Retrograde trans-synaptic retinal ganglion cell loss identified by optical coherence tomography

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There is experimental evidence of trans-synaptic retrograde degeneration of retinal ganglion cells following retrogeniculate visual pathway lesions in primate studies. Retinal nerve fibre loss in congenital homonymous hemianopia in humans is well recognized from clinical observation but the findings in acquired lesions have been controversial. Forty-eight persons were recruited and divided into three groups. Two groups were patients with retrogeniculate lesions. In the first group, the occipital damage had occurred during childhood or in adult life whilst the lesions in the second group were congenital. Inclusion criteria for the retrogeniculate lesions included: age >18 years at time of testing; homonymous hemianopia; no other ophthalmic or neurological disorder; and neuroimaging demonstration of occipital lobe damage. The third group had normal visual function. Measurement of the thickness of the peripapillary retinal nerve fibre layer by optical coherence tomography has been carried out in both eyes of each subject. The primary outcome is the peripapillary retinal nerve fibre layer thickness (RNT) in microns. The mean RNT in the eyes with temporal hemianopia (here called the ‘crossing-fibre defect’ eyes) is 79.8μ (SD = 35.1μ) in the acquired and 72.7μ (SD = 33.2μ) in the congenital. The mean RNT in eyes with nasal hemianopia (here called the ‘non-crossing-fibre defect’ eyes) is 83μ (SD = 29.5μ) in the acquired and 73.4μ (SD = 26μ) in the congenital. In the control group, the RNT measured 101.4μ (SD = 36.6μ) for the left eyes and 100.8μ (SD = 35.4μ) for the right eyes. In both crossing-fibre defect eyes and non-crossing-fibre defect eyes the mean RNT is significantly greater in the controls than in the hemianopia groups (P<0.001). These data confirm that there is thinning of the retinal nerve fibre layer following both congenital and acquired lesions of the retrogeniculate visual pathway in humans. This is most likely to represent retinal ganglion cell loss in both congenital and acquired groups. Furthermore the magnitude of the thinning is similar in both groups despite the fact that clinical observation has consistently found evidence of RNT thinning in cases of congenital but not in cases of acquired pathology. The data have also been analysed in 12 sectors around the optic disc: it has been shown that the RNT thinning follows the known trajectories of the crossing and non-crossing retinal ganglion cell axons approaching the disc.

Keywords: retrograde trans-synaptic degeneration; retrogeniculate lesions; retinal nerve fibre layer; optical coherence tomography; band atrophy

Abbreviations: CI = confidence interval; LGN = lateral geniculate nucleus; MRI = magnetic resonance imaging; OCT = optical coherence tomography; RNT = retinal nerve fibre layer thickness
Introduction

It is well established that retrograde trans-synaptic degeneration can occur in the human central nervous system. For example, degeneration of the cells of the inferior olive has been demonstrated following a lesion of contralateral cerebellar cortex (Holmes and Stewart, 1908) and chromatolytic change in Betz cells in area 4 following limb amputation (Campbell, 1905). Van Buren in 1963 observed atrophy of the right lateral geniculate nucleus (LGN) as well as retinal ganglion cell loss following right occipital lobectomy in a primate (Van Buren, 1963). This finding was later confirmed by other studies (Cowey, 1974; Cowey et al., 1999; Johnson and Cowey, 2000). Clinical observations in humans with acquired disease have been at variance with those in animals. In a case report of a patient who had suffered a cerebrovascular accident resulting in complete left homonymous hemianopia 57 years previously, the optic discs appeared healthy bilaterally (Miller and Newman, 1981), which argued against such a change in humans. However, optic disc pallor and band atrophy have been described in patients with congenital occipital lesions (Fletcher et al., 1988) and also in patients with unilateral periventricular leucomalacia (a type of brain injury associated with premature birth; Ragge et al., 1991). Small optic discs and increased cup-to-disc ratio suggesting axonal loss were found in children with congenital periventricular leucomalacia associated with haemorrhage (Jacobson et al., 2003). A MRI study of the brain in children with periventricular leucomalacia; bilateral occipital infarction; and neuroaxonal dystrophy showed signal hyperintensity in the LGN in T2-weighted images, indicating gliosis (Uggetti et al., 1997). A pathological investigation revealed changes in the LGN but the retinal ganglion cells were not studied (Beaty et al., 1982). Cowey showed retinal nerve fibre layer thinning in a single case of longstanding (childhood onset) hemianopia using optical coherence tomography (OCT) (Cowey, 2004). In 2005, Metha and Plant reported thinning of the peripapillary retinal nerve fibre layer in congenital occipital lesions using OCT (Metha and Plant, 2005a, b).

The retinal nerve fibre layer is made up primarily of the axons of retinal ganglion cells. The subsequent course of these axons is in the optic nerve, the optic chiasm and the optic tract. The majority of these axons (around 90%) synapse in the LGN, the remainder project to the pretectal region of the midbrain. The optic nerve fibres of the retinal ganglion cells in the nasal hemiretinae subserve the temporal visual hemifield and cross (decussate) at the optic chiasm. The optic nerve fibres of the retinal ganglion cells in the temporal hemiretinae subserve the nasal visual hemifield and do not cross at the optic chiasm. Damage to the optic chiasm or the optic tract will result in loss of retinal ganglion cells by Wallerian degeneration. It is well established from ophthalmoscopic clinical observation that loss of the crossing fibres in an eye, whether from a lesion at the optic chiasm (which will affect both eyes similarly) or at the optic tract (which affects the crossing fibres of the contralateral eye and non-crossing fibres of the ipsilateral eye) results in a pattern of atrophy known as ‘band atrophy’ or ‘bow-tie atrophy’ (Hoyt and Kommerell, 1973). This is seen because at the nasal and temporal equatorial poles of the disc all (or almost all) fibres are lost, whereas elsewhere there is thinning only. In the eyes with the nasal hemianopic defect in cases of homonymous hemianopia due to damage of the optic tract, there is at no location around the optic disc total loss of fibres; there is thinning of all sectors with the exception of the nasal and temporal sectors to which the non-crossing fibres do not contribute (Fig. 1). The occurrence of band atrophy in the eye with temporal hemianopia in cases of optic tract lesion and also in cases of congenital post-chiasmal lesions is a commonplace clinical observation.

OCT (Huang et al., 1991) is a non-invasive technique that can be used to measure the cross-sectional thickness of the retinal nerve fibre layer in vivo. We have employed this technique to look for evidence of thinning of this layer which occurs owing to the loss of retinal ganglion cell axons.

In the analysis of results in the hemianopia cases, the eyes with loss of the temporal visual hemifield, whether the left or right eye, have been considered together as the ‘crossing-fibre defect’ eyes. As stated above in these eyes, the affected retinal ganglion cells are located in the nasal hemiretinae. The axons of these retinal ganglion cells, which decussate at the optic chiasm, are distributed in all 12 sectors studied but crossing fibres occur exclusively in the temporal and nasal sectors. The homonymous nasal field defect in the fellow ‘non-crossing-fibre defect’ eyes involves the axons of ganglion cells in the temporal hemiretinae which course in all sectors towards the optic disc with the exception of the temporal and nasal sectors. These axons do not decussate in the optic chiasm.

Methods

Forty-eight cases were recruited to the present study. There were three groups. The first pathological group, comprising 19 cases, had acquired homonymous hemianopia; the median duration of disease was 3.5 years (range 0.3–67 years) and the median age was 65 years (range 30–83 years). The second pathological group, comprising seven cases, had congenital homonymous hemianopia; the median age was 31 years (range 17–59 years). The control group consisted of 22 volunteers with normal vision and no ophthalmic disorder. The median age of this group was 45 years (range 23–61 years).

Visual field analysis was carried out using the Humphrey® visual field analyser. Patients were recruited if there was substantial visual loss in either the left or right homonymous hemifields using the 24-2 protocol. The deficit ranged from a mean deviation loss of −11 dB to a loss of −19.1 dB for all cases. Hence a wide range of visual deficit was represented ranging from a total failure to detect any stimuli within the affected hemifield to incomplete loss. Thirty-three percent of patients had macular sparing. The relationship between regional visual field loss and the pattern of nerve fibre layer thinning (including macular sparing and quadrantanopia) will be the subject of a future report.

The peripapillary nerve fibre layer thickness was measured in each eye and in each of 12 sectors around the optic nerve head (Fig. 2). The Stratus™ Optical Coherence Tomography version 4 (Carl Zeiss Meditec, Inc.) was used in every case. The aim was to investigate the possibility of loss of axons corresponding to the fibre trajectories of ganglion cells located in the right and left hemiretinae, respectively. This study utilized a paradigm that measures the cross-sectional retinal nerve fibre layer thickness (RNT) of a circle of 3.4 mm diameter.
centred on the optic disc. The circle was slightly larger than the optic disc in all cases. The measurements are given in microns, according to the calibration provided by the manufacturers. The study was approved by the local Ethics Committee and informed consent was obtained from all subjects who participated in the study.

Figure 1 The pattern of nerve fibre loss is shown diagrammatically for a case of left homonymous hemianopia secondary to a lesion of the right optic tract with total loss of affected retinal ganglion cell axons. The retinal ganglion cells subserving the unaffected hemifield (nasal retina of right eye: temporal retina of left eye) and the axons of those retinal ganglion cells are shown diagrammatically in white. In the eye with the temporal hemianopia (the crossing-fibre defect eye; on the right in the illustration) there is a total loss of fibres at the nasal and temporal equatorial poles of the optic disc (giving the appearance of band atrophy) and thinning of the remaining nerve fibre layer (the arcuate bundles). In the eye with the nasal hemianopia (the non-crossing-fibre defect eye; on the left in the illustration) there is a loss of fibres travelling to the disc in the arcuate bundles from the temporal hemiretina. Nowhere in the disc circumference of this eye are nerve fibres lost totally: there is thinning of the arcuate bundles only. (modified from Hoyt and Kommerell, 1973 with permission). In our cases, the nerve fibre loss has occurred following damage to the post-geniculate pathway (trans-synaptic degeneration) but the pattern of loss is identical to that occurring with an optic tract lesion (Wallerian degeneration).

Figure 2 For each eye of each subject the RNT was measured in a circular cross-section centred on the disc. The circle was divided into 12 equal sectors. T = temporal, N = nasal, I = inferior, S = superior. The labelling is mirror symmetric in the two eyes.

Statistical analysis

We report the estimated overall mean RNT in each eye and the ratio of the two measurements with confidence intervals for every group. The difference in the group means among the three groups, i.e. between the control and acquired, between the control and congenital and between the acquired and congenital, are shown together with 95% CI and P-values. We performed hierarchical repeated measures ANOVA (analysis of variance) separately for each variable, with cases nested in groups and sectors representing the repeated measures. Bonferroni post hoc comparisons were made if the group means were significantly different in the ANOVA. If there was a significant interaction between groups and sections in the ANOVA, a one-way ANOVA to compare the group means was performed separately for each sector. The assumptions underlying every analysis were checked by a study of the residuals and were verified for the non-crossing-fibre defect eyes and the ratio of crossing- to non-crossing-fibre defect eyes. In order for the constant variance assumption to be satisfied for the crossing-fibre defect eyes, the ANOVA was performed on the logarithm of the RNT. A significance level of 0.025 was used rather than the conventional level of 0.05 as an additional safe guard against spurious results arising from multiple testing.
Correlations were also calculated between the mean magnitude of the RNT and the loss of visual sensitivity (as measured by automated perimetry).

Results

Results are presented as the mean together with the 95% CI of each measurement. In non-crossing-fibre eyes the estimated mean RNT was 83 $\mu$m (CI 79–87 $\mu$m) in the acquired group; 73.4 $\mu$m (CI 67.7–79 $\mu$m) in the congenital group; and 101.4 $\mu$m (CI 97–105.8 $\mu$m) in the left eyes of the control group. The overall ANOVA indicated that there were significant differences in RNT between the group means and the sector means and a significant interaction between groups and sectors. Post hoc comparisons indicated that there were significant differences in all three group means as follows: (control–acquired) difference in means = 18.4 $\mu$m, (CI 14.3–22.4 $\mu$m, $P < 0.001$); (control–congenital) difference in means = 28 $\mu$m, (CI 22.2–33.5 $\mu$m, $P < 0.001$); and (acquired–congenital) difference in means = 9.7 $\mu$m, (CI 4–15.3 $\mu$m, $P < 0.001$). One-way ANOVA comparing the groups in each sector, followed by post hoc comparisons, demonstrated significant differences in the group means in Sectors S, I, IT, TI, TS and ST between the congenital and control groups and in Sectors S, SN, IT, TI, TS, and ST between the acquired and control groups (see Fig. 2 for explanation). There were no statistically significant differences in the group means in sectors NS, N, NI nor T. A significant difference in the group means was detected only in Sector I between the acquired and congenital groups. The group means for all sectors are plotted in Fig. 3.

Results in the crossing-fibre eyes were as follows: the mean values for RNT were 79.8 $\mu$m (CI 75–84.5 $\mu$m) in the acquired group; 72.7 $\mu$m (CI 65.5–79.9 $\mu$m) in the congenital group; and 100.8 $\mu$m (CI 96.6–105.1 $\mu$m) in the right eyes of the control group. The overall ANOVA on the log values indicated that there were significant differences in RNT between the group means and between the sector means but that there was no significant interaction between the groups and sectors. Post hoc comparisons indicated that there was a significant difference between the acquired and control group means and between the congenital and control group means: there was no significant difference between the acquired and congenital group means. To conform with the presentation of the results for the non-crossing-fibre defect eyes, the difference in the means of the raw data are presented: (control–acquired) difference in means = 21 $\mu$m, (CI 16.9–25.2 $\mu$m, $P < 0.001$); (control–congenital) difference in means = 28 $\mu$m, (CI 22.5–33.8 $\mu$m, $P < 0.001$); and (acquired–congenital) difference in means = 7 $\mu$m, (CI 1.3–12.9 $\mu$m,

Figure 3 Mean RNT for non-crossing-fibres eyes is plotted for each of 12 sectors. In both pathologic groups, there was no significant thinning of RNT in sectors NS, N, and NI, and T, compared with controls.
Using the log data, one-way ANOVA comparing the groups means in each sector, followed by post hoc comparisons, demonstrated significant differences in the group means in all sectors except ST, I and IT between the congenital and control groups; and in all sectors except NI, IN, I and IT between the acquired and control groups. No significant differences in the group means were detected in any sector when the acquired and congenital groups were compared. The group means (raw data) for all sectors are plotted in Fig. 4.

In order to better illustrate the different pattern of retinal nerve fibre layer thinning between the crossing- and non-crossing-fibre defect eyes, the same data employed to generate the results shown in Figs 3 and 4 were analysed as the ratio of the group means. In Fig. 5 the ratio crossing-fibre eye: non-crossing-fibre eye is plotted for the congenital and acquired groups and the ratio right eye: left eye for the control group. The results indicate a broad area on the nasal side of the disc where there is predominantly a loss of crossing fibres (sectors SN, NS, N, NI and IN) but only a solitary sector temporally (T). This pattern is identical for both the congenital and the acquired groups.

There was no significant linear correlation between the mean magnitude of the RNT and the magnitude of the loss of visual sensitivity (as measured by automated perimetry). This was the case for all sectors and for both eyes of both the congenital and the acquired groups.

Discussion

In general terms, these results show that acquired unilateral damage to the occipital lobe resulting in homonymous hemianopia leads to nerve fibre layer thinning as measured by OCT. This thinning is very similar in magnitude to that which occurs in cases of congenital damage. It is clear from previous studies that OCT is a more sensitive indicator of peripapillary retinal nerve fibre layer thinning or other changes than is clinical observation. Therefore, lack of clinical evidence of nerve fibre layer thinning in acquired occipital lesions can be explained in part because OCT shows greater sensitivity. If this were the entire explanation, it would be expected that the changes in acquired would be less than in congenital cases whereas, in the series reported herein, the changes are similar.

Our results can be compared with findings in primate studies. Naito, using a tracer technique, studied the arrangement of retinal...
ganglion cell axons entering the optic disc with respect to retinotopic origin (Naito, 1989). Naito showed that the projection of crossing fibres from nasal retina makes up exclusively a larger sector on the nasal than on the temporal equatorial pole of the disc. This finding is in accord with the results shown in Fig. 4 where the predominantly crossing fibre loss extends over 4–5 sectors nasally but only one sector temporally. Thus, there is considerably greater intermingling of crossing and non-crossing fibres on the temporal than on the nasal side of the disc.

The finding has important implications for the study of trans-synaptic retrograde degeneration following damage to other CNS pathways and provides a model whereby the time-course of such loss and the effect of neuro-protective strategies can be studied directly rather than indirectly by other imaging techniques (e.g. tractography or functional imaging).

These findings have significant implications also for proposed rehabilitation strategies in patients with homonymous hemianopia (Sabel et al., 2005; Sahraie et al., 2006) as retinal ganglion cell loss might be expected to reduce the potential for recovery. The authors suggest that the potential impact of retinal ganglion cell loss should be taken into account in such studies.

A remaining question relates to the lack of clinical evidence for nerve fibre loss in acquired as opposed to congenital hemianopia. This suggests that the process does not lead to the same visible changes at the optic disc and the explanation of this will be the subject of future investigation.

**References**


