Axon deviation in the human lamina cribrosa

James E Morgan, Glen Jeffery, Alexander J E Foss

Abstract

Aims—To examine the course taken by individual retinal ganglion cell axons through the human lamina cribrosa.

Methods—Retinal ganglion cell axons were labelled using the retrograde tracer horseradish peroxidase applied directly to the optic nerve in two normal human eyes removed during the course of treatment for extraocular disease.

Results—A majority of axons took a direct course through the lamina cribrosa but a significant minority, in the range 8–12%, deviated to pass between the cribrosal plates in both central and peripheral parts of the optic disc.

Conclusions—It is postulated that these axons would be selectively vulnerable to compression of the lamina cribrosa in diseases such as glaucoma in which the intraocular pressure is increased.

In the human eye the lamina cribrosa consists of a series of collagenous plates with several hundred perforations through which retinal ganglion cell axons pass to enter the optic nerve. The course taken by these axons is central to our understanding of diseases such as glaucoma in which raised intraocular pressure is associated with distortion of the lamina cribrosa and subsequent axon compression. Axoplasmic transport may be disrupted thereby predisposing to retinal ganglion cell death.

Detailed knowledge of the axon paths through the lamina cribrosa is essential if we are to understand the mechanisms by which axon compression may occur. Within the lamina, axon bundles appear to follow a relatively direct course as they pass through successive pores running with their neighbours towards the optic nerve head to be grouped in a retinotopic arrangement behind the eye. The course taken by individual axons within these bundles remains to be defined. Evidence from other systems suggests that this may not be entirely direct. For example, axons can deviate from their expected topographic pattern in both the vertical and horizontal aspects of the nerve fibre layer and the optic nerve.

Indeed, some axon deviation might be expected since the number of cribrosal pores increases in the more posterior layers, resulting in the division of fascicular bundles. Such deviations might result in retinal ganglion cell axons passing between the plates of the cribrosa, rendering them vulnerable to the compressive effects of increased intraocular pressure.

In order to investigate this possibility, the course of axons through the lamina cribrosa of the human optic nerve head was traced in two human eyes obtained from mature male subjects following exenteration for extraocular orbital disease which had not compromised the eye or the optic nerve head. Axons were labelled using horseradish peroxidase (HRP) transported retrogradely from injections made in the cut end of the optic nerve.

Methods

Neuroanatomical investigations were carried out on two eyes removed from adult males (ages 44 and 86 years) and conformed to the ethical standards requirements of Moorfields Eye Hospital, London. Both eyes were removed in the course of treatment for advanced malignant neoplasia of the ocular adnexa. In neither case had tumour growth compromised the optic nerve or undergone intraocular extension. Intraocular pressures as measured by Goldmann tonometry just before surgery, were within the normal range (less than 21 mm Hg).

Each globe was rapidly removed following transection of the optic nerve. The remaining 1–2 cm stump of optic nerve was then carefully trimmed under microscopic observation to 1–2 mm. The anterior segment was excised and the globe placed in a solution consisting of 0.21 g/l sodium bicarbonate, 0.18 g/l sucrose, and 0.89% Ames medium (Sigma), saturated with a mixture of 95% oxygen and 5% carbon dioxide. The vitreous was gently removed and a glass micropipette tip (outside diameter 100 µm) coated with HRP (Sigma type VI, 70% solution in distilled water) placed into the superior and inferior quadrants of the globe placed in a solution consisting of 0.21 g/l sodium bicarbonate, 0.18 g/l sucrose, and 0.89% Ames medium (Sigma), saturated with a mixture of 95% oxygen and 5% carbon dioxide. Cuts in the retina were used to preserve retinal orientation. The vitreous was gently removed and a glass micropipette tip (outside diameter 100 µm) coated with HRP (Sigma type VI, 70% solution in distilled water) placed into the supero-temporal and inferior quadrants of the sectioned end of the optic nerve. A single penetration was made at each site. The pipette was left in place for approximately 5 minutes to allow diffusion of the HRP into the optic nerve and the globe returned to the incubation medium between injections.

The globes were incubated for approximately 12 hours at 37°C in the gassed medium. The retinae were then fixed by...
immersion in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), placed in 25% sucrose in phosphate buffer overnight, embedded in gelatin and cut as frozen sections at 60 µm. Sections were reacted for HRP using DAB as the chromogen, mounted on subbed slides, dehydrated, cleared, and stained with cresyl violet.

**Results**

High quality retrograde axonal labelling was achieved in both optic nerve heads. Figure 1 summarises the histological findings from one eye; similar axon trajectories were found in the other eye. Axons could be traced from the retrolaminar part of the optic nerve, through the lamina cribrosa and, in many cases, several hundred micrometres into the peripapillary retinal nerve fibre layer. The plates of the lamina cribrosa were easily discerned in unstained sections as zones that formed a regular meshwork devoid of blood vessels. The location of these zones was confirmed with the Nissl counterstain. The majority of axons passed through the lamina cribrosa with little deviation. However, a number were seen to pass around and between the plates of the lamina cribrosa before entering the retrolaminar region of the optic nerve.

The line diagram of the optic nerve head in Figure 1 shows the areas that are outlined in greater detail. Shaded areas correspond to the plates of the lamina cribrosa. Blood vessels (BV) could easily be identified by the presence within their lumen of red blood cells. In Figure 1A axons course fairly directly through the

**Figure 1** Camera lucida drawing showing the path taken by individual HRP labelled axons through the optic nerve head. Transverse section. Optic nerve showing the location of sample areas a, b, and c. Scale bar 1 mm. A, B, and C are areas in the region of the lamina cribrosa drawn at higher magnification. Stippled areas show regions corresponding to the location of the cribrosal plates. BV = blood vessel (filled areas in all figures). Scale bar 50 µm.

**Figure 2** Photomicrograph from the optic nerve head showing deviation of HRP labelled axons in the vicinity of a cribrosal plate (asterisk). Arrows highlight the axon of interest. The top of the image represents more anterior optic nerve. Scale bar 2 µm.

**Figure 3** Photomicrograph of a single deviating axon in the vicinity of a cribrosal plate taken from the optic nerve in Figure 1. The top of the image represents more anterior optic nerve. Arrows highlight deflections in the axon of interest. Scale bar 2 µm.
optic nerve stump was made under micro-
HRP injection sites and final trimming of the
transection sites were at least 1 cm from the
moval of the eye. The initial (surgical)
reflects trauma induced during surgical re-
(Fig 1B). It is also unlikely that the deviation
axons passing through the same cribrosal pore
able to observe both straight and deviated
Axon misdirection could be seen before dehy-
think that this is unlikely for several reasons.
distortion during histological processing. We
deviation may have resulted from tissue
nar region of the optic nerve. An important
diagram in those areas with heavy axon
labelling. In areas of lighter labelling where all
axons in a field could be identified, the
labelling. In areas of lighter labelling where all
axons in a field could be identified, the
labelling. In areas of lighter labelling where all
axons in a field could be identified, the
labelling. In areas of lighter labelling where all
axons in a field could be identified, the
labelling. In areas of lighter labelling where all
labelled axons were only drawn
the plates of the lamina cribrosa. In the exam-
larval eyes1 one implication of our
findings is that these interlaminar axons, which
were found in both central and peripheral parts
of the optic nerve will be particularly vulner-
able to the compressive effects of raised
intraocular pressure. Such damage would be in
addition to any shearing effect that would
occur in eyes with advanced glaucomatous
damage where peripheral regions of the lamina
cribrosa rotate with respect to the axon stream
and are displaced both posteriorly and peripher-
ally.9 In central parts of the cribrosa
this rotation would be less marked but
deviating axons would still be vulnerable to
compression. In view of the considerable
evidence that larger axons are selectively dam-
aged in glaucoma,11 12 it would be important to
know whether the deviating axons have axon
diameters larger than the mean for the optic
nerve. In the present material, we were unable
to measure directly the diameters of the deviat-
ing axons. However, such axons were found
throughout the nerve and did not cluster in the
periphery, where the larger axons from more
peripheral retinal ganglion cells might be found.4 11
The simplest explanation for the axon devia-
tion is that it is the result of topographic
imprecision in the retinogeniculate pathway as
seen in other species.4 Alternatively, it is possi-le that it represents an attempt by axons to
achieve the correct topographic location in the
retrolaminar part of the optic nerve. In the pri-
mate (macaque) retinal nerve fibre layer, the
axon of peripheral retinal ganglion cells run
anterior (superficial) to those arising from
more central retinal ganglion cells.9 Since these
axons lie more peripheral in the optic nerve1
some rearrangement would be needed to
match these topographic patterns. Studies of
axon paths in prelaminar optic nerve provide
evidence that this occurs.1 In the present study
this process may have been obscured because of
the density of axon labelling. In addition, in
the human, axon rearrangement may be
constrained by the tight organisation of astro-
cytes in the prelaminar region of the optic
nerve.14 By contrast, in the lamina itself, astro-
cytes are relatively sparse in the regions
between the cribrosal plates,14 so allowing for
some axon deviation.
Our findings add support to the concept that mechanical axonal compression may be a factor in diseases such as glaucoma. The cause of axon misdirection remains unclear and raises the need for further studies of axon trajectory in the lamina cribrosa.

JEM was supported in part by the International Glaucoma Association.

We are grateful for the assistance of Phil Luthert and to Dave West for advice on the incubation medium.


