PERSPECTIVE

Selective cell death in glaucoma: does it really occur?

J E Morgan

Glaucoma is associated with retinal ganglion cell death and consequent deterioration in visual field. However, the association between these events is not straightforward since a substantial proportion of the ganglion cell population may be lost before visual field defects can be detected using conventional perimetric methods.^{1,2} Clinically, ganglion cell loss manifests as optic disc cupping and defects in the nerve fibre layer³ before the onset of visual field changes. In recent years a number of studies have suggested that some ganglion cells may have a greater susceptibility to the damaging effects of glaucoma and this idea has become central to a number of strategies designed to detect the earliest changes in this disease. Such ideas rest on the fact that the retinal ganglion population is not homogeneous. It comprises cell groups with different physiological and anatomical properties that enable the complex parallel visual analysis thought to occur in the primate.^{4–7} Cells can be divided broadly into those of the parasol type that comprise the M visual pathway and those of the midget type that comprise the P pathway. For simplicity, in this review, I will refer to the respective retinal ganglion cells as M or P cells.⁸ M cells form approximately 10% of the retinal ganglion cell population, have high luminance and contrast sensitivity, large receptive fields, and lack spectral selectivity. P cells have lower luminance sensitivity, smaller receptive fields, and do show spectral sensitivity.^{9–11} Anatomically, at any retinal eccentricity M cells have larger cell soma, axons, and dendritic fields than the P cells and project almost exclusively to the magnocellular laminae of the lateral geniculate nucleus. P cells project to the more dorsal parvocellular laminae.⁵ It has been reported that cells with a larger soma and axon diameter may be selectively damaged early in glaucoma^{12–15} raising the exciting prospect that psychophysical tests that are sensitive to and specific for the properties of M cells may be able to detect the ‘silent’ cell death occurring in the early phase of the disease.^{16,17}

The hypothesis that ganglion cell death is selective in early glaucoma is attractive and has become widely accepted in the ophthalmic community. Large cells are more susceptible to glaucomatous damage than small cells and, since cell size correlates with type, it follows that this indicates a selective vulnerability of cells of a particular physiological class (the M cells). There are two important aspects to this hypothesis. Firstly, that glaucomatous cell damage is selective for cell size and, secondly, that it is selective for cell type. The evidence in favour of cell size related change is persuasive though there are some caveats that should be considered when reviewing the data. The effects that would be expected on particular cell types are more complicated and require close analysis in the light of recent anatomical and psychophysical data. I will consider both of these aspects in turn.

The earliest anatomical indications of selective loss came from the analysis of optic nerve axon counts in both the human and monkey that showed, in glaucomatous subjects, loss of axons with diameters greater than the mean when compared with normal optic nerves.^{12,13} Corresponding loss was also seen in the ganglion cell layer in flat mounted retinal

preparations^{2,14,18} where, away from the fovea, cell size histograms indicated a reduction in the number of cells at the upper end of the cell diameter range. A similar effect was subsequently observed in tissue taken from the macular zone and examined in the transverse plane.¹⁹ It seemed reasonable to assume that these changes in the axon and cell diameter distributions would be reflected as a change in the proportion of the types of remaining ganglion cells.

The first aspect of these cell size distributions to consider is whether they result from a mechanism of cell death that is influenced by cell size. Taken on its own, this point is important since it has implications for the mechanism of cell death in glaucoma.²⁰ There are a number of possible explanations for the published distributions that have been considered. Cell shrinkage, for example, has been considered as a mechanism to account for the cell size distributions¹⁴ and it is instructive to consider the arguments concerning this mechanism. Cell shrinkage, in its simplest form could occur non-selectively throughout the entire ganglion cell distribution. If all cells underwent the same *percentage* shrinkage the absolute effect of this change would be greater for the larger cells. It is conceivable, therefore, that this may generate cell size distributions in which larger cells appear to have been selectively affected. In Figure 1 the size frequencies for a hypothetical cell population have been plotted whose mean size and standard deviation approximate that in the monkey model. The cell distribution is Gaussian and as such is an oversimplification of actual distributions. In Figure 1A all the cells from which this distribution has been derived underwent 5% cell shrinkage. The frequency distribution for this population was then replotted and, to model the effect of cell death, the number of cells in each bin reduced by 20% (a percentage cell death value for which cell distributions have been published¹⁴). The decrease in the height on the glaucomatous distribution is obvious but there does not, at first glance, appear to have been selective death of the larger cells. However, if the number of remaining cells in the shrunken distribution is plotted on the same graph, it can be seen that this effect is quite marked for the larger cells. An important argument against cell shrinkage is that this might in the early stages be reflected in a left shift in the cell population. Such a shift has not been seen to occur in the macaque monkey model though there is a suggestion that it occurs in some of the cell size distributions in the human (see Quigley *et al.*² figures 7, 8, and 11). Figure 1B shows the sort of left shift that might be expected with a higher degree of cell death where cells have undergone 10% shrinkage and 20% cell death. The percentage cell death at each cell size is also superimposed on this plot and quite clearly there is a strong trend for cell loss as a function of cell size. The third graph (Figure 1C) shows how this left shift may be hidden where the proportion of cell death in relation to cell shrinkage is increased. In this plot the tendency for cell loss to increase with cell size is marked and there appears to be little tendency for the distribution to shift beyond the lower limits of the normal distribution. The important point to stress about these models is that they do

Figure 1 (A) Hypothetical cell size distributions approximating those seen in the human and monkey. Broken line shows the normal cell size distribution. Continuous line shows the cell size distribution when cells in the normal population all undergo 5% cell shrinkage and 20% cell death. ■ Indicate the percentage cell death as a function of cell size. The continuous straight line shows the best straight line fit to these points. (B) Cell size distribution for a cell population that has undergone 10% shrinkage and 20% cell death. Conventions as for (A). (C) Cell size distribution for a cell population that has undergone 15% cell shrinkage and 50% cell death. Conventions as for (A).

not require selective action of either cell death or cell shrinkage as a function of cell size.

The methods used to quantify the ganglion cell population raise further problems for the interpretation of cell size distribution data. Ganglion cells in both the human and monkey examples were identified on the basis of Nissl stains^{13,14} in which ganglion cell distinction from amacrine cells requires some subjective judgment. The distinction between these cell types may be more difficult in glaucomatous eyes where one is dealing with a diseased cell population and the criteria that are used in normal subjects may not be applicable. In any primate study, ganglion cell identification, particularly of the smaller cells, is confounded by the presence of displaced amacrine cells in the ganglion cell layer as well as the displacement of some ganglion cells in the amacrine cell layer.⁹ The published amacrine cell size distribution¹⁴ suggests that overlap between retinal ganglion and amacrine cells soma size may be slight. Recent evidence, however, points also to the presence of some amacrine cell populations whose size overlaps extensively with that of retinal ganglion cells.²¹ Details of the retinal eccentricities of all the sampled areas are also essential for accurate interpretation of cell soma size data, but these are missing in several of the published figures where cell populations have been pooled on the basis of percentage cell death. Given the wide variation in cellular morphology with respect to eccentricity,²² this may have significant effects on the shape of a cell size distribution.

As yet, there is no evidence that cell shrinkage occurs in glaucomatous damage and the foregoing discussions are entirely theoretical. Cell shrinkage has been observed as a prelude to cell death and can be a manifestation of apoptosis.^{23,24} Recent work suggests that apoptosis occurs in the monkey glaucoma model²⁵ but also that it is a rapid process, unlikely to generate large populations of shrinking cells. Cell shrinkage and atrophy have been widely studied in development²⁶ and in models of transneuronal degeneration.²⁷ Significantly, cell shrinkage can occur in retinal ganglion cells in transneuronal degeneration with the preservation of the dendritic characteristics that differentiate cell type.²⁷ From the models of shrinkage and death presented in the previous section, the degree of shrinkage would not have to be that great to generate cell size distributions suggesting the occurrence of selective cell death.

The axon and cell size distribution histograms on which the conclusions of selective cell loss are based must be interpreted with even more caution when considering whether cell death is selective for particular cell types. A decrease in the number of cells in a particular part of the cell size distribution does not translate simply into selective loss of one particular cell class. In Figures 2 and 3 the mean and standard deviation of the M and P class ganglion cells have been represented graphically and include the subset of larger P cells that are thought to represent the blue (ON) ganglion cells.²⁸ In both cases there is considerable overlap of all three cell groups. In terms of size, the blue (ON) cell distribution in the monkey lies between those for the M and P cells. Selective cell damage would, in this species, correlate well with the finding that short wave sensitivity loss can be an early predictor of glaucomatous damage.^{29,30} However, this explanation is less convincing in the human where the size distribution of blue (ON) cells and P cell population shows greater overlap. Of course the assumption in this argument is that retinal ganglion cell death is the only mechanism by which a particular physiological pathway may manifest dysfunction and result in psychophysical defects. Reliable conclusions about the effects on various cell classes can only be made when the relevant cells have been unequivocally identified on the basis of their dendritic and soma characteristics.

In an attempt to clarify these issues, Dandona *et al*¹⁵

Figure 2 Cell size distributions based on known values of the mean and standard deviation for ganglion cells comprising the P and M systems in the monkey (from Dacey²⁸). S denotes the cells involved in the blue (ON) centre system. The relative contributions to the entire ganglion cell populations have been estimated at 80% for P cells of which 8%, at most, would be S cells, and 10% for M cells. There appears to be less overlap between the S and P populations in the monkey compared with the human.

studied the anterograde labelling of retinal ganglion cells in normal and glaucomatous monkeys. Since M and P cells project almost exclusively to the magnocellular and parvocellular laminae of the lateral geniculate nucleus (LGN)^{9,31} any selective damage to these populations should manifest as a decrease in labelling at these laminae. The finding that in the glaucomatous monkeys the density of labelling was more reduced in the magnocellular layers was interpreted as a strong argument for selective loss of the M cell population. However, this result can be explained without the need to invoke mechanisms of selective cell death. The relation between the density of anterograde label in the LGN and the retinal ganglion cell population is complex. Tritiated amino acids are often used in such studies and some idea about the density of innervation of a particular part of the brain can be based on the densities of silver grain deposition.³² For a single cell, the amount of tritiated amino acid transported anterogradely to the LGN will be determined not only by the number of axons projecting to that part of the LGN but also by the area and the density of the terminal axonal arborisation.^{32,33} Anterograde tracing studies have provided valuable information about terminal arbors but can give quite misleading ideas about the parent cell population.³⁴ For example, the terminal area of a retinal ganglion cell axon may undergo shrinkage and retraction before cell death resulting in a

Figure 3 Cell size distributions based on known values of the mean and standard deviation for ganglion cells comprising the P and M systems in the human (from Dacey²⁸). S denotes the cells involved in the blue (ON) centre system. These distributions do not reflect possible skew in any of the cell populations. The size of each distribution reflects the contribution made by each cell type to the entire retinal ganglion cell population. Thus P cells would comprise approximately 80% of the population, M cells 10%. S cells would comprise, at most 6% of the P population. Note the considerable overlap between all cell class size distributions.

decrease in anterograde labelling in the geniculate laminae. Although the affected geniculate laminae would be less densely labelled than unaffected areas, it does not necessarily follow (at least in the early stages of glaucoma) that this reflects greater cell death among the cells giving rise to those axons. Consideration of the probable innervation densities of the primate LGN suggests possible alternative interpretation of anterograde labelling data. The ratio of P to M ganglion cells in the primate retina is approximately 8:1 compared with 4:1 for that of the P to M laminar volumes.^{35,36} Not surprisingly, this disparity is reflected in the different terminal arborisation patterns for the two ganglion cell classes.³¹ P cell terminal (axonal) fields are cylinder-like with an average width of 40 μm whereas M terminal field are much larger and more spherical with mean diameters of 125 μm . If all arbors in the LGN underwent the same percentage shrinkage then, in absolute terms, the effect would be greater for the projection to the magnocellular laminae generating a disparity in the density of innervation of the M and P laminae.

Recent anatomical studies of the human LGN also suggest that cell death may be selective.³⁷ Postmortem analysis of cell densities in the geniculate laminae of glaucomatous patients has revealed a significantly lower cell density in the magnocellular laminae, but not parvocellular laminae, when compared with normal subjects. The authors conclude that by comparison with the parvocellular laminae the cells in the magnocellular layers have been selectively lost in glaucoma. Unfortunately, such conclusions are flawed when made without reference to the volume of the remaining laminae. Anterograde degeneration certainly results in cell loss in the geniculate nucleus but it can also cause a reduction in laminar volumes. Quantitative analysis in the adult macaque monkey following enucleation^{35,38} has shown that shrinkage can occur in denervated laminae to a greater extent than cell death with a consequent *increase* in cell density. The lower density in the magnocellular lamina of the glaucomatous LGN is the result of cell death but the statement that this is selective relies on the evidence that less cell death has occurred in the parvocellular laminae. At first glance, the lack of any change in the parvocellular laminae seems to support this view. However, it is also what would have been expected if laminar shrinkage had kept pace with cell death, thereby maintaining a 'normal' cell density. Indeed, it is difficult to believe that the unchanged cell density in the parvocellular layer reflects a relatively undamaged parvocellular cell population in view of the marked glaucomatous damage in some of the subjects studied. Once again, the interpretation of apparently straightforward anatomical data is fraught with difficulties.

If we are to understand the pattern of ganglion cell death in glaucoma, unequivocal identification and characterisation of cell populations must be achieved. In the monkey glaucoma model where retinal ganglion cells have been labelled with horseradish peroxidase (HRP) injected into the optic tract and LGN, preliminary reports suggest that selective ganglion cell death does not occur.³⁹ However, any interpretation of these data must reflect the fact that the disruption of axonal transport mechanisms in glaucoma may introduce sampling bias. If, for example, transport mechanisms in larger cells were selectively affected then this might, artefactually, suggest greater death among this cell population. With regard to cell type, a promising approach has been to address some property such as neurofibrillar structure that appears to correlate with cell type.⁴⁰ Recent work in which neurofilament proteins were labelled immunologically suggests that these cells may be selectively vulnerable in the monkey model of glaucoma.⁴¹ However, the correlation between neurofibrillar staining and cell type is somewhat indirect and it should be noted that non-M cell types also stain positively for neurofibrils (though less dramatically⁴⁰). Work in other species has to be compared with caution with the macaque

monkey and human models. In the cat, where cells belonging to the larger α ganglion cell class are easily distinguished on the basis of HRP staining⁴² the smaller (β) cells appear to be more susceptible to raised intraocular pressure.⁴³ α Cells were relatively well preserved, even in areas of severe cell loss and, significantly, underwent cell soma and dendritic tree shrinkage. However, it should be noted that the cells in this study were subjected to relatively short periods of extremely high intraocular pressure and this may result in changes that are unrepresentative of those occurring in chronic glaucoma. Interestingly, direct external pressure to the intraorbital optic nerve in the cat results in selective degeneration of the larger Y type axons.⁴⁴

In spite of these difficulties with the interpretation of the anatomical evidence for selective cell death, many psychophysical studies have produced results that seem to be in its favour. For example, in the monkey model, low spatial frequency losses can be demonstrated using the pattern electroretinograph and visual evoked potentials⁴⁵ and, in the human, motion detection thresholds are raised.^{16,17} These deficits are to be expected if the M pathway is affected early in glaucoma. However, the conclusion that they are selective implies that, at a given stage in the disease process the properties of the P cells are affected to a lesser extent. The demonstration that pathways are differentially affected requires the employment of tests that have comparable specificity and sensitivity for the properties of the P and M pathways. This problem is epitomised in the paper by Silverman *et al.*,¹⁶ where the M pathway function was measured with a random dot display and the P pathway function using the Pelli-Robson chart; it is not at all clear whether these techniques allow an unbiased assessment of the relative damage to the M and P pathways.¹¹ If the two pathways are assessed using the common criterion of spatial frequency⁴⁶ 80% of patients have high spatial frequency attenuation (with 60% of these having attenuation also at the lower spatial frequencies). High spatial frequency loss tended to be seen in those cases with mild visual field loss whereas the lower spatial frequencies are involved in more advanced disease (see also Sample *et al.*⁴⁷). The results from a number of recent studies that short wavelength (chromatic) sensitivity is decreased early in glaucoma and ocular hypertension⁴⁸ undermine the strength of the argument for selective visual function loss early in glaucoma. Chromatic selectivity is a property of the parvocellular pathway^{49,50} with the magnocellular system having a broad band response to light of differing wavelengths. The implication therefore is that the parvocellular system sustains damage early in glaucoma. It does not follow that this damage is any greater than that in the magnocellular system. Indeed, electrophysiological studies in glaucomatous monkeys are consistent with similar damage to the two pathways since the reduction in the frequency with which M and P cells are encountered is reduced to the same extent in their respective laminae.⁵¹ Results from single tests can be misleading. When ocular hypertensive or early glaucoma patients are followed it has been found that defects in both blue/yellow and temporal modulation occurred concurrently and preceded defects in white on white perimetry,⁵² arguing against cell loss that is specific for cells of either the M or P pathway.

Given the problems with the interpretation of the anatomical data, the concept of selective cell death requires clarification. Any doubts about selective cell death with regard to cell type can only be laid to rest on the basis of anatomical studies in which ganglion cells have been unequivocally identified and classified using retrograde tracers (see Zamber *et al.*³⁹) a technique that could also dispel any theories regarding cell shrinkage. It must be stressed that the current data are consistent with both the non-selective and selective models and I have not attempted to discuss which may be the more

important. It is conceivable that both patterns of cell loss may occur in different parts of the retina at different times.⁵³ The precise mechanisms remain unclear and further work is required if we are to understand the mode of ganglion cell death in glaucoma and develop tests that are sensitive and specific for the earliest changes in this disease.

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Commentary

The review by Morgan considers evidence from our laboratory that chronic human glaucoma and experimental glaucoma in monkeys cause a more rapid loss of larger retinal ganglion cells. This work has now been confirmed in chronic glaucoma tissues by three other research groups (his references 18, 37, 41). The evidence includes measurements of ganglion cell body size by three different histological techniques, measures of axon diameter, and quantitative patterns of axonal transport and trans-synaptic effects in the lateral geniculate body (LGN). Morgan suggests alternative explanations for our data. However, each of the possible explanations that he considers have been adequately discussed in our previous publications. I am grateful to the editor of the *British Journal of Ophthalmology* for allowing me to comment.

Morgan invokes cell shrinkage to explain selective large cell loss, pointing out that we considered this 'as a mechanism to account for the cell size distributions'. We did discuss but dismissed shrinkage as an explanation, since it is an implausible event that does not fit the data. Only with a contrived match of shrinkage in *all* ganglion cells to cell loss is it possible to model our data to simulate no selective effect. In addition, the amount of putative shrinkage has to differ with various degrees in cell loss in exactly the correct proportion, or the result does not match observed data. Why would the amount of shrinkage change as percentage cell loss increases? Furthermore, we have recently reported that cell death by apoptosis occurs in glaucomatous ganglion cells (his reference 25), but this is 'unlikely to generate large populations of shrinking cells' – in fact, the number of cells dying at any point in time by apoptosis is very small, since it occurs so rapidly. Hence, shrinkage as a confounder is not only 'entirely theoretical', it is unsupported by data on primary ganglion cell degeneration.

Morgan suggests that we confused amacrine cells for ganglion cells, citing 'subjective judgment'. I have studied inner retinal anatomy for over 20 years and this research included quantitative studies of cell size and cytology in masked tissues of glaucoma, optic nerve transection, and normal specimens. While no experiment is perfectly objective, our reported ganglion cell body data do not include cells of the same size or morphology as amacrine cells that normally

reside in the ganglion cell layer. The numerical data to demonstrate this have been published.

Morgan has interpreted our LGN data to imply that anterograde axonal transport is a poor indicator of cell body health. We found a selectively greater, statistically significant decrease in axonal transport to magnocellular layers compared with that to parvocellular layers. His shrinkage argument cannot explain the data, since we controlled the observations by comparing LGN transport from the experimental glaucoma eye with those from control and acute glaucoma eyes. His presentation therefore, on the ratio of cells, laminar volumes, and arborisation patterns cannot explain the data. Either there was less transport by larger cells or the terminal arborisations of large cells were drastically altered compared with smaller cells. Both conclusions are compatible with a selectively greater susceptibility to chronic experimental glaucoma among the magnocellular projecting cells. Furthermore, the topography of transport decrease shows it to be greatest in LGN areas corresponding with the mid retina, sparing the macular and nasal peripheral projection zones. The damage pattern is exactly what would be expected from the loss of ganglion cells in the upper and lower optic nerve (from the arcuate retina) and supports the relevance of anterograde transport decrease to glaucomatous damage.

Among the possible explanations for our findings and those of others, it is most likely that larger ganglion cells are selectively susceptible to injury. Morgan seems to suggest that other possibilities have not been considered. This is far from the case, as can be seen from reading the discussion sections of our work.

Our group and others continue to study the details of glaucoma damage to ganglion cells, particularly effects on the many cells that are not M cells. M cells are thought to comprise only 10% of all primate ganglion cells. If glaucoma causes loss of one third or one half of the optic nerve, the clinical findings are still mild. But, many of the cells that are dead at this stage must be non-M types. We have recently reported central ganglion cell damage in glaucoma that may be relevant to blue-yellow sensitivity loss.

As to the implications of larger ganglion cell loss for