickness in the overall cohort (dark blue) and in patients without prior ON (light blue). The Jülich probabilistic mask for Optic Radiations (OR, represented in yellow) was used to evaluate the volume of lesion clusters within OR. Results of VBM analysis were analyzed at the cluster level with familywise error correction ($p < 0.05$, uncorrected threshold at voxel level of $p < 0.001$). Resulting clusters are represented in axial, coronal, and sagittal planes of 1mm per the Montreal Neurological Institute template .

OR. In addition, in each of these analyses, to control for the effect of previous ON, we first included prior ON as an independent variable in the analysis of the overall cohort. Then we performed separate subanalyses in cases without prior ON and in cases with prior ON. The directionality implied in this statistical approach would potentially evaluate the possible presence of retrograde trans-synaptic degeneration in the visual pathway. We first evaluated the association of RNFL thickness with visual cortex volume in the overall cohort. Accordingly, we found that visual cortex volume was significantly associated with average RNFL thinning ($\beta = +0.601$, 95% confidence interval [CI] = +0.04 to +1.16, $p = 0.036$) independently of other confounders, especially ON (Table 3). Our regression model (Equation) explained nearly half of the variance in the change in RNFL thickness (as indicated by the $R^2 = 0.43$), indicating that a decrease of $1cm^3$ in visual cortex volume predicts a reduction of $0.6\mu m$ in RNFL thickness (independent of other variables):

$$\begin{aligned}\text{RNFL thickness} &= 74.630 + 5.178\ \text{sex (1 female)} \\ &- 0.080\ \text{age (years)} - 12.295\ \text{ON (1 for previous ON)} \\ &- 1.009\ \text{EDSS score} + 0.601\ \text{VCV}\ (cm^3) \\ &- 2.551\ \text{VolOR}\ (cm^3)\end{aligned} \quad (1)$$

where VCV stands for the volume of the visual cortex and VolOR indicates the lesion volume inside the OR.

TABLE 3. Association of Posterior and Anterior Visual Pathway Damage by Multiple Linear Regressions at Study Inclusion

	All Cases,n = 100		MS-NON, n = 62		MS-ON, n = 38	
	β (95% CI)	R^2	β (95% CI)	R^2	β (95% CI)	R^2
Visual cortex volume, cm^3						
RNFLT, μm	+0.601(+0.041 to +1.161)[a]	0.429	+0.552(−0.243 to +1.348)	0.218	+0.416(−0.391 to +1.224)	0.526
MV, mm^3	+0.011(−0.008 to +0.030)	0.208	+0.012(−0.013 to +0.037)	0.114	−0.003(−0.030 to +0.036)	0.284
NAA in visual cortex, ppm						
RNFLT, μm	+1.075(+0.190 to +1.961)[a]	0.451	+1.717(+0.499 to +2.935)[a]	0.353	−0.015(−1.370 to +1.340)	0.500
MV, mm^3	+0.036(+0.007 to +0.065)[a]	0.270	+0.050(+0.014 to +0.086)[a]	0.255	+0.008(−0.048 to +0.064)	0.258
Lesion volume within optic radiations, cm^3						
RNFLT, μm	−2.551(−3.910 to −1.192)[a]	0.429	−1.969(−3.711 to −0.226)[a]	0.218	−4.171(−6.492 to −1.850)[a]	0.526
MV, mm^3	−0.074(−0.120 to −0.028)[a]	0.208	−0.059(−0.114 to −0.004)[a]	0.114	−0.121(−0.216 to −0.025)[a]	0.284

Results are shown as R^2 values, the beta regression coefficients, and 95% CIs.
[a]$p < 0.05$.
CI = confidence interval; MS = multiple sclerosis; MS-NON = MS cases without prior optic neuritis; MS-ON = MS cases with prior optic neuritis; MV = mean bilateral macular volume; NAA = N-acetyl aspartate; RNFLT = mean bilateral average retinal nerve fiber layer thickness.

In line with these findings, when we analyzed the association between NAA levels in the visual cortex and retinal atrophy, we found that the decay in the absolute NAA levels in the visual cortex was significantly associated with average RNFL thinning in the overall cohort, and especially in patients without prior ON (see Table 3).

Furthermore, when we analyzed the association between WM lesion volume inside the OR and the retinal thickness by multiple linear regression analysis, we found that the lesion volume inside the OR was significantly associated with the average RNFL thickness and MV in the overall cohort, as well as in the patients with and without prior ON (see Table 3).

Evaluation of Anterograde Trans-Synaptic Degeneration

We evaluated the effect of prior ON on visual cortex atrophy by general linear models with the data obtained at study inclusion. The specificity of this association was evaluated by assessing the effect of ON on the atrophy of another cortical area unrelated with afferent visual pathway (precentral cortex). The multivariable-adjusted mean of the visual cortex volume was estimated and compared between 3 groups: no prior ON (control group; n = 62), mild prior to ON (RNFL thickness $\geq$ 78μm and visual field mean deviation $>$ −3.18dB; n = 20), and severe prior to ON (RNFL thickness $<$ 78μm and visual field mean deviation $<$ −3.18 dB; n = 18). After adjustment for sex, age at inclusion, disease duration, EDSS score, and lesion volume inside the OR, the mean visual cortex volume was significantly lower for MS patients who previously experienced severe ON compared to the control group (MS patients without ON; adjusted difference = −3.01, 95% CI = −5.08 to −0.95; Table 4). Similarly, MS patients with mild prior ON had a lower mean visual cortex volume than the controls, although the adjusted differences compared with MS patients without prior ON were not statistically significant (see Table 4). Lastly, when we analyzed MS patients with history of ON and compared mean visual cortex volume between cases with mild

TABLE 4. Multivariable-Adjusted Means and Differences in Patients with Severe and Mild ON by General Linear Models at Study Inclusion

	Severe MS-ON, n = 18, Mean (95% CI)	Mild MS-ON, n = 20, Mean (95% CI)	MS-NON, Reference, n = 62, Mean (95% CI)
Visual cortex volume, cm^3	39.53(37.71 to 41.36)	41.52(39.81 to 43.22)	42.55(41.58 to 43.52)
Adjusted difference vs reference, cm^3	−3.01(−5.08 to −0.95)[a]	−1.03(−3.02 to +0.95)	—
Precentral cortex volume, cm^3	34.57(32.18 to 36.97)	36.79(34.66 to 38.93)	34.87(33.65 to 36.09)
Adjusted difference vs reference, cm^3	−0.30 (−3.03 to +2.43)	+1.92 (−0.56 to +4.40)	—

Multivariable-adjusted means and corresponding CIs for visual and precentral cortex volume in cases with severe and mild ON (subjects of interest) and for cases with no prior ON (reference subjects) by general linear models (R^2 of this model is 0.196).
[a] $p < 0.05$.
CI = confidence interval; MS = multiple sclerosis; MS-NON = MS cases without prior optic neuritis; MS-ON = MS cases with prior optic neuritis.

and severe prior ON; we found that cases with mild ON had higher visual cortex volume (mean = 41.81, 95% CI = 40.12 to 43.50) compared with those with severe ON (mean = 39.99; 95% CI = 38.14 to 41.84), although differences were not significant (adjusted difference = −1.81, 95% CI = −4.50 to +0.88, $p = 0.183$). To confirm the specificity of this association, we evaluated the effect of ON on the atrophy of precentral gyrus using the same statistical approach and did not find significant differences (see Table 4).

Longitudinal Analysis after 1 Year of Follow-up

After 1 year follow-up (n = 94), mean visual cortex volume decreased $0.63cm^3$ (1.5%), mean lesion volume in OR increased $0.01cm^3$ (0.7%), mean average RNFL thickness decreased 0.4μm (0.4%), and macular volume increased $0.04mm^3$ (0.5%; see Table 1 in Supplementary File 2). To confirm our results at study inclusion, we repeated the analysis for retrograde damage with the data obtained after 1 year of follow-up. We observed again that visual cortex atrophy and higher lesion volume in OR were associated with retinal atrophy (see Table 2 in Supplementary File 2). We selected the retrograde model, because analyzing the anterograde model with conditions similar to baseline analyses requires the presence of a group of incident ON, and we have detected only 1 patient with incident ON after 1 year of follow-up. We also evaluated the association of the change of retinal measures with mean visual cortex volume change from baseline to year 1. As expected, considering the small observed changes between both time points, the study had insufficient power to detect longitudinal changes.

Discussion

In this study, we have found an association between the damage in the anterior and posterior visual pathway, having controlled for the presence of axonal damage within plaques (eg, ON or lesions in the OR). The direction of this association has been further corroborated in a longitudinal analysis performed in the same cohort after 1 year of follow-up. Therefore, our findings suggest the presence of trans-synaptic degeneration in the brain of patients with MS. First, we demonstrated by VBM that posterior visual pathway damage was specifically associated with retinal atrophy, highlighting the suitability of this region to analyze transgeniculate degeneration of the retina. More specifically, the association of visual cortex damage (visual volume and NAA decay) with RNFL atrophy in MS patients with no prior ON suggests the presence of retrograde transgeniculate degeneration in the retina. Conversely, the presence of more intense atrophy in the visual cortex of MS patients who had previously suffered ON provides indications of the anterograde transgeniculate degeneration. The direction of these findings was further confirmed in the analysis of the same cohort after 1 year of follow-up. To gain insight into the weight of this phenomena, our models explained more than half of the variance (as indicated by the R^2 value of the model = 0.43) in the change in RNFL thickness, and they indicate that a reduction of $1cm^3$ in visual cortex volume predicts a reduction of nearly 0.6μm in the RNFL thickness (retrograde trans-synaptic damage in the retina). Conversely, the presence of severe ON predicts a reduction of $3cm^3$ (7%) in visual cortex volume (anterograde trans-synaptic damage of the visual cortex). If we

generalize these results to the entire brain, these findings suggest that trans-synaptic degeneration may not be a minor element in the overall neurodegenerative process in MS, and for this reason it should be monitored and targeted by therapies for this disease.

In a previous study, we demonstrated retrograde and trans-synaptic degeneration in the posterior visual pathway using a nonaprioristic approach (VBM), showing that lesions in the OR explained almost 30% of the atrophy of the LGN.[10] However, we could not differentiate whether there was trans-synaptic retrograde degeneration within the OR. Our current results extend such studies, supporting the influence of trans-synaptic degeneration as a mechanism of axonal damage in MS. Previous studies in animal models have demonstrated the existence of trans-synaptic degeneration after focal brain damage,[23–26] revealing the potential spreading effect of focal WM lesions along WM tracts and through networks within the brain. Such a phenomenon has also been studied in the human visual pathway after focal damage of a different nature in either the visual cortex or optic nerve, providing evidence of trans-synaptic degeneration between the anterior and posterior visual pathway.[2,27–31] With new imaging approaches, it was found that MS patients who had suffered ON developed abnormalities in the OR (evident by diffusion tensor imaging and tractography)[11,13,14,32] or in the cortex (assessed by magnetic transference imaging or brain spectroscopy),[12,33] even some time after focal injury, indicative of trans-synaptic damage. Our study is consistent with these previous findings, entering into more detail by allowing retrograde and anterograde damage to be assessed.

Our study has several limitations. First, the short follow-up time of our cohort leads to small changes in OCT and MRI metrics, and thus an insufficient statistical power to detect longitudinal changes in our model of trans-synaptic degeneration, which may entail years of waiting.[3] Future longitudinal analyses of the MS-VisualPath cohort with longer follow-up times are planned to validate longitudinally the presence of trans-synaptic degeneration in MS. Conversely, we have made use of the same cohort to analyze retrograde and anterograde degeneration. Considering that the number of eyes with previous ON is lower than that of non-ON eyes and that half of axons cross in the chiasm, power to detect anterograde degeneration is smaller, and for this reason current results would require validation in prospective studies with patients suffering acute ON. Also, we have not analyzed each retinal layer of the macula separately. In previous studies of glaucoma using spectral domain OCT, a condition in which trans-synaptic degeneration has also been reported, the contribution of retinal ganglion cell atrophy to inter-retinal degeneration seems to be limited.[34] In addition, we screened for prior ON based on medical records and clinical criteria from the Optic Neuritis Treatment Trial.[16] However, no reliable criteria have yet been established to detect subclinical ON unambiguously. To increase the accuracy of classifying patients with prior (clinical or subclinical) ON, we further analyzed cases by detailed ophthalmological examination, OCT, perimetry, and MRI. However, this issue should be addressed in future studies applying multifocal visual evoked potentials, which are very sensitive in detecting subclinical ON.[35] The LGN is a very tiny structure that is difficult to segment, and for this reason it was quantified embedded in the thalamus. New segmentation algorithms and sequences may provide better opportunities for analyzing this critical deep GM structure.[36] Regarding segmentation of OR, current diffusion tensor imaging protocols are inaccurate and achieve only partial reconstruction, missing the Meyer loop. For this reason, we decided to use a probabilistic atlas from a healthy control reference population as the best approach to identify OR. Development of tractography algorithms would allow an individualized and improved segmentation of this tract. In addition to axonal degeneration in the WM due to MS plaques, there is also evidence that chronic and progressive "slow-burn" axonal injury and degeneration affects the GM, possibly due to chronic activity of microglia, lack of oligodendrocyte support, and the presence of a microenvironment that is deleterious for neurons.[37] We did not address the role of chronic degeneration of the GM (including the retina), which seems to be a diffuse process throughout the brain. Finally, our cohort was mainly composed of RRMS patients and as such our results will mainly apply to this subtype. We hypothesize that patients with progressive disease may experience significantly more trans-synaptic degeneration, although several other confounding factors in this subgroup might limit this analysis, such as a lower WM lesion volume, increased brain atrophy, or a more significant effect of age.

Better understanding of trans-synaptic degeneration would enhance our understanding of the degenerative component of MS and/or the development of new neuroprotective therapies. Here we provide a model to quantify the extent of trans-synaptic degeneration in the visual pathway that could serve to monitor disease course, or as a marker of the effects of neuroprotective or regenerative therapies being tested in MS. Given the significant interest in defining biomarkers of neurodegeneration and neuroprotection for clinical trials,[38] longitudinal studies of visual pathway degeneration are warranted to confirm the results presented here.

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Potential Conflicts of Interest

E.H.M.-L.: travel expenses, Biogen, Bayer. S.L.: speaking fees, Teva, Biogen, Novartis; travel expenses, Teva, Novartis, Biogen, Merck-Serono. M.S.: paid educational presentations, Biogen, Teva; travel expenses, Biogen. A.S.: grants/grants pending, Instituto Salud Carlos III; speaking fees, travel expenses, Biogen. S.O.: no conflicts to disclose. B.S.-D.: speaking fees, paid educational presentations, Novartis; travel expenses, Alcon, Bausch + Lomb. P.V.: board membership, Roche, Novartis, Neurotec Farma, Bionure Farma; consultancy, Novartis, Roche, TFS, Heidelberg Engineering, MedImmune, Digna Biotec, Neurotec Pharma; grants/grants pending, European Commission, Instituto Salud Carlos III, Marato TV3, Novartis, Roche; patents, Digna Biotec, Bionure Farma; stock/stock options, Bionure Farma; travel expenses, Novartis.

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