RESEARCH ARTICLE

# Structure-function relationships in the visual system in multiple sclerosis: an MEG and OCT study

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## Abstract

**Background**: We conducted a multi-modal optical coherence tomography (OCT) and magnetoencephalography (MEG) study to test whether there is a relationship between retinal layer integrity and electrophysiological activity and connectivity (FC) in the visual network influenced by optic neuritis (ON) in patients with multiple sclerosis (MS). **Methods**: One hundred and two MS patients were included in this MEG/OCT study. Retinal OCT data were collected from the optic discs, macular region, and segmented. Neuronal activity and FC in the visual cortex was estimated from source-reconstructed resting-state MEG data by computing relative power and the phase lag index (PLI). Generalized estimating equations (GEE) were used to account for intereye within-patient dependencies. **Results**: There was a significant relationship for both relative power and FC in the visual cortex with retinal layer thicknesses. The findings were influenced by the presence of MSON, *particularly* for connectivity in the alpha bands and the outer macular layers. In the absence of MSON, this relationship was dominated by the lower frequency bands (theta, delta) and inner and outer retinal layers. **Conclusion**: These results suggest that visual cortex FC more than activity alters in the presence of MSON, which may guide the understanding of FC plasticity effects following MSON.

## Introduction

Many patients with multiple sclerosis (MS) suffer from impairment of visual function, particularly following MS–associated optic neuritis (MSON).[1,2] Patients rate visual impairment as the second most relevant disability that reduces their quality of life.[3] The severity of subjective visual impairment is however not always explained by the severity of damage to the optic pathways; some patients with very severe damage following MSON perform relatively well in their daily activities, while other patients with only mild unilateral MSON suffer from severe difficulties with visual impairment. It is possible that part of the symptoms reported by patients may be related to success or failure of local or long distance communication within the visual cortex.

The occipital network of the human visual system receives input from about 100 million rod photoreceptors and 4.6 cone photoreceptors per eye via pathways that traverse the dorsal lateral geniculate nucleus. The analogue input from the outer retinal layers (ORL) is modulated by exactly 12 types of bipolar cells residing in the inner nuclear layer (INL) and transformed into an "on/off" digital signal fed to the inner retinal layers (IRL).[4] Further signal processing is performed in the retinal ganglion cell layer (GCL) from where about 1,158,000 axons arise to synapse with second-order visual system neurons in the human central nervous system (CNS).[5] In a healthy subject, the fovea's of both eyes are centered on the

relevant visual stimulus such that binocular vision becomes possible. The strict representation of the retinal structure in the primary visual cortices, called retinotopic mapping, is important to the functioning of the fine-tuned cytoarchitecture of the receiving cortical neurons.[6] The higher visual cortex is capable of a remarkable degree of plasticity which permits a rapid adaptation to an ever changing visual environment.[7] Optic neuritis is a pragmatic model to study this adaptation and these structural-functional relationships.[1]

Lesions to the visual pathway and retrograde transsynaptic axonal degeneration are thought to cause atrophy of the IRL.[8] Importantly, retrograde transsynaptic atrophy in the retina stops at the level of the INL.[8–11] The preservation of the INL following MSON is understood to be due to multiple afferent inputs through a network of horizontal and amacrine cells.[8,12,13] Another less well understood, but consistent, observation in the retina is the thickening of the ORL following MSON.[8,12] We have previously hypothesized that long-term thickening of the ORL might be related to plastic remodeling within the retina and in the visual cortex.[8] This is in contrast to the short-term ORL thickening thought to be caused by inflammation-related edema.[2] In this first multi-modal MEG and OCT study in MS, we tested the hypothesis that there is a relationship between structural changes in the retinal layers and function of the visual cortex.

Function depends upon both activity and functional connectivity within the visual cortex. Activity corresponds to relative spectral power within a region, whereas functional connectivity corresponds to statistical interdependencies between activity profiles of two separate regions within the visual cortex. The former is assumed to reflect local communication within an area, whereas the latter is assumed to reflect long distance connections between cortical areas. Having said that, we assumed that the structure-function relationship would be influenced by the presence of MSON.[2,14] Because of the balanced signal requirements for human binocular vision,[15] it was assumed that the structural-functional relationships would be influenced differently by unbalanced signaling (unilateral MSON, with the better/dominant eye providing most of the information) from the eye to the visual cortex, compared to either bilateral symmetrically reduced signaling (bilateral MSON) or bilateral normal signaling (bilateral MSNON). To test this hypothesis, we investigated patients with MS who (1) never suffered from MSON (MSNON), (2) suffered from unilateral MSON or (3) bilateral MSON.

## Methods

In total, 102 MS patients were included for analysis based on a previous study, and the MEG data used in this study have been published in another context,[16] separately from the OCT data.[8] Patients were recruited from the Amsterdam MS cohort, at the VUmc MS center. The study protocol was approved by Medical Ethical Review Committee of the VU University Medical Center, whose ethics review criteria conformed to the Helsinki declaration. All subjects gave written informed consent prior to participation (protocol number ethics 2010/336, R&D CWO/10-25D).[8,16] A detailed description of the full cohort, recruitment, and eligibility procedure can be found elsewhere.[16]

## Retinal OCT imaging

All images were obtained using spectral domain OCT (Spectralis®, Heidelberg Engineering Inc, Heidelberg, Germany, Software version 1.7.1.0) with the eye-tracking function enabled. All OCT scans were obtained at the same site, by three different experienced technicians. Room light conditions were dimmed and no pharmacological pupil dilation was used. Retinal thickness data were obtained by means of quality controlled automated segmentation of the optic disc (12°ring scan) and macula (20 × 20°, 25 B-scan composite volume, using the mean thickness of the 1, 3, 6 mm grid).[18,19] Segmentation of the IRLs consisted of the cumulative thickness from the inner limiting membrane to the outer border of the inner plexiform layer. The outer plexiform and outer nuclear layer made the ORLs.). Scans were excluded from the analyses if they did not fulfill the revised quality control criteria (OSCAR-IB), which led to a rejection rate of 18%.[18,20] All raters were blinded to all other clinical data or paraclinical information.

## MEG data acquisition

Data acquisition is described in detail in previous work.[16] The MEG data were recorded using a 306-channel whole-head MEG system (Elekta Neuromag, Oy, Helsinki, Finland). Participants were in supine position in a magnetically shielded room (Vacuumschmelze, Hanau, Germany). Fluctuations in magnetic field strength were recorded during a no-task, eyes-open, resting-state condition for 3 min (not analyzed here) and eyes-closed condition for five consecutive minutes with a sample frequency of 1250 Hz. An anti-aliasing filter of 410 Hz and a high-pass filter of 0.1 Hz were applied online and other artifacts were removed offline using the temporal extension of Signal Space Separation (tSSS) in MaxFilter software (Elekta Neuromag Oy, version 2.2.10),[21] with a 10 sec sliding window and a correlation limit of 0.9. Artifactual noisy channels were identified by automatic and visual data inspection [PT, AH] and removed prior to tSSS. The number of excluded channels varied between 1 and 12,

and was similar for MS patients and healthy controls (Mann–Whitney, *P* > 0.05). In addition, the participant's head position relative to the MEG sensors was recorded continuously using the signals from four head-localization coils. The head-localization coil positions were digitized, as well as the outline of the participant's scalp (~500 points), using a 3D digitizer (Fasttrak, Polhemus, Colchester, VT, USA). Scalp surfaces of all subjects were coregistered with their structural MRIs using a surface-matching procedure, with an accuracy of about 4 mm.[22] A single sphere was fitted to the outline of the scalp as obtained from the coregistered MRI, which was used as a volume conductor model for the beamformer approach described below.

## Estimation of MEG activity and functional connectivity

An atlas-based beamforming approach was adopted to map MEG data from sensor level to source space.[23] The exact beamforming procedure has been described in previous work[16] and can also be found in the Data S1. For every subject this procedure results in one time-series for every cortical region of interest (ROI) in the automated anatomical labeling (AAL) atlas. The frequency bands of interest were: delta (0.5–4 Hz), theta (4–8 Hz), lower alpha (8–10 Hz), upper alpha (10–13 Hz), beta (13–30 Hz), and lower gamma bands (30–48 Hz). Subsequently, representative time-series for regions within the visual cortex were considered for further analysis (Fig. 1; see SI for regions).[24]

Activity in the visual cortex was defined as relative spectral power in a ROI in the visual cortex. To quantify

activity, we computed the absolute value of the Fast Fourier Transform of time-series of each AAL region within the visual cortex, which was normalized by the power over the whole spectrum to obtain a measure for relative power. All individual spectra for all visual regions were averaged across regions to obtain one spectrum per subject. From this spectrum, we quantified the relative power for each frequency band and the peak frequency (defined as the highest peak in the 4–13 Hz range). To quantify functional connectivity, we performed frequency filtering for every time-series followed by computation of the phase lag index (PLI)[25] for each pair of ROIs in the visual network. The PLI is a phase synchronization metric that quantifies the asymmetry of the distribution of the phase differences between the time-series and is relatively insensitive to signal leakage. The rationale to use this functional connectivity metric is that it has proven to be a very sensitive metric to distinguish between healthy subjects and MS patients in a clinically meaningful way.[16,24] PLI values between visual regions were averaged in order to obtain a single PLI value per subject.

## Clinical assessments

A diagnosis of MSON was based on medical history, according to consensus criteria,[2] that is based on patient interviews and chart reviews. At this stage no perimetry was performed. Patients' eyes were dichotomized into those with and those without MSON. High contrast visual acuities were recorded and clinical disability documented on the expanded disability status scale (EDSS). The disease course was classified into relapsing remitting (RR), primary (PP), and secondary progressive (SP).[26]
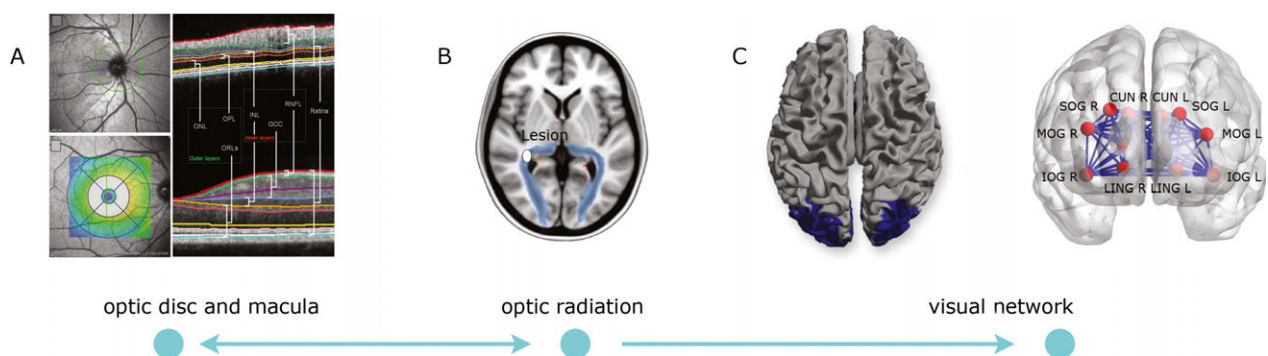


**Figure 1.** The structural-functional relationship between the retina and the functional network in the visual cortex. (A) shows the OCT scan location at the optic disc and the macula. The colored lines in (A) are the result of the automated retinal layer segmentation. (B) shows the optic radiation and a lesion in these tracts that could lead to retrograde axonal degeneration. (C) illustrates the MEG visual resting-state network as projected on a template mesh of the cortical surface viewed from the top (left) and from the back (right). Red dots correspond to visual regions, and blue lines to connections between these visual regions. The light blue arrows indicate how damage in one structure may influence other structures in the visual pathway. Abbreviations: superior occipital gyrus (SOG), medial occipital gyrus (MOG), inferior occipital gyrus (IOG), cuneus (CUN), lingual gyrus (LING).

## Statistical analyses

All statistical analyses were performed using SAS software Cary, United States of America (version 9.4). Patient grouping was based on presence or absence of MSON. Demographic and clinical data for the groups were compared using general linear models (GLM). Because OCT data from two eyes per subject was used, the hypothesis to be tested was that there is a relationship between integrity of the eye and relative power and PLI in the visual cortex. To test this, we used generalized estimation equations (GEE) in order to account for intereye within-patient dependencies using the exchangeable correlation structure as recommended by Balcer and coworkers.[27] We tested the relationship between retinal layer thickness (atrophy or augmentation of IRL, INL, ORL) and band-specific power/functional connectivity in the visual cortex. Relevant covariates for the GEE analyses were age, gender, visual acuities, and extent of MS lesions in the optic radiations (note that lesion segmentation methods and results for this cohort have been described earlier[17,28]). Models were built by forward selection. Two-sided tests were used throughout for these exploratory analyses. Because of the exploratory nature of the study, we accepted a $P < 0.05$ as significant and indicated those comparisons which also retained their level of significance for a Bonferroni-corrected $P$-value in bold.

## Result

The baseline characteristics of the patients are summarized in Table 1. The demographic data (age, gender) were comparable between the groups. For disease duration, there was a significant group effect (GLM, $P = 0.0064$). Post hoc analysis showed that patients with evidence for bilateral MSON had a significant longer disease duration compared to those with only unilateral MSON ($P = 0.02$) or MSNON ($P = 0.0015$). The degree of disability in terms of EDSS and high contrast visual acuity were comparable between groups. The majority of the patients (90%) with bilateral MSON had a RR/SP disease course. As expected, clinically only one out of 10 patients with a PP disease course experienced MSON.

## Retinal layer thickness is related to relative power in the visual cortex

Note that thicker outer retinal layers in most groups corresponded to higher alpha (alpha1 and/or alpha2) band relative power (Table 2), or likewise thinner outer retinal layers was related to lower relative power in these frequency bands. In the bilateral MSON group, this relationship was not found for relative power, but instead a strong positive relationship between outer retinal layers and the peak frequency (predominantly located in the alpha band) was found. These associations between outer retinal layers and relative power were only found for the macula, and not for the optic disc where these layers are much less substantial. Relative power in two other frequency bands (delta and beta) also showed significant relationships with retinal layer thickness. Relative power in the delta band was negatively associated with inner retinal layers in the unilateral MSON group. Lastly, in the pooled group of all MS patients, relative power in the beta band was negatively associated with inner retinal layers in the macula (see all results in Table 2). Notice that significant relationships between relative power and

**Table 1.** Baseline characteristics of patients with multiple sclerosis.

|  | MS patients (pooled) | MSNON (bilateral) | MSON (unilateral) | MSON (bilateral) |
|---|---|---|---|---|
| N | 102 (100%) | 48 (47.1%) | 38 (37.3%) | 16 (15.7%) |
| Age (years) | 54.3 (±10.0) | 54.9 (±10.9) | 52.8 (±8.8) | 55.9 (±8.5) |
| Gender (% female) | 66 (64.7%) | 27 (56.3%) | 29 (76.3%) | 10 (62.5%) |
| MS type (N [%]) |  |  |  |  |
|   RR | 68 (66.7%) | 30 (62.4%) | 26 (68.4%) | 12 (75.0%) |
|   SP | 24 (23.5%) | 9 (18.8%) | 12 (31.6%) | 3 (18.8%) |
|   PP | 10 (9.8%) | 9 (18.8%) | 0 (0%) | 1 (6.2%) |
| Disease duration (years) | 18.2 (±6.7) | 16.7 (±6.5) | 18.2 (±5.8) | 22.8 (±7.6)[1] |
| EDSS (median [range]) | 4.1 [1.0–8.0] | 4.1 [1.0–7.5] | 4.1 [2.5–8.0] | 4.0 [2.0–6.0] |
| Visual acuity | 0.8 (±0.2) | 0.8 (±0.2) | 0.8 (±0.2) | 0.8 (±0.2) |

Data are shown for the entire cohort and for those MS patients who never experienced an episode of MS–associated optic neuritis (MSNON), and those with bilateral or unilateral MSON. Numbers (%), mean (±SD) or median [range] are shown. RR, relapsing remitting MS; SP, secondary progressive MS; PP, primary progressive MS.
[1]Patients with bilateral MSON had a significant longer disease duration compared to patients with unilateral MSON ($P = 0.02$) and MSNON patients ($P = 0.0015$).

**Table 2.** Functional–structural relationships between MEG frequency band activities and OCT retinal layer thicknesses.

| Patient group | OCT location | MEG relative power (frequency band) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | δ | θ | α1 | α2 | β | γ | Peak latency |
| MS (pooled) | Disc | – | – | – | | – | – | – |
| | Macula | – | – | ORL(+)* | – | **INL(-)**\*\* | – | – |
| MSNON (bilateral) | Disc | – | – | – | – | – | – | – |
| | Macula | – | – | ORL(+)* | ORL(–)* | – | – | – |
| MSON (unilateral) | Disc | INL(–)* | – | – | – | – | – | – |
| | Macula | IRL(–)* | – | – | **ORL(+)**\*\* | – | – | INL(–)* |
| MSON (bilateral) | Disc | – | – | – | – | – | – | – |
| | Macula | – | – | – | – | – | – | **ORL(+)**\*\*\* |

General estimating equations (GEE) were used for logistic regression prediction of frequency band–specific activity, adjusting for age, VA, gender, and extent of MS lesions in the optic radiations. Significant relationships were indicated as *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, – = no significant relationship. The signs (+/−) behind the significant relationships denote whether the association was positive (i.e., denoting a significantly thicker retinal layer in patients with higher visual cortex activity) or negative (–). Inner nuclear layer (INL), outer retinal layer (ORL), inner retinal layer (IRL). Significant findings which remain after Bonferroni correction ($P < 0.007$) are shown in bold.

**Table 3.** Functional–structural relationships between MEG functional connectivity in different frequency bands and OCT retinal layer thicknesses of the optic disc or macula.

| Patient group | OCT location | MEG functional connectivity (frequency band) | | | | | |
|---|---|---|---|---|---|---|---|
| | | δ | θ | α1 | α2 | β | γ |
| MS (pooled) | Disc | IRL(+)*, INL(+)*, ORL(+)* | – | – | – | INL(+)* | – |
| | Macula | – | – | – | – | – | IRL(+)* |
| MSNON (bilateral) | Disc | IRL(+)*, INL(+) *, ORL(+)* | – | – | – | – | – |
| | Macula | – | INL(–)* | – | – | – | IRL(+)* |
| MSON (unilateral) | Disc | – | – | – | – | – | – |
| | Macula | – | – | – | – | – | – |
| MSON (bilateral) | Disc | INL(–)*, ORL(+)* | **ORL(+)**\*\* | ORL(–)** | – | – | – |
| | Macula | – | – | ORL(+)* | **ORL(+)**\*\*\* | – | – |

General estimating equations (GEE) were used for logistic regression of predicting high connectivity, adjusting for age, VA, gender, and extent of MS lesions in the optic radiations.

Significant relationships were indicated as *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, – = not significant. The signs (+/−) behind the significant relationships denote whether the association was positive (+ and denoting a significantly thicker or thinner retinal layer in patients with higher functional connectivity in the visual cortex, respectively). Inner nuclear layer (INL), outer retinal layer (ORL), inner retinal layer (IRL). Significant findings which remain after Bonferroni correction ($P < 0.008$) are shown in bold.

retinal layers thickness were predominantly found for the macula where cones predominate.

## Retinal layer thickness is related to functional connectivity in the visual cortex

The results for connectivity analysis are summarized in Table 3. Most of the significant relationships between retinal layer thickness and average PLI were found for the bilateral MSON patients (Table 3). In this group, outer retinal layer thickness was positively related to average PLI in the visual cortex for different frequency bands, but most prominently for the alpha bands. In the unilateral MSON group, there were no significant relationships between retinal layer thickness and average PLI for this

unilateral MSON group. For MSNON patients quite different results were observed, where a significant positive structural-functional relationship was found between all retinal layer compartments at the optic disc and delta band average PLI in the visual cortex.

For the pooled cohort of all MS patients, a significant positive structural-functional relationship was also revealed between all retinal layer compartments at the optic disc and delta band functional connectivity in the visual cortex (Table 3). A significant structural-functional relationship for the pooled group was also found between beta band connectivity and the INL thickness. Furthermore, there was a significant positive association between gamma band connectivity and the inner retinal layer thickness at the macula in the pooled cohort. Given the

results in the MSNON group and the fact that the MSNON group was the biggest group in terms of subjects, it is likely that the results in the pooled group are driven by the MSNON group. Lastly, in contrast to *activity*, notice that significant relationships between *connectivity* and retinal layers thickness were found for both the macula and the optic disc.

## Discussion

We conducted a multi-modal MEG/OCT study to test the hypothesis that there is a relationship between retinal layer thickness and electrophysiological activity and connectivity in the visual network influenced by the presence of optic neuritis. Results for activity show that low alpha band activity is related to thinner outer retinal layers in the macula for most groups, weakly influenced by the presence of optic neuritis. A stronger distinction between MSON and MSNON groups was found for functional connectivity, where results showed that in MS patients without optic neuritis, the thickness of all retinal layers was positively related to functional connectivity in especially the lower frequency bands. In contrast, patients with bilateral MSON showed a strong positive relationship of especially outer retinal layers with alpha band connectivity.

A consistent observation for the different clinical groups for neuronal activity was a positive association between outer retinal layer thickness in the macula and alpha band activity. This association was present in both MSON and MSNON groups, although stronger in the MSON group. A potential explanation for the relationship between of the outer retinal layers and alpha band activity is that the relationship is linked to antero- and retrograde axonal degeneration in the visual pathway and therefore mediated by white matter integrity. The observed relationship between alpha band activity/peak frequency and outer retinal layers as seen in bilateral MSON patients may support this, given the fact that these patients generally have more damage in white matter tracts. A more extensive mediation analysis with white matter integrity could confirm this in a future study. The present analysis considered only presence of lesions in the optic radiations.

With respect to functional connectivity there was greater distinction between the bilateral MSON and the bilateral MSNON group. This study demonstrated that patients with MSNON, who suffer from a similar reduction in high contrast visual acuity compared to MSON patients (both averaging at 0.8, Table 1) seem to have a structural-functional relationship for all retinal layers that is dominant in the lower frequency band (delta).

Patients with bilateral MSON showed, in addition, a strong structural-functional relationship in the higher frequency bands (lower and upper alpha). The outer retinal layer thickness in the latter group was positively related to alpha band connectivity. A potential explanation may again be that optic neuritis causes damage to white matter tracts, which are especially known to be mediating alpha band oscillations.[29] For the MSNON group, we found that delta band connectivity was related to thickness of the outer retinal layers. This may also be the effect of a global pathological and/or degenerative process since decreased delta band connectivity in the group was found widespread across the cortex,[16] and is generally more found in frontal regions than occipital regions. The observed relationship between delta band connectivity and outer retinal layer thickness in the MSNON may therefore be an indirect effect.

For interpretation of these data one needs to remember that all analyses were corrected for the extent of damage to the optic radiations by MS lesions, which might also be responsible for an asymmetric signal feed to the occipital visual network. Limitations of this study are:[1] no electrodiagnostic tests were performed;[2] an important neuro-anatomical relay station in the visual pathway, the volume of the lateral geniculate nucleus, was taken into account as covariate given the difficulty to segment this brain region. However, this thalamic structure plays an important role in the hierarchical feed-forward system from the retinal layers to the visual cortex. Future studies should include longitudinal data on the electroretinogram (ERG) and visual evoked potentials (VEP)/visual evoked magnetic fields (VEF) following guidelines incorporating the ISCEV standards.[2,30,31] In addition, future studies should study outer retinal layers in relationship to white matter tract integrity, neuronal activity and connectivity during the acute phase and after this phase of an optic neuritis episode, in order to elucidate what mechanisms in the visual system allow for recovery of visual acuity. Together with modeling and animal studies this may also elucidate why different relationships for visual activity and connectivity can be found with the macula and the optic disc.

In conclusion, this study suggests that resting-state connectivity and activity is related to retinal layer thickness, which is influenced by the presence of optic neuritis. In the presence of MSON, there seems to be a strong relationship between outer retinal layers and alpha band connectivity, whereas for MSNON, the structure-function relationship was more pronounced in the delta band. This exploratory work could guide us to understand how connectivity changes in MSON patients during and after an episode of optic neuritis.

## Conflict of Interest

None declared.

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## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Data S1.** The atlas-based beamforming approach is explained in the supplementary text S1.