The visual pathway: A long pathway to study multiple sclerosis

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Abstract: Patients with multiple sclerosis (MS) almost always experience effects in the visual pathway; and thus, visual dysfunction is not only common but also highly relevant. The visual pathway represents a model of acute focal central nervous system (CNS) damage, through acute optic neuritis and retinal periphlebitis, as well as a model of chronic, diffuse CNS damage through chronic retinopathy and optic neuropathy. The optic pathway can be accurately evaluated in detail, due to the availability of highly sensitive imaging techniques (e.g. magnetic resonance imaging or optical coherent tomography) or electrophysiological tests (multifocal visual evoked potentials or electoretinography). These techniques allow the interactions between the different processes at play to be evaluated, such as inflammation, demyelination, axonal damage and neurodegeneration. Moreover, these features mean that the visual pathway can be used as a model to test new neuroprotective or regenerative therapies.

Keywords: Axonal damage, demyelination, inflammation, multiple sclerosis, neurodegeneration, optical coherent tomography, optic neuritis, review, visual evoked potential, visual pathway

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The visual pathway is made up of 4-neuron chains that convey visual information from the retina to the primary visual cortex (Figure 1). Photoreceptor cells, bipolar cells and retinal ganglion cells (RGCs) represent the first, second and third order neurons of the anterior visual pathway; however, the retina includes two additional neural types that modulate the activity of the other retinal neurons, namely amacrine and horizontal cells. This network is important to modulate the signals to the cortex and some authors suggest that it provides some degree of neuroplasticity to counteract retrograde neurodegeneration in multiple sclerosis (MS).

The axons from RGCs constitute the retinal nerve fiber layer (RNFL), the optic nerve, the chiasm and the optic tracts; and ultimately, they form synapses with neurons of the lateral geniculate nucleus (LGN). Axons from the RGCs of the macula are small (parvoganglion cells) and they travel along the papillomacular bundle of the RNFL to the temporal quadrant of the optic disk.

In addition to the LGN, the posterior visual pathway contains the optic radiations (ORs) and the primary visual cortex (V1). The LGN is part of the thalamus and it modulates visual information before projecting it to the visual cortex. Compared to the retina, there are 300 to 400-fold more neurons in the visual cortex and LGN, which may explain the neuroplasticity that provides certain tolerance to axonal damage after optic neuritis (ON).

Tests of visual function

The integrity of the visual pathway can be assessed by tests of visual function that assess high-contrast visual acuity (HCVA), also known as best corrected visual acuity (BCVA), low contrast visual acuity (LCVA), contrast sensitivity, color vision acuity (CVA) and visual fields (VFs). Snellen and retro-illuminated Early Treatment for Diabetic Retinopathy Study (ETDRS) charts are sufficiently sensitive to identify HCVA deficits after ON, but not to capture visual impairment in MS.

The LCVA measured with Sloan charts (2.5% or 1.25% contrast) and contrast sensitivity measured with Pelli-Robson charts correlates well with the...
Figure 1. The visual pathway as a model for MS.
(a) The acute phase of optic neuritis: Gadolinium enhancement in optic nerve, thickening in the RNFL and reduction in the amplitude of the afferent visual pathway conduction can be used to monitor acute inflammation along optic nerve. Delay in latency, MTR reduction and RD increase represents demyelination. Axonal damage can be tracked with mfVEP, because decrease in amplitude indicates not only conduction block due to inflammation, but also axonal damage; and with DWI MRI modalities, because a decrease in AD represents axonal damage. Finally, inflammation, demyelination and axonal damage are responsible for visual function impairment that can be quantified by high and low contrast visual acuity, color vision test and visual field. (b) The subacute and chronic phase of optic neuritis: Shortening of latency in mfVEP during the follow-up, MTR increase and RD decrease represent remyelination after an acute optic neuritis. Retrograde axonal degeneration can be monitored by RNFL and especially by GCL thinning in the retina, using OCT. Decreased mfVEP amplitude represents axonal damage along the entire afferent visual pathway. OCT is also useful to track transynaptic retrograde degeneration that is represented by thickening and MME in INL. MRI quantifies transynaptic anterograde degeneration due to ON. Thalamic and visual cortex atrophy and NAA decay represents transynaptic grey matter damage and decreased AD; and increased RD in OR represents transynaptic white matter damage.

AD: axial diffusivity; DWI: diffusion weighted imaging; GCL: ganglion cell layer; Gd (+): gadolinium enhancement; INL: inner nuclear layer; LGN: lateral geniculate nucleus; mfVEP (a): multifocal visual evoked potentials amplitude; MME: microcystic macular edema; MRI: magnetic resonance imaging; MTR: magnetization transfer ratio; NAA: N-acetyl aspartate; OCT: optical coherence tomography; ON: optic neuropathy; OR: optic radiations; PVC: pericalcarine visual cortex; RD: radial diffusivity; RNFL: retinal nerve fiber layer.
health-related quality of life of MS patients. These LCVA measures are sensitive methods to evaluate visual disability in ON and especially, in MS. There is evidence for a good structure-function correlation between LCVA and RNFL thickness, ganglion cell complex (GCC) thickness and brain magnetic resonance imaging (MRI) lesion load in visual areas. Finally, LCVA measures seem to be sensitive to the effects of treatments. Abnormal color perception is common in ON and MS, regardless of any prior history of ON. Moreover, regardless of any history of ON, dyschromatopsia is associated with a deterioration in disability and grey matter atrophy during the follow-up period.

VF defects are frequent in ON and MS, even in the absence of previous ON and visual symptoms. Scotomas may correspond to focal MS lesions in the visual pathway; and general VF depression, which is the most common finding in MS, might indicate more diffuse damage. Nevertheless, very little is known about the utility of VFs in monitoring the course of MS and the response to therapy. Indeed, multifocal visual evoked potentials (mfVEPs) seem to be more sensitive to capture subclinical damage and as they are not prone to subjective errors, they may supersede the use of VFs in the future.

Electrophysiological assessment

Full-field (pattern) VEPs measure the cortical response to monocular stimulation in 3–8 central degrees of the visual field; and the potential evoked is a single global response that is heavily biased towards the macular region, due to its cortical overrepresentation. The mfVEP provides information about local responses of small areas occupying up to 24 central degrees of the visual field. Mild abnormal local responses and scotomas are detectable through mfVEPs, with a strong agreement with perimetry. A delayed latency of the VEP indicates demyelination, while a shortening of the latency during the follow-up implies remyelination. Similarly, decreased amplitudes are indicative of impaired conduction and/or axon damage. The mfVEPs also reveal diffuse chronic damage of the visual pathway in the patient’s fellow eyes.

The full-field electroretinogram (ERG) provides information about the general function of the outer retinal layers (photoreceptors); but in contrast to full-field VEP, it is biased towards the peripheral retina, because the macula represents only 3 mm² of the retina. Full-field ERG reveals outer retinal layer dysfunction in MS, while pattern ERG reflects the function of RGCs and can indicate RGC dysfunction in MS, when correlated with structural damage. By contrast, multifocal ERG provides information about focal dysfunction of the retina, including the optic nerve head.

Optical coherence tomography

Optical coherence tomography (OCT) can demonstrate that ON eyes have a thinner RNFL and smaller macular volume (MV) than non-ON eyes, in patients with MS. Regarding changes in the retinal layers, the GCC has emerged as the most interesting layer to monitor MS. Both RNFL thickness and GCC thickness have been used to monitor damage after acute ON; however, measuring GCC thickness has some advantages, because the GCC is not affected by edema, so that its thinning is not masked by early inflammation, and because it is less severely affected by astrogliosis, which frequently appears in the inner retinal vessels. Finally, the GCC is better correlated with visual function tests than RNFL thickness, making it easier to assess meaningful changes in terms of clinical relevance.

Through trans-synaptic effects due to chronic diffuse damage, retinal neurodegeneration may also occur as a consequence of chronic grey matter damage in MS. Because the effects of both mechanisms are captured by OCT, it can be used to monitor diffuse neurodegeneration in MS. Such processes may be responsible for the moderate associations between RNFL and MV measures and neurological disability, brain atrophy and lesion load.

Regarding the changes in other layers, increased baseline inner nuclear layer (INL) is associated with a greater risk of developing new T2- and gadolinium-enhancing lesions, and of disease progression and new relapses, independent of any history of ON. Pathological studies have found that inflammation and microglial activation are present within the parenchyma of the inner retina, in MS patients. Hence, increased INL thickness may reflect the retinal inflammation that parallels brain inflammatory activity in MS. Microcystic macular edema (MME) of the INL is found in some MS patients, especially those with prior ON; as well as in other inflammatory, degenerative and compressive optic neuropathies. Although MME was thought to represent the breakdown of the blood-retinal barrier due to retinal inflammation, this wide clinical spectrum suggests that MME is a sign of optic neuropathy, irrespective of its etiology. Müller cell nuclei are found within the INL and play a critical role in regulating ion and water.
homeostasis. A thickening of the INL may reflect edema, either due to retinal inflammation or as a consequence of impaired fluid clearance in the INL, due to Müller cell dysfunction.

In conclusion, OCT offers the opportunity to track axonal and neuronal degeneration in the retina of MS patients as a consequence of both acute and chronic diffuse damage. The retina is less affected by astrogliosis and the pseudoatrophic effects of central nervous system (CNS) inflammation; and OCT is a less expensive, non-invasive technique that produces data that can be processed more rapidly than that obtained by MRI.

**Magnetic resonance imaging**

MRI can be used to quantify regional atrophy in the visual pathway, such as optic nerve atrophy after ON, atrophy of the thalamus (which includes the LGN) or atrophy of the visual cortex. The magnetization transfer ratio (MTR) and diffusion weighted imaging (DWI) are parameters that are sensitive to microstructural damage. Accordingly, eyes with prior ON have a reduced MTR in their optic nerves, which is associated with visual function. Regarding DWI analysis, increased radial diffusivity and decreased axial diffusivity in an early phase of ON is correlated with visual recovery and surrogate markers of axonal damage, such as RNFL thickness and VEP amplitude, 6–12 months later. Finally, DWI parameters in the non-ON eyes of MS patients are well correlated with visual function tests and retinal atrophy, suggesting the presence of chronic, diffuse damage.

N-acetyl aspartate (NAA) levels within the visual pathway can be measured by magnetic resonance spectroscopy and are lower in MS patients whom have abnormal VEPs. Decreased NAA levels in the visual cortex have been related to retinal injury, suggesting there is trans-synaptic degeneration in MS. Moreover, functional MRI has been used to evaluate neuroplasticity in the visual cortex after ON, revealing increased activation and delayed activity, in order to compensate for visual pathway dysfunction.

**Patterns of damage in the visual pathway in MS**

The visual pathway is very susceptible to damage in MS; and retinal inflammation and atrophy have been found in the eyes of most MS patients. ON is the onset syndrome in 20–30% of cases and it occurs in nearly 70% of MS patients, during the course of the disease. In addition, the ORs and primary visual cortex are a common site of damage in MS. Moreover, visual pathway damage is frequent during the development of MS from early to progressive MS. Visual dysfunction has a major impact on a patient’s quality of life, with visual function weighted as the second priority after lower limb function, for MS patients.

There are some biological issues that favor the visual pathway as a suitable model for MS. First, it is a well-defined neural pathway with few neurons and long tracts, which facilitates the quantification of myelin, as well as axon and neuron damage. The second advantage is its retinotopic organization throughout all tracts that permit any damage to be fine mapped, from the retina to the occipital cortex. Third, visual information goes from the retina to the primary visual cortex through only one synapse in the LGN, differing from pathways like the primary motor pathway, where information is not only modulated by the thalamus, but also by the basal ganglia, cerebellum and spinal cord. These last two factors help to assess the structure-function correlations that are important to evaluate the clinical relevance of any damage. Fourth, there are two structures, namely the INL and the primary visual cortex, which offer some degree of neuroplasticity to the visual system.

Neuroplasticity may counteract damage, thereby influencing the functional prognosis of the disease. One of the most important biological advantages is that the retina lacks myelin. This is a key issue, because it provides an ideal model to evaluate the interplay between inflammation and axonal damage, without the interference of myelin. Finally, inflammation is also present in the retina as retinal periphlebitis, a perivascular inflammatory infiltrate around the retinal vessels that is similar to that we found in the brains of MS patients.

**Acute CNS focal damage**

ON represents acute focal damage of the anterior visual pathway. Demyelination in ON can be assessed by the delayed latencies of the mfVEPs, and by MTR and DWI. Axonal damage can be evaluated by assessing RNFL thinning by OCT, given that these parameters represent retrograde neurodegeneration due to ON, or by studying the changes in the mfVEP amplitude. The close correlation between delayed latency and RNFL reduction in ON suggests that demyelination and neurodegeneration are directly related. A threshold of 75 µm of the RNFL is reported to predict permanent visual dysfunction after ON.
Retinal periphlebitis represents another opportunity to study focal damage in the retina of patients with MS. Its presence is directly associated with the existence of gadolinium-enhancing lesions. When combined with pathological studies, imaging findings suggest that retinal periphlebitis and acute MS plaques have a similar pathological substrate. We previously analyzed the association between the existence of retinal periphlebitis in a given quadrant and thinning of the RNFL in the same quadrant over the following 6 months; however, because retinal periphlebitis may induce mild axonal damage and OCT cannot analyse the peripheral retina where such lesions occur, this may have impeded identifying the damage to the retina produced by retinal periphlebitis.

Chronic diffuse CNS damage in MS
There is some evidence suggesting that diffuse and chronic degenerative processes affect neural components of the retina in a similar manner as normal-appearing grey matter in MS. First, full-field ERG has demonstrated outer retinal layer dysfunction in MS. Second, in a group of primary progressive MS (PPMS) patients, OCT has shown that INL and ONL thinning is predominant, with relative sparing of RGCs and abnormalities in the mfERGs, although these observations still require validation. Third, patients with PPMS appear to have greater INL thinning, compared to relapsing patients, consistent with pathological studies describing INL atrophy as being predominantly present in progressive MS. The bulk of the available data also suggests that primary chronic inflammatory retinopathy may exist in MS. Finally, the visual pathway of non-ON eyes represents a model to evaluate the dynamics between inflammation, chronic demyelination and neurodegeneration, because it reproduces findings similar to those in apparently normal grey and white matter. Moreover, OCT and mfVEPs reveal the impact of sub-clinical inflammatory demyelination in promoting diffuse axonal loss in the visual pathway.

Trans-synaptic degeneration: Spreading neurodegeneration through the CNS
Trans-synaptic degeneration may propagate neurodegeneration after acute lesions, such as ON. Damage in the ORs was seen to be greater in patients with a prior history of ON than for non-ON patients, suggesting the existence of anterograde trans-synaptic damage. We found an association between axonal damage in the posterior visual pathway (e.g. visual cortex volume or levels of NAA) and the RNFL thickness, supporting the occurrence of retrograde trans-synaptic degeneration. We also witnessed how an acute lesion in the anterior visual pathway due to ON affects the volume of the primary visual cortex. More recently, this association between atrophy of the visual cortex in patients with prior history of ON was corroborated. Overall, the data suggest that trans-synaptic degeneration contributes to neurodegeneration in MS.

Neuroplasticity: Counteracting optic pathway damage in MS
The functional prognosis of a patient after a neurological insult is defined by the equilibrium between the damage caused and the neuroplasticity that minimizes its impact. Recovery of VEP amplitudes in ON eyes in the post-acute phase, even in the presence of RNFL thinning, provides evidence that neuroplasticity in the primary visual cortex probably contributes to improved functional prognosis after ON. Evaluation of neuroplasticity in the visual pathway in MS using fMRI highlights reduced activity in visual areas during the acute phase, coupled with compensatory extrastriatal and LGN activity. The resting-state activity of the visual network in MS patients also indicates there is compensatory activity after damage to the visual pathway; thus, the visual pathway does appear to be suitable to evaluate neuroplasticity in MS.

The visual pathway for the development of neuroprotective and regenerative therapies for MS
Even though the retina does not contain myelin, it is possible to assess the effects of myelin loss and recovery on the visual pathway with reasonable sensitivity. Indeed, mfVEPs have been successfully used to track improvement in velocity conduction after ON, and MTR has been used to monitor demyelinating and remyelinating processes, following acute ON. Accordingly, both these measurements can be used to evaluate the efficacy of myelin repair therapies in ON (Table 1). OCT can accurately and reproducibly monitor neuronal and axonal degeneration after ON; therefore, ON seems to be an appropriate model to evaluate neuroprotection due to acute lesions, even with relatively few samples. OCT can also be used to monitor related neurodegeneration in chronic MS; however, the average yearly thinning of RNFL is around 2 microns/year and thus, longer follow-up periods (more than 2–3 years) and larger sample sizes...
may be needed than previously expected, to witness any effects.41

Future directions
The visual pathway is suitable to evaluate both focal acute and diffuse chronic damage in MS. Moreover, this pathway can be studied with different electrophysiological and imaging techniques that allow evaluation with high sensitivity of the interplay of different mechanisms in MS: inflammation, demyelination and neurodegeneration. Moreover, it offers a model that can be used to evaluate inflammatory neurodegeneration, without the confounding factor of demyelination; however, the visual pathway has some shortcomings. First, it is necessary to extend the use of LCVA and color vision, and to better clarify its translation into clinically relevant changes. Second, OCT provides structural information about the retina, but it cannot distinguish between different pathogenic processes. Developing other molecular imaging techniques to study the retina, such as Raman spectroscopy,42 will help analyze the biological processes specific to the anatomical and functional changes observed in these pathological situations.

Conflict of interest
None declared.

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Table 1. Sample sizes in randomized clinical trials of the afferent visual pathway, treated with myelin regeneration and neuroprotective therapies.

<table>
<thead>
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<th>Trial aim</th>
<th>Drug</th>
<th>Indication</th>
<th>Primary outcome</th>
<th>Alpha error</th>
<th>Power</th>
<th>Effect size</th>
<th>Samples/trial</th>
<th>Drop-out rate</th>
<th>Ref/NCT</th>
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<td>Myelin repair</td>
<td>Anti-LINGO mAb (BIIB033)</td>
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<td>80%</td>
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<td>40</td>
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<tr>
<td>Myelin repair</td>
<td>MS</td>
<td>MTR</td>
<td>80%</td>
<td>50%</td>
<td>48 (20–96)</td>
<td>0%</td>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuro-protectn.</td>
<td>MS</td>
<td>MTR</td>
<td>80%</td>
<td>50%</td>
<td>13–17</td>
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<tr>
<td>Neuro-protectn.</td>
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| GCC: ganglion cell complex; ON: Optic Neuritis; LINGO: Leucine rich repeat and Ig domain containing 1; mAb: monoclonal antibody; MS: multiple sclerosis; MTR: magnetic transfer ratio; NCV: nerve conduction velocities from visual evoked potentials; NCT: national US clinical trials, ClinicalTrials.gov; neuro-protectn.: neuroprotection; Ref: reference; RNFL: retinal nerve fiber layer.

References
8. Martinez-Lapiscina EH, Ortiz-Perez S, Fraga-Pumar E, et al. Colour vision impairment is associated with...


