Serial magnetization transfer imaging in acute optic neuritis

S. J. Hickman,1,2 A. T. Toosy,1,2 S. J. Jones,3 D. R. Altmann,1,4 K. A. Miszkiel,5 D. G. MacManus,1 G. J. Barker,1,6 G. T. Plant,2 A. J. Thompson1 and D. H. Miller1

1NMR Research Unit, Department of Neuroinflammation, Institute of Neurology, University College London, 2Department of Neuro-Ophthalmology, Moorfields Eye Hospital, 3Department of Clinical Neurophysiology, National Hospital for Neurology and Neurosurgery, 4Medical Statistics Unit, London School of Hygiene and Tropical Medicine, 5Lysholm Radiological Department, National Hospital for Neurology and Neurosurgery, and 6Institute of Psychiatry, Kings College London, London, UK

Summary
In serial studies of multiple sclerosis lesions, reductions in magnetization transfer ratio (MTR) are thought to be due to demyelination and axonal loss, with later rises due to remyelination. This study followed serial changes in MTR in acute optic neuritis in combination with clinical and electrophysiological measurements to determine if the MTR changes over time mirror the picture in multiple sclerosis lesions, further validating MTR as a marker of tissue integrity. Twenty-nine patients were recruited who had acute optic neuritis for a median of 13 days (range 7–24 days) since the onset of visual symptoms. A clinical examination and measurement of visual evoked potentials (VEP) was performed on each patient. Their optic nerves were imaged with a fat-saturated fast spin echo (FSE) sequence and a magnetization transfer sequence. Twenty-one had multiple subsequent examinations over the course of 1 year. In addition, 27 control subjects had their optic nerves imaged up to three times over 1 year. A blinded observer segmented the optic nerves from the MTR maps. Lesions were defined on the acute FSE images and, from the coordinates, the ratio of mean lesion MTR : healthy nerve MTR (lesion ratio) was calculated for each dataset. The time-averaged mean MTR in control optic nerves was 47.7 per cent units (pu). In diseased optic nerves, baseline mean MTR was 47.3 pu, with a mean lesion ratio of 0.98. The diseased optic nerve MTR and lesion ratio declined over time with a nadir at about 240 days at a mean MTR value of 44.2 pu and mean lesion ratio of 0.91. Subsequently, diseased optic nerve MTR appeared to rise; after 1 year the diseased optic nerve mean MTR was 45.1 pu (mean lesion ratio 0.93), although the difference was not significant compared with the nadir value. For each 0.01 increase in time-averaged lesion ratio logMAR visual acuity recovery improved by 0.03 (95% CI, 0.002, 0.08, P = 0.02). Time-averaged VEP central field latency was shorter by 6.1 ms (95% CI 1.5, 10.7, P = 0.012) per 1 pu rise in time-averaged diseased optic nerve MTR. The early fall in diseased optic nerve MTR is consistent with demyelination and Wallerian degeneration of transected axons. The late nadir compared with studies of multiple sclerosis lesions may have been due to slow clearance of myelin debris. Remyelination may have influenced subsequent MTR changes. The observations support using MTR to monitor symptomatic demyelinating lesions.

Key words: optic neuritis; multiple sclerosis; MRI; magnetization transfer ratio

Abbreviations: CI = confidence interval; FSE = fast spin echo; GE = gradient echo; MT = magnetization transfer; MTR = magnetization transfer ratio; ROI = region of interest; VEP = visual evoked potential

Introduction

The defining clinical feature of relapsing–remitting multiple sclerosis is the presence of clinical exacerbations (relapses) due to new inflammatory demyelinating plaques within the CNS. Most relapses are followed by near-complete recovery of symptoms. Clinical recovery is thought to be due to a number of processes including resolution of inflammation and the consequent nerve conduction block (Youl et al., 1991), proliferation of sodium channels along axonal segments denuded of myelin (Moll et al., 1991; Felts et al., 1998), remyelination (Smith et al., 1979, 1981; Smith and McDonald, 1999), and cortical adaptation (Werring et al., 2000).

MRI provides a means for in vivo study of multiple sclerosis. Conventional imaging techniques are very sensitive at detecting multiple sclerosis lesions, but the correlation between lesion load and disability is limited (Miller et al., 1998) and identification of lesions responsible for individual symptoms can rarely be achieved in the brain and spinal cord (Behan et al., 2000). More pathologically specific imaging sequences are needed.

Magnetization transfer (MT) imaging provides a means by which tissues can be examined in more detail (Wolff and Balaban, 1989), going beyond the more conventional MRI T1 and T2 characteristics (McGowan, 1999; Barker, 2000; van Buchem and Tofts, 2000). Use of MT allows the otherwise invisible ‘bound’ water associated with myelin sheaths to be examined. It also provides a means of producing quantitative images by calculating parameters related to the degree of exchange between bound and free protons; in particular the magnetization transfer ratio (MTR).

In multiple sclerosis, a combined radiological–histopathological examination showed correlations between MTR and both axonal density ($r_S = 0.71$) and myelin density ($r_S = 0.45$) in active demyelinating lesions (van Waesberge et al., 1999). MTR may also be able to follow the remyelination process in vitro and in vivo. In a lysolecithin-induced demyelination model in the rat corpus callosum there was a significant negative correlation between the decrease in MTR and the percentage of remyelinated axons in lesions ($r_S = -0.793$, $P < 0.001$) (Dolio-Grassin et al., 2000). Dousset et al. (1998) performed serial imaging over a 1-year period in four multiple sclerosis patients. A common characteristic was an early decrease in MTR in 13 out of 15 lesions during the first 2 months after lesion appearance, the majority of the decrease occurring at lesion appearance. In subsequent months the MTR in five lesions had increased back to the normal range (three of these disappeared on T2-weighted imaging), eight lesions showed smaller increases and two lesions had progressive decline in MTR. The increase in MTR was considered to be due to resolution of oedema, remyelination and gliosis. The progressive decline may have been due to ongoing demyelination and axonal loss.

In an animal model of Wallerian degeneration Lexa et al. (1994) surgically ablated the visual cortices from one hemisphere of 10 cats. MT imaging was then performed at varying intervals with histological analysis. In the first 2 weeks following the ablation, the MTRs in the optic radiations were higher on the ablated side compared with the contralateral hemisphere ($P < 0.02$) associated with the early phase of Wallerian degeneration: axonal collapse, increased axoplasmic density and collapse of myelin tubes. Over the next 2 weeks MTR fell as, histologically, demyelination occurred with gliosis.

Taken together, these studies suggest that: (i) early changes due to Wallerian degeneration may be associated with an early rise in MTR, possibly due to exposure of myelin fragments to extracellular tissue increasing the exchange of magnetization; (ii) low MTR results from a decreased capacity for exchange due to oedema, demyelination and gliosis; (iii) axonal degeneration combined with demyelination results in even greater falls in MTR; (iv) remyelination may result in restoration of MTR.

Optic neuritis is a good model for the study of multiple sclerosis relapses as there are accurate clinical tests of visual function, conduction in the optic pathways, a marker of myelination, can be measured using visual evoked potentials (VEPs) (Halliday et al., 1973) and as MRI, with appropriate fat saturation techniques, enables the symptomatic lesion in optic neuritis to be visualized (Gass et al., 1996).

Previous cross-sectional studies have detected decreased MTR in both acute and chronic optic neuritis. In a study of 39 patients with acute optic neuritis mean intra-orbital MTR was 41.1 pu in control optic nerves, 30.6 pu in 22 optic nerves in which either a high signal lesion was present on T2-weighted images or contrast-enhancement was seen and 36.3 pu in 12 of the remaining 18 patients, who had no high signal lesion but who demonstrated a reduction in MTR (Boorstein et al., 1997). Thorpe et al. (1995) in a study of 20 patients between 3 months and 16 years following optic neuritis observed that the mean MTR in one slice in the intra-orbital optic nerve was 49 pu in controls, 48 pu in healthy contralateral optic nerves and 42 pu in diseased optic nerves ($P < 0.005$ versus unaffected nerves and control nerves). The MTR was significantly correlated with VEP latency ($r_S = -0.554$, $P < 0.01$) but not Snellen visual acuity. The inverse correlation observed between MTR and VEP latency supports the idea that MTR reduction is due to demyelination and the lack of correlation of MTR with clinical measures was concordant with the complex relationship between demyelination and vision that had previously been shown in VEP studies of optic neuritis (Halliday et al., 1973). However, a subsequent study of patients with multiple sclerosis and previous optic neuritis yielded the opposite result: MTR correlated with visual acuity ($r_S = 0.49$, $P = 0.01$) but not VEP latency ($r_S = -0.10$) (Inglese et al., 2002). A possible explanation for the latter finding was that the cohort was biased towards those with a limited visual recovery, over 50% having a visual acuity worse than 20/25.
The above studies used two-dimensional gradient echo (GE) imaging and relied on the manual placement of a region-of-interest (ROI) of 4–8 voxels for segmentation. These segmentation techniques risk the exclusion of important information if the optic nerves are swollen, as in acute optic neuritis, but also the inclusion of partial volume pixels containing CSF and orbital fat in the presence of optic nerve atrophy. It would be desirable to use a three-dimensional (3D) sequence to minimize partial volume effects by having the highest resolution possible. Partial volume voxels at the junction between the nerve and surrounding CSF cause a particular problem for MTR measurement. The optic nerve has a much greater surface area : volume ratio than the brain and therefore a greater proportion of partial volume voxels. This proportion increases with atrophy, which is known to occur following optic neuritis (Youl et al., 1996). There is, therefore, a danger that decreases in MTR following optic neuritis will be due to increased measurement of these partial volume voxels rather than due to changes in the nerve substance itself. Both the acquisition sequence and segmentation technique need to be selected with care. The optic nerves are not large enough for meaningful histogram analysis, hence the need for ROI analysis.

The temporal evolution of MTR changes after an acute attack of optic neuritis and the relationship with imaging, clinical and electrophysiological parameters has not been previously studied. This report presents the results of a prospective serial study of optic nerve MTR using a high resolution 3D GE sequence in a cohort of patients with acute unilateral optic neuritis. The key aims of the present study are: (i) to quantify the MTR changes over time due to a symptomatic acute inflammatory demyelinating lesion; (ii) to determine if the MTR changes over time mirror the electrophysiological and histopathological observations which suggest demyelination followed by remyelination in acute multiple sclerosis lesions [It would be desirable to confirm by imaging the electrophysiological observation of remyelination in the recovery process (Brusa et al., 2001); such an outcome would support using MTR as a tool to investigate remyelination in the optic nerve and elsewhere in the CNS.]; (iii) to explore whether the MTR measures either acutely, or during recovery, are associated with the degree of visual recovery.

**Patients and methods**

Twenty-nine patients with their first episode of acute unilateral optic neuritis (10 males, 19 females; median age 30 years, range 19–53 years) were recruited from the Neuro-Ophthalmology clinic, Moorfield’s Hospital, London. The median delay from onset of visual symptoms to the first examination was 13 days (range 7–24 days). Three of the patients had clinically definite multiple sclerosis, five had clinically probable multiple sclerosis and 21 had clinically isolated optic neuritis (Poser et al., 1983). All the patients were examined acutely and most after 2, 4, 8, 12, 26 and 52 weeks (n = 21, with some missing time points for some of the patients).

MRI was performed on a Signa 1.5-T imager (General Electric, Milwaukee, WI, USA). The patients had their optic nerves imaged with a coronal 3D GE sequence [repetition time (TR) 23.1 ms, echo time (TE) 5.6 ms, number of excitations (NEX) 2, flip angle 12°, 256 × 192 matrix, 19 × 14.25 cm field of view, in-plane resolution 0.75 × 0.75 mm, 60 × 1.5 mm contiguous slices, acquisition time 18 min] both with and without a pre-pulse to saturate the broad resonance of immobile macromolecular protons (offset frequency 2 kHz, equivalent on-resonance flip angle 500°) and a fat-saturated dual echo fast spin echo (FSE) sequence (coronal-oblique; TR 2300 ms, TE-effective 58/145 ms, ETL (echo-train length) 8, NEX 2, 512 × 384 matrix, 24 × 18 cm field of view, in-plane resolution 0.5 × 0.5 mm, 16 × 3 mm interleaved contiguous slices, 11 min acquisition time).

In addition, 24 of the patients had their optic nerves imaged at baseline before and after intravenous administration of 0.03 mmol/kg dimeglumine gadopentate (triple-dose gadolinium) with a coronal-oblique fat-saturated T1-weighted spin echo sequence (TR 600 ms, TE 20 ms, 1 excitation, 256 × 192 matrix, 24 × 18 cm FOV, 16 contiguous 3 mm slices, 3 min acquisition time). An experienced radiologist (K.A.M.), blinded to the lesion side and severity of visual loss, identified and measured the length of any enhancing optic nerve lesions on the post-gadolinium images. Serial imaging following triple-dose gadolinium was performed on 15 of the patients after 2, 4, 8 and 12 weeks until enhancement was deemed to have ceased. The duration of enhancement was noted.

Twenty-seven control subjects (11 male, 16 female, median age 32 years, range 21–58 years) were also imaged. Eight of the controls were imaged once, four were imaged twice and 15 were imaged on three separate occasions spread out over the course of 1 year.

A quadrature birdcage head coil was used as both transmitter and receiver coil. Subjects were asked to close their eyes and avoid any deliberate eye movements during image acquisition. Between imaging sessions, care was taken to accurately reposition the imaging field of view. In particular, the line from the anterior commissure to the posterior commissure on sagittal localizer images was used to ensure that the subjects’ head angles were the same at each session (Hickman et al., 2002b).

At each visit the patients were examined. Best visual acuity with appropriate spectacle or pinhole correction was measured using a retro-illuminated ETDRS (Early Treatment Diabetic Retinopathy Study) chart and recorded as the 4 m logMAR acuity (Ferris et al., 1982). When no letters could be correctly identified a score of 1.7 was assigned (Optic Neuritis Study Group, 1991). The central 30° of the visual field was analysed using the 30-2 program on the Humphrey field analyser (Allergan-Humphrey Inc., San Leandro, CA, USA). Wide-angle lenses were used to correct refractive errors where necessary. The overall field mean deviation was
compared with a reference field derived from control data provided by the manufacturer. A mean deviation of −35 dB was assigned when vision was too poor to attempt the test (Kupersmith et al., 2002). The above two parameters were chosen because they give continuously variable measures that are amenable to statistical analysis.

In addition, at baseline, 4, 12 and 52 weeks whole-field and central-field pattern-reversal VEPs were measured on each patient (Brusa et al., 2001). Ethical approval was obtained for the study from the joint ethics committee of the Institute of Neurology and the National Hospital for Neurology and Neursurgery; informed consent in writing was obtained from each subject, according to the Declaration of Helsinki.

From the images, MTR was calculated on a voxel-by-voxel basis from the expression: 100 × (M₀ − Mₛ)/M₀ per cent units (pu) where Mₛ and M₀ represent signal intensities with and without the saturation pulse, respectively. The calculated MTR maps were displayed on workstations (Sun Microsystems, Mountain View, CA, USA) using the DispImage display tool (Plummer, 1992). An observer, experienced in interpreting optic nerve images, but blinded to subject identity and acquisition order, segmented the optic chiasmata and the optic nerves, from all the slices on which the nerves were visible, using threshold-based contouring with the threshold fixed at 30 pu to include voxels left out with fixed-size ROIs in optic nerve swelling but exclude partial volume voxels that might occur with optic nerve atrophy (Hickman et al., 2002a). While defining the ROI directly on the MTR maps (rather than on an unrelated, but spatially registered image such as that of M₀) could potentially leads to bias in region size and placement, the MTR maps were used in this case as the intra-orbital optic nerve could not be identified on the M₀ images due to high signal and chemical shift artefact from orbital fat. Since fat has a low MTR the signal from orbital fat was suppressed on the MTR maps. The measurements made on the baseline imaging of the controls, plus 20 of the patients’ scans (selected at random), were repeated so that the measurement reproducibility could be assessed.

Lesions were identified on the acute FSE images (intrinsically registered to the MT images as they were acquired together) by the radiologist (K.A.M.) and from their coordinates the part of the nerve occupied by a lesion could be identified on the MTR maps. The mean lesion and post-lesion MTR (i.e. the MTR in the section of the nerve between the lesion and the optic chiasm) could then be calculated. Formal co-registration was not possible due to the small size of the optic nerves and the fact that the nerves can move (semi-)independently of the surrounding tissue. Instead, at each subsequent time-point the same parts of the nerve were identified by using the optic chiasm as the reference point and counting the same number of slices from the anterior part of the chiasm.

In controls, the optic nerve mean MTR varied with slice position from the optic chiasm reaching a peak in approximately the location of the optic canal (Fig. 1). For analysis of lesion and post-lesion MTR, the ratio of the symptomatic optic nerve lesion mean MTR or post-lesion mean MTR to the corresponding part of the healthy contralateral optic nerve mean MTR was therefore used because the lesion length and position along the optic nerve varied between patients. This also had the effect of smoothing out any effects due to varying movement artefact between sessions, as any movement artefact due to head motion and conjugal eye movements tends to affect both eyes equally.

**Statistical methods**

To assess measurement reproducibility the within- and between-subject standard deviations (and hence coefficient of variation and intra-class correlation coefficient) were obtained from random effects one-way analysis of variance (Bland and Altman, 1996). Variations in MTR variables over time were examined using random intercept (Goldstein, 1995) regression models with fixed linear and quadratic terms in time (there was no evidence that random slopes in time were examined using random intercept (Goldstein, 1995) regression models with fixed linear and quadratic terms in time (there was no evidence that random slopes in time improved the fit). Baseline (time 0, the day of onset of visual symptoms) and 365-day values were estimated from these models, and patient versus control difference in gradient assessed, including a subject status by time interaction term. Time to nadir (minimum of the quadratic curve) and value at nadir were functions of the linear and quadratic parameters, for which confidence intervals (CIs) were obtained using a non-parametric bias-corrected bootstrap with 1000 replicates (Carpenter and Bithell, 2000). Bootstrap CIs were also obtained where normality of regression residuals could not confidently be assumed. Simple regression was used to examine relationships between ‘time-averaged’ variables (with one value per subject averaged over available time-points); a subject status indicator was used to compare patients and controls (this is equivalent to a t-test).
visual recovery levels were estimated for each patient as asymptotes of exponential models in time (Snedecor and Cochran, 1989). All analyses were carried out in Stata 7.0 (Stata Corporation, College Station, TX, USA) except for random slopes models which were examined in MLwiN 1.10 (Centre for Multilevel Modelling, Institute of Education, London, UK).

One patient had bilateral recurrence of optic neuritis after 12 weeks and two patients had recurrences in their previously healthy contralateral optic nerves, one patient at both 12 and 52 weeks and one patient at 26 weeks. The data collected on these subjects after these events were excluded from the analysis.

Results
Measurement reproducibility figures (coefficients of variation ranges 0.3–0.5%) are given in Table 1. The estimated mean MTR in control optic nerves at baseline was 47.8 pu (95% CI 47.4, 48.2). This value showed no evidence of a systematic change according to the date of acquisition, nor was there evidence that mean MTR in controls changed significantly over 1 year; the estimated rate of change over the year was −0.00065 pu/day (95% CI −0.0024, 0.0011), \( P = 0.462 \) (Fig. 2). The time-averaged mean MTR in controls was 47.7 pu (95% CI 47.4, 48.0).

The estimated mean MTR from healthy contralateral optic nerves from patients at baseline was 47.9 pu (95% CI 47.5, 48.3). The gradient of change over 1 year was +0.00092 pu/day (95% CI −0.0012, 0.0030), \( P = 0.383 \), again suggesting that there was no significant change in MTR in the healthy contralateral eyes over 1 year. There was no evidence of any difference in time-averaged mean MTR between the healthy contralateral nerves and controls (patient−control difference in means = 0.2813, 95% CI −0.2843, 0.8470, \( P = 0.323 \)).

The characteristics of mean MTR in diseased optic nerves and lesion MTR ratio are given in Table 2 and illustrated in Figs 3 and 4. The significant positive quadratic coefficients indicate a U-shape: an initial decline in MTR reaching a nadir at about 240 days followed by a slight increase. The estimated 365-day values remained significantly less than both baseline and control values although the increases compared with the nadir values were not significant (as shown by the appropriate 95% confidence limits).

At the first assessment there was no significant difference in MTR between diseased optic nerves and control optic nerves (mean MTR in patients 47.3 pu, mean MTR in controls 47.7, difference −0.4, 95% CI −1.2, 0.4, \( P = 0.354 \)). There was, however, a significant difference in the time-averaged mean MTR from diseased optic nerves and control optic nerves (46.2 pu versus 47.7 pu, difference in means −1.5, 95% CI −0.8, −2.1, \( P = 0.0001 \)).

The mean MTR ratios from post-lesion segment of diseased optic nerves and the mean MTR results from the optic chiasmata are given in Table 3. A linear model gave the best fit for these two variables and indicated a decline in both over time.

In controls there was no evidence of change of chiasmal MTR over time (gradient −0.0013 pu/day, 95% CI −0.0038, 0.0012, \( P = 0.304 \)). However, there was no statistical evidence of a difference in gradient between patients and controls, probably through insufficient power. At first
assessment the chiasmal MTR was significantly less in patients than controls (47.1 pu versus 48.3 pu, \(P = 0.023\)). There was also a significant difference between patients and controls for the time-averaged mean chiasmal MTR (46.8 pu versus 48.0 pu, \(P = 0.004\)); and the 365-day estimated value for patients (46.0 pu, 95% CI 45.3, 46.8), was lower than the control time-averaged mean (48.0 pu, 95% CI 47.3, 48.7).

There was no evidence of association between the acute lesion length (measured on either FSE or triple-dose gadolinium enhanced images) and any of the MTR variables. The median duration of gadolinium enhancement was 63 days (range 0–113 days). Again, the duration of enhancement was not associated with any of the MTR variables.

The relationship between the MTR variables and the clinical variables was complex. There was no linear relationship between any of the diseased optic nerve MTR variables and logMAR acuity or visual field mean deviation at baseline or 1 year. Although there appeared to be an association within patients between the MTR variables and vision (with the MTR variables appearing to fall as vision improved), this seemed to be an artefact of time; once time was entered into the model, the within-person MTR versus vision relationship lost statistical significance. However, eventual visual recovery level was improved in patients with higher time-averaged ratios of diseased : healthy optic nerve MTR; this was true for the whole optic nerve, for the lesion segment and for the post-lesion segment (Table 4).

There were no significant direct linear relationships by cross-sectional analyses between the MTR variables and electrophysiological measurements. There was evidence that patients with higher time-averaged MTR values had shorter time-averaged VEP latencies. Whole field latency was lower

### Table 2 Whole optic nerve and lesion MTR results in patients

<table>
<thead>
<tr>
<th></th>
<th>Estimated time '0' value (95% CI)</th>
<th>Linear coefficient* (95% CI)</th>
<th>Quadratic coefficient (95% CI)</th>
<th>Time to nadir(^1) (95% CI)</th>
<th>Nadir value (95% CI)(^2)</th>
<th>Estimated 365 day value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseased optic nerve MTR (pu)</td>
<td>47.4 (46.8, 48.1)</td>
<td>-0.027 (-0.035, -0.019)</td>
<td>5.7 × 10^{-5}, (3.6 × 10^{-5}, 7.8 × 10^{-5})</td>
<td>239 (216, 277)</td>
<td>44.2 (43.7, 44.8)</td>
<td>45.1 (44.4, 45.8)</td>
</tr>
<tr>
<td>Ratio of diseased: healthy optic nerve MTR</td>
<td>0.99 (0.97, 1.00)</td>
<td>-0.00051 (-0.00072, -0.00030)</td>
<td>1 ± 10^{-6}, (5 ± 10^{-7}, 1.6 ± 10^{-6})</td>
<td>246 (214, 313)</td>
<td>0.92 (0.91, 0.94)</td>
<td>0.94 (0.92, 0.95)</td>
</tr>
<tr>
<td>Ratio of lesion: healthy optic nerve MTR</td>
<td>0.98 (0.97, 1.00)</td>
<td>-0.00058 (-0.00083, -0.00033)</td>
<td>1.2 ± 10^{-6}, (5.6 ± 10^{-7}, 1.8 ± 10^{-6})</td>
<td>241 (215, 289)</td>
<td>0.91 (0.90, 0.93)</td>
<td>0.93 (0.91, 0.95)</td>
</tr>
</tbody>
</table>

*Linear coefficient can be interpreted as the rate of daily decline at time '0'. \(^1\)Days from time '0' (day of onset of visual symptoms). \(^2\)Bootstrap derived confidence intervals.

![Fig. 3 Mean MTR over time for all diseased optic nerves with fitted curve in bold.](image)

![Fig. 4 Ratio of MTR of the lesion: corresponding portion of healthy contralateral optic nerve over time with fitted curve in bold.](image)
The mean MTR in the healthy optic nerve varied with slice position, being highest in the optic canal, probably due to the more densely packed structure of the optic nerve within the confines of the bony canal (Fig. 1). The decline in the orbital portion may be due to a looser structure as well as movement artefact in this portion of the optic nerve. There was also variation in length of the optic nerve between individuals leading to larger confidence intervals at the most anterior slices.

A quadratic model was used for both diseased optic nerve MTR and lesion MTR ratio because inspection of the raw data suggests that, on average, both declined to nadirs at around 240 days followed by small increases (Table 2; Figs 3 and 4). The 1-year values, however, were not significantly higher than the nadir values.

At the onset of optic neuritis, when visual impairment was at its worst, MTR values were unchanged. This finding is in contrast with acute brain lesions in multiple sclerosis where there is a rapid initial decrease in MTR. The normal MTR value in acute optic neuritis may result from a balance of two factors. First, in the first 2 weeks after axonal transection, ‘acute’ Wallerian degeneration may transiently increase MTR, probably due to increasing exposure of myelin fragments (Lexa et al., 1994). Secondly, breakdown of the blood–optic nerve barrier, depicted using gadolinium enhancement, results in an inflammatory cellular infiltrate and an element of vasogenic oedema; the increase in interstitial fluid will decrease MTR. The optic nerve has a more compact structure with subdivision into fascicles of axons by fibrous septa and a surrounding optic sheath (Williams et al., 1989). Vasogenic oedema may therefore be limited in the optic nerve, lessening the initial effects of oedema on MTR changes in the nerve. In comparison, acute multiple sclerosis lesions in the brain may show much larger initial reductions in MTR values (Dousset et al., 1998; Silver et al., 1998) because there is a much greater amount of interstitial fluid due to vasogenic oedema.

During the most rapid phase of visual recovery, mean MTR was gradually declining—indeed this decline did not reach a nadir until about 8 months. This discordance suggests that (i) there are factors leading to early visual recovery that are not reflected using MTR; and (ii) a continuing breakdown in tissue structure is occurring for several months following visual recovery. The initial rapid recovery of vision may have

### Table 3 Post-lesion and chiasmal MTR results from patients

<table>
<thead>
<tr>
<th>Ratio of post-lesion: healthy optic nerve MTR</th>
<th>Estimated time ‘0’ value (95% CI)</th>
<th>Linear coefficient* (95% CI)</th>
<th>Estimated 365-day value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiasmal MTR (pu)</td>
<td>47.2 (46.7, 47.7)</td>
<td>-9 × 10⁻⁵</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1.5 × 10⁻⁴, -3 × 10⁻⁵)</td>
<td>P = 0.01^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(±0.0033, ±0.0007)</td>
<td>P = 0.013^b</td>
</tr>
</tbody>
</table>

*This is interpreted as the estimated rate of change per day in the MTR variable. ^Bootstrap derived CIs and P-value.

### Table 4 The relationship between time-averaged MTR ratios and visual recovery level

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Linear coefficients* (95% CI) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LogMAR visual acuity recovery</td>
</tr>
<tr>
<td></td>
<td>Visual field mean deviation recovery, dB</td>
</tr>
<tr>
<td>Diseased optic nerve:</td>
<td></td>
</tr>
<tr>
<td>healthy optic nerve MTR</td>
<td>0.95 (-0.01, -0.08) P = 0.007</td>
</tr>
<tr>
<td>MTR</td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Lesion: healthy optic nerve MTR</td>
<td>0.46 (-0.02, -0.08) P = 0.02</td>
</tr>
<tr>
<td>P = 0.04</td>
<td></td>
</tr>
<tr>
<td>Post-lesion: healthy optic nerve MTR</td>
<td>0.79 (-0.03) P = 0.03</td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Interpreted as estimated change in eventual visual recovery level for each 0.01 rise in time-averaged MTR ratio. ^Bootstrap derived confidence intervals.

by 5.3 ms (95% CI 0.2, 10.3, P = 0.043) and central field latency was lower by 6.1 ms (95% CI 1.5, 10.7, P = 0.012) per 1 pu rise in time-averaged diseased optic nerve MTR. There were no associations between time-averaged amplitude results and any time-averaged MTR variables.

### Discussion

The use of a fixed threshold of 30 pu enabled the optic nerves to be segmented with high reproducibility, although it should be noted that as the results reflect the mean MTR over the whole optic nerve, variations will tend to be, to an extent, smoothed out. The high threshold was chosen to exclude partial volume voxels which, in patients, might be expected to have a differential effect later in the study where optic nerve atrophy might be developing (Hickman et al., 2002a). This may also have limited the proportionate decrease in MTR values seen compared with previous studies in both multiple sclerosis and optic neuritis, by minimizing the risk of a spurious reduction in MTR due to partial volume effects with inclusion of CSF in the optic nerve sheath. However, absolute MTR values between studies should not be directly compared as they vary according to the imager and sequence used.
been due to (i) disappearance of inflammatory mediators of conduction block (e.g. nitric oxide) (Youl et al., 1991); (ii) insertion of sodium channels along the inter-nodal membrane to restore axonal conduction (Moll et al., 1991; Felts et al., 1998); (iii) early remodelling of visual function and utilizing redundant capacity at the level of central synaptic networks (Frisen and Quigley, 1984; Werring et al., 2000). Possibly none of these processes had a material effect on MTR.

The optic nerve mean MTR continued to decline beyond the time when gadolinium enhancement, indicating inflammation, was last detected. The continued decline in MTR might be due to several factors: (i) ongoing demyelination, as reflected by the relationship between time-averaged MTR and mean VEP central field latency; (ii) gradual clearance of myelin breakdown products (that continue to act as structural elements whilst present) resulting either from initial inflammatory myelin breakdown per se (Adams et al., 1989) or as part of the prolonged degenerative phase of Wallerian degeneration (Lexa et al., 1994). The decline in MTR continued for much longer in this study than studies of multiple sclerosis brain lesions (Dousset et al., 1998; Silver et al., 1998), possibly due to the different ultrastructure of the optic nerve compared with white matter tracts in the brain slowing clearance of myelin debris. In this context, it is noteworthy that, in a rat optic nerve model of Wallerian degeneration, myelin debris could be detected 22 months post-enucleation (Ludwin, 1990).

The decline in MTR appeared to reach a nadir after about 8 months, which may reflect arrest of demyelination and clearance of myelin debris. Remyelination in the lesion may have contributed to the subsequent trend for MTR to increase, a view supported by the significant relationship observed between time-averaged MTR and mean VEP central field latency. However, further follow-up is needed to determine whether or not the increase in MTR is significant and sustained.

Over the whole period, lower time-averaged MTR ratios were associated with poorer visual outcome, which suggests that, although the relationship between MTR and vision is complex, the longer the time during which the affected nerve MTR was lower than that of its fellow nerve (implying tissue disruption most likely due to demyelination and axonal loss) the worse the prognosis.

The continued linear decline in post-lesional and chiasmal MTR is probably solely due to Wallerian degeneration in the axons transected in the acute inflammatory lesion. The initial post-lesional MTR was not abnormally high, unlike that which was seen in the case of the experimental transection study by Lexa et al. (1994), possibly due to inflammation in these regions not apparent on conventional imaging depressing MTR slightly. Also, Wallerian degeneration is likely to be much less extensive in the optic nerve following optic neuritis than in the above lesion study, probably affecting only about 10% of fibres compared with 100% in the nerve section model.

If, in future trials of remyelination therapies, the optic nerves are chosen, then optic nerve MT imaging in combination with conventional imaging, VEP and clinical measures may be useful in assessing the response to treatment. The potential advantage of using optic nerve MT imaging to assess optic nerve structure over VEP measurement is the ability to visualize the optic nerves and to assess different parts, including both the lesion and post-lesion segments. Electrophysiological measures can only give an indication of conduction in the whole optic pathway which may be influenced by changes in the optic tract, lateral geniculate body and optic radiation. MTR measures may also have a role in detecting remyelination of multiple sclerosis lesions elsewhere in the CNS.

Acknowledgements

S.J.H. is supported by The Wellcome Trust. A.T.T. is supported by the Brain Research Trust. The NMR Research Unit is supported by the Multiple Sclerosis Society of Great Britain and Northern Ireland, who also supported G.J.B. during the course of this study.

References

Filippi M, Rocca MA, Martino G, Horshfield MA, Comi G. Magnetization transfer changes in the normal appearing white matter precede the