A major objective in multiple sclerosis therapeutics is to develop strategic targeting of specific injury pathways to provide neuroprotection and potentially even restoration. Here we underscore the potential utility of the anterior visual system for the purpose of modeling neuroprotection in response to novel therapies.

One of the formidable challenges for modern neurobiology with respect to neurodegenerative disorders such as multiple sclerosis (MS) has been to better understand those processes that contribute to the varied targets of the disease process (e.g., myelin, oligodendrocytes, axonal integrity, neuronal cell bodies, and the promotion of astrocytic proliferation). Although monumental progress has been made in understanding cellular trafficking mechanisms and their therapeutic manipulation in MS, we have not generated a commensurate level of knowledge concerning the underpinnings of neurodegeneration and how to manipulate such processes for the purpose of neuroprotection or restoration.

The retinal nerve fiber layer (RNFL), the innermost layer of the retina containing ganglion cell axons that compose the optic nerve, provides a unique opportunity to measure (using ocular imaging techniques such as optical coherence tomography [OCT] as described later) a central nervous system (CNS) structure that consists of isolated axons. This is true because axons within the RNFL are not myelinated until they pass through the lamina cribrosa to compose the retrobulbar portion of the optic nerve. This structural specialization of the RNFL makes it an ideal tissue to examine for the purpose of understanding neurodegenerative processes within the CNS because quantitation of RNFL thickness reflects the burden of axons without potential structural effects of myelin degeneration. Here we describe the application of novel techniques to model and quantify changes in tissue architecture within the neuroretina that are directly related to the pathophysiology of MS and its related clinical consequences.

### MECHANISMS OF INJURY AND MODELING NEUROPROTECTION IN MS

Neuronal cell death and axonal degeneration are now well-recognized characteristics of MS pathology and are likely the substrate of permanent disability. Neuroprotection refers to any therapeutic intervention that results in the protection of...
neurons and/or axons. In discussing neuroprotective treatment strategies, it is useful to consider inflammatory and noninflammatory mechanisms of neuronal and axonal injury, although in reality both are likely occurring at the same time to some degree in all patients. Strategies to broadly or even selectively target cells or activation markers on cells of the peripheral immune system may offer some benefit.

To effectively model a pathological process in MS, it is important to identify specific syndromes that are the result of a discrete anatomical localization with corresponding and measurable physiological changes. Optic neuritis is one such model and typically results in clinically stereotypic abnormalities, such as pain, reduction in high- and low-contrast letter acuity and sensitivity, color desaturation, visual field loss, prolongation in visual evoked potential (VEP) latencies, occasional decrements in VEP amplitudes, pupillary defects, and evidence of inflammation (gadolinium enhancement) in the optic nerve on magnetic resonance imaging (MRI), when assessed at the time of the acute syndrome.

AFFERENT VISUAL PATHWAY IN OPTIC NEURITIS

The optic nerve obtains its myelin behind the eye at the lamina cribrosa and comprises approximately 1.2 million ganglion cell axons that are positioned within the eye at the RNFL. The 3 principal tract systems that are transmitted to the CNS from the retina include the retinogeniculocalcarine (for visual processing) (Figure 1), the retinomesencephalic (for the pupillary light reflex) (Figure 2), and the retinohypothalamic (for the regulation of the circadian clock contained within the suprachiasmatic nucleus) (Figure 3).

The RNFL represents the most anterior part of the afferent visual pathway; because it lacks myelin, this structure provides a unique opportunity to observe the neurodegenerative effects within the retina as a consequence of retrobulbar damage within the optic nerve. Lesions affecting the optic nerve along its retrobulbar course can cause retrograde axonal damage, which manifests as diffuse or localized atrophy within the RNFL.

The ability to detect RNFL atrophy is dependent on both the pattern of RNFL loss and the zone in which the loss has occurred. Experimental models have shown that in eyes with total optic nerve transection, the disappearance of normal RNFL striations begins at 1 month and is essentially complete by 2 months. Furthermore, the visible detection of RNFL atrophy with traditional ophthalmic techniques requires a loss of about 50% of neural tissue in an affected retinal area. Defects of the RNFL have been well described in the context of MS.

ANTEOR VISUAL PATHWAY AS A MODEL FOR INVESTIGATION OF NEUROPROTECTION IN MS

Visual impairment occurs in 80% of patients with MS, and up to 50% of these patients experience visual loss as an initial presenting symptom. The most commonly reported symptoms of visual dysfunction in MS include blurring and distortion. Even patients with visual acuities of 20/20 OU or better may note these symptoms. Such patients frequently demonstrate subtle disturbances of afferent visual function by clinical or psychophysical testing, including low-contrast letter acuity, contrast sensitivity, color vision, visual fields, and VEPs. Although visual symptoms in MS may precede, occur coincidentally with, or follow the development of other neurologic manifestations, they often represent the most significant symptoms of MS from the patient’s point of view.

The most common and extensively studied visual manifestation of MS is demyelinating acute optic neuritis (AON). It is a common cause of visual loss among young and middle-aged adults. Occurring at some time during the course of MS in 50% of patients, AON is a common cause of MS-related visual loss and is a first clinical sign of MS in 15% to 20% of patients. While recovery is generally regarded as favorable, visual deficits persist over the long term in approximately 60% of patients and are associated with significant reductions in quality of life. Incomplete recovery is also common among patients whose visual acuities improve to 20/20 or better with residual symptoms and abnormalities that are detectable by testing contrast sensitivity and low-contrast letter acuity. These observations, based primarily on data from the Optic Neuritis Treatment Trial, indicate that tests of contrast sensitivity (Pelli-Robson chart) are the most sensitive clinical measures for demonstrating persistent visual abnormalities following AON. Further clinical trials in AON represent an opportunity to improve visual outcomes, particularly among patients at risk for incomplete recovery, and will provide a disease model that is well suited to the evaluation of the next generation of therapies, neuroprotective agents.

While acute demyelination is an important contributor to visual dysfunction in MS and AON, the pathophysiology of permanent visual deficits is now recognized to be due to irreversible losses of myelin and axons with secondary neuronal degeneration. Axonal loss in the optic nerves has been confirmed in patients with MS and AON by pathologic studies, MRI techniques, and, most recently, imaging of the peripapillary RNFL with ocular imaging techniques such as OCT (as described later). Axonal densities are reduced in the optic nerves and optic tracts of patients with MS compared with age-matched control subjects at autopsy (30%-45% fewer axons), particularly in eyes with history of AON. To the extent that axonal and neuronal loss underlies irreversible visual loss in all forms of optic neuropathy, the identification and testing of agents that protect axons and myelin and preserve neuronal integrity (neuroprotective agents) are essential next steps in improving visual outcomes.

Acute optic neuritis represents an ideal in vivo model for investigation of neuroprotective agents in MS for the following reasons: (1) AON is a common demyelinating event, is easily identifiable by clinical criteria, and produces symptoms of visual dysfunction that prompt patients to seek evaluation early; (2) sensitive and reliable visual function tests with standardized protocols for administration and quantitative scoring have been validated for assessment of clinical outcomes in optic neuritis and MS; (3) duration to outcome is relatively short

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(6 months) and natural history of recovery is well defined; (4) with current treatments, a high proportion of patients (60%) have persistent deficits at 6 months using standard outcome measures; (5) OCT imaging, a non-invasive biomarker and a potential surrogate end point for MS trials, provides a unique opportunity to measure CNS axons in the absence of myelin (RNFL thickness, 70% abnormal at 6 months) and to quantify degeneration of retinal ganglion cells (macular volume); and (6) the anterior visual pathways represent a unique CNS site in MS for which involvement of specific structures (optic nerves, retina) is correlated directly with a specific function (vision).

**OPTIC NERVE MRI IN MS**

**Conventional MRI**

Conventional optic nerve MRI can help in diagnosing AON by detecting the acute inflammatory lesion but also may detect other diseases that can mimic AON, such as compressive lesions.17 Gadolinium enhancement, evaluated on
fat-saturated T1-weighted images, is a consistent feature in AON and should be considered for the evaluation of patients with suspected AON (Figure 4), although dedicated orbital imaging is usually not required for diagnostic confirmation in uncomplicated cases of AON.

**Unconventional MRI Measures**

In AON, there is usually optic nerve swelling that later evolves into atrophy due to the development of Wallerian degeneration of axons transected in the acute inflammatory lesion. Atrophic changes within the optic nerve following AON can be quantified (Figure 5). Further, during the acute phase of inflammatory demyelination, magnetization transfer ratios in affected optic nerves have been observed in one study to decline and stabilize, with a possible late rise in these ratios up to 1 year after the acute episode, which may represent an imaging correlate of remyelination. Diffusion-weighted and diffusion tensor imaging techniques may provide more spe-
cific measures of optic nerve axonal integrity, although these methods are time-consuming and technically demanding.20,21

OPTIC DISC AND RNFL IMAGING TECHNOLOGIES

For centuries, physicians relied solely on the direct ophthalmoscope to visualize the optic disc and RNFL. During the last decade, several new technologies have emerged that can be considered quantitative ophthalmoscopes. Although all of these competing devices measure the same structures, the optic disc topography and RNFL thickness, they all depend on different bioengineering technologies.

Heidelberg Retinal Tomography

Using confocal laser scanning ophthalmoscopy, the Heidelberg Retinal Tomograph II (Heidelberg Engineering, Vista, California) scans the retinal surface to calculate reflectance from the vitreoretinal interface. Serial optical slices are then assembled to produce a height map of the targeted anatomical region. The Heidelberg Retinal Tomograph II constructs a reference plane beneath the papillomacular bundle height. The region below the reference plane is regarded as the optic cup and the region above the reference plane represents the neuroretinal rim. The RNFL thickness is calculated by determining the distances between the reference planes. The technique has the advantage of being a rapid assessment...
strategy. However, the RNFL thickness and macular volume measures are not as accurate as those achieved with other technologies.

Scanning Laser Polarimetry

In scanning laser polarimetry, light reflects from a birefringent medium (the retinal pigment epithelium) back to the device. This technology quantifies the shift in polarization of the light projected through the RNFL, with the RNFL acting as a polarizing filter. The amount of shift of the polarized light is measured, and this shift (or retardation) is directly proportional to RNFL thickness. The newer version of the device (GDx-VCC; Carl Zeiss Meditec, Dublin, California) estimates the birefringence of the cornea and then subtracts the corneal birefringence from the peripapillary birefringence to produce a measurement of the shift produced by the peripapillary RNFL. The device yields a measure of the RNFL thickness, designated the nerve fiber index, and can also produce deviation plots to identify the regional distribution of retinal thinning. One advantage of this technology is that a confounding effect of edema on the measure of RNFL is avoided. A disadvantage is that studied patients must be able to adequately fixate on a target within the device without confirmation by the examining technician. As such, patients with severe visual loss cannot be evaluated with this technique.

Optical Coherence Tomography

Optical coherence tomography is comparable to B-scan ultrasonography but uses light instead of sound to create images based on the different optical reflectivities of the ocular tissues. It uses a Michelson interferometer to obtain images with a resolution of 10 µm (advanced devices with a resolution of about 2.5 µm are in development).

During OCT, a light beam is split into 2 components. One beam travels to a reference mirror and is reflected back. The other beam enters the eye and is then reflected back (backscatter). The interference pattern of the recombined light beams produces information about the distance and thickness of the optic disc and retina.
The currently available OCT systems depend on time-domain technology. This technique provides data not only about the peripapillary RNFL thickness but also concerning the thickness of the fovea and macula. Ultimately, OCT provides histologically accurate images of the fovea, macula, RNFL, retinal cellular layers, retinal pigment epithelium, and choroid (Figure 6). It has the advantage that the technician can assure adequate fixation to achieve a high-quality image. However, a potential confounder with this technology is that edema can on occasion lead to an overestimation of the RNFL thickness.

STUDIES OF OCT IN MS AND OPTIC NEURITIS

A noninvasive biomarker and potential end point for MS trials, OCT imaging provides a unique opportunity to measure central nervous axons in the absence of myelin (RNFL thickness) and to quantify degeneration of retinal ganglion cells (macular volume). While some of the earliest investigations of OCT in MS were performed nearly 10 years ago,22 data concerning the impact of AON on RNFL thickness, macular thickness, and macular volume have now begun to emerge on a larger scale.23

In series of patients with a history of AON, decrements in RNFL thickness are correlated with high-contrast visual acuity, visual field mean deviation, and color vision.21-24 Trip et al5 showed that macular volumes were decreased in affected eyes of patients with AON when compared with fellow and disease-free control eyes, suggesting the occurrence of retinal ganglion cell degeneration in addition to axonal loss.

To determine how changes in RNFL thickness following AON may predict visual recovery, Costello et al24 studied 54 patients using OCT and computerized static perimetry. In this cohort, 40 of 54 patients (74%) demonstrated thinning of the RNFL within 3 to 6 months following the acute episode. The mean (SD) RNFL thickness for affected eyes examined at 3 or more months was 78 (30) µm, whereas unaffected eyes were not as significantly reduced in terms of degrees of RNFL thinning (mean [SD] RNFL thickness, 100 [33] µm) compared with published values from disease-free control subjects (mean [SD] RNFL thickness, 105 [12] µm).25-26 Patients with incomplete recovery of visual fields demonstrated greater RNFL loss after AON; analyses demonstrated a threshold of RNFL thickness (75 µm), below which RNFL measurements at 3 or more months predicted persistent visual dysfunction. Serial analyses of eyes with AON (n=27 patients) performed by Sergott25 showed that reductions in RNFL thickness can occur despite recovery of visual acuity.

Optical coherence tomography techniques may also be useful in clinical trials of more heterogeneous MS cohorts. In an investigation designed to examine the validity of low-contrast letter acuity, a new MS clinical trial visual outcome measure, Fisher et al27 demonstrated that while RNFL thickness changes are greatest among eyes in patients with MS and a history of AON (n=63 eyes; mean [SD] RNFL thickness, 85 [17] µm), eyes in patients with MS without similar history also had significant reductions in RNFL thickness (n=108 eyes; mean [SD] RNFL thickness, 96 [14] µm) compared with eyes of disease-free control subjects (n=72; mean [SD] RNFL thickness, 105 [12] µm) (P=.03 for comparison with eyes of patients with MS without AON).25,26 Similar findings were noted when macular volume, an OCT parameter that captures mainly retinal ganglion cells, was examined in eyes of patients with MS with vs without AON.3

Using normative data included in the OCT-3 with OCT 4.0 software (Carl Zeiss Meditec), only 40 of 180 eyes (22%) in the patients with MS in the cohort studied by Fisher et al27 had abnormal values of RNFL thickness. However, because the OCT 4.0 normative database considers the fifth percentile for age to be the cutoff for abnormal values, RNFL thickness abnormalities are likely to be of substantially greater prevalence in eyes of patients with MS and AON. In this study, the average RNFL thickness in fellow eyes of patients with MS with a history of AON (n=28; mean [SD] RNFL thickness, 99 [13] µm) was similar to that in eyes of patients with MS without AON (n=108 eyes; mean RNFL thickness, 95 µm) (P=.31, generalized estimating equation models accounting for age and adjusting for within-patient, inter-eye correlations). Generalized estimating equation models are regression models that allow the investigator to account for potential intercorrelations with groups or, in this case, between eyes of the same patient. This study also demonstrated that RNFL thickness correlates significantly with low-contrast letter acuity scores (P<.001) and reflects overall MS disability (P=.02 for linear trend across Expanded Disability Status Scale tertiles and disease duration; P=.02 accounting for age). These results not only support a role for OCT in studies of AON but also indicate that OCT may be useful in MS trials as a noninvasive structural biomarker of axonal loss.

PUPILLARY LIGHT REFLEXES

The damage caused by optic neuritis is sustained at the afferent part of the anterior visual system and interrupts the signaling pathway of the pupillary light reflex. Relative afferent pupillary defects, differences in direct and consensual reaction to light in the same eye, manifest frequently in those with optic nerve lesions. Demyelination and potentially loss of axons of the optic nerve represent the pathological substrate responsible for the relative afferent pupillary defects (although abnormalities of the efferent limb, mediated by cranial nerve III, must also be considered).28,29 The short- and long-term retinal axonal damage occurring after optic neuritis can be quantified by measures of pupillary function and monitored during disease progression. We are currently investigating the relationship between visual loss, thinning of the RNFL, and pupillary dynamics (eg, latency, constriction velocity, and percentage of change in pupillary diameter) as measured by quantitative infrared pupillometry. Objective measures of pupillary function may serve as potential biomarkers for both neurodegeneration and neuroprotection.

MULTIFOCAL VEPs

Conventional VEPs provide information on whole optic nerve conduction properties. Multifocal VEPs provide
Figure 6. The capability of optical coherence tomography to delineate the various layers of the retina. A, The retinal structures including the ganglion cell bodies and their corresponding axons within the retinal nerve fiber layer (RNFL). IPL indicates inner plexiform layer; OPL, outer plexiform layer; PRL, photoreceptor layer; and RPE, retinal pigment epithelium. B, A typical optical coherence tomographic image shows the retinal structures, including the RNFL, with the uppermost layer in red. The red box shows the transverse section of the retinal layers at the fovea (depicted on the left in A), and the yellow box shows the transverse section of the retinal layers between the fovea and optic disc (depicted on the right in A). C, A cutout of the retina showing the demarcation of the myelin-free axonal zone, anterior to the lamina cribrosa.
similar domains of information but on a broader array of divisible test zones (more than 50). Recent data suggest that identification of latency abnormalities at the time of AON as a clinically isolated demyelinating syndrome is a strong predictor for conversion to clinically definite MS. Alternately, normal latencies argue against this diagnosis, at least in the short-term horizon. A powerful hypothesis is that multifocal VEPs may be capable of identifying zones of tissue damage that correspond to specifically identified sectors of axonal (RNFL) and cellular (macular volume) degeneration, thereby providing yet another piece of evidence to corroborate the dynamic process of tissue damage in MS that can be modeled within the retina.

FUTURE DIRECTIONS

The third projection pathway of the retina is the retinohypothalamic system. Retinal ganglion cells destined to project to the hypothalamus contain melanopsin and innervate the suprachiasmatic nucleus. This nucleus serves as the circadian clock, mediates processes that are important for sleep-wake cycle transitions, and likely has influences over neuroendocrine relays and the regulation of mood. Disruption within this system may be related to central causes of chronic fatigue, the singular most common symptom in patients with MS and a frequent cause of disability retirement. Although little is currently known about the impact of MS on this circuitry, there is some evidence to suggest impairment of the hypothalamic-pituitary-adrenal axis as a result of tissue pathology in these patients.

CONCLUSIONS

A relationship between visual dysfunction and changes within the retina has now been established and sets the context for landmark studies focused on the use of this system to model the process of neurodegeneration, neuroprotection, and potentially even restoration. A remaining and central objective of current research initiatives in this area will be to demonstrate that specific changes in the retina constitute a biomarker for predicting similar changes within the CNS in general.

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