PERSPECTIVE

Glaucoma: squaring the psychophysics and neurobiology

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Advances in our understanding of the pathophysiology of retinal ganglion cell death in glaucoma are providing important insights into the functional changes occurring in retinal ganglion cells in the early stages of the disease. These exciting new findings may help us develop psychophysical tests to monitor early retinal ganglion cell damage, possibly before neurons are committed to the process of cell death.

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Primary open angle glaucoma (POAG) is diagnosed by examining the optic disc and visual field and measuring intraocular pressure (IOP). The lack of sensitivity of standard automated perimetry (SAP) in the early detection of POAG has triggered intensive research into the evaluation of alternative psychophysical techniques.

Much of the basic science and clinical research that provides insights into visual processing is psychophysical in nature. Examples of psychophysical tests in routine clinical use include visual acuity, refraction, visual fields, and colour vision testing. Therefore, it is essential for the clinician to have a basic understanding of psychophysical theory and methodology in order to perform these tests and interpret the results meaningfully.

The development of psychophysical tests for early diagnosis of glaucoma is based on our current understanding of functional channels in vision. Primate retinal ganglion cells can be classified according to the layer of projection in the dorsal lateral geniculate nucleus (LGNd). Ganglion cells are classified as M cells if they project to the magnocellular layers and P cells if they project to the parvocellular layers. They are both morphologically and physiologically distinct. Accordingly, there is much anatomical and physiological evidence to support the idea of independent primary visual pathways for the processing of visual information.[1–3] M cells respond to high temporal and low spatial frequencies, high luminance contrast, and movement. P cells respond best to high spatial and low temporal frequencies and are colour opponent.[4] This functional dichotomy has led to the development of psychophysical tests that isolate the M or P cell channels and several studies have been performed to establish which tests are superior in terms of early glaucoma diagnosis.

FUNCTIONAL PROPERTIES OF RETINAL GANGLION CELLS

The retinal ganglion cell (RGC) is the output neuron of the retina and there are 0.7–1.3 million RGCs in each human retina.[5] The response properties of RGCs result from transformations of photoreceptor responses and are mediated by interactions in the outer and inner plexiform layers of the retina. The pattern of inputs from all the cell types in the different layers of the retina defines the receptive field of the RGC, which is the area of retina monitored by the RGC. Spatial summation refers to the ability of RGCs to pool excitatory effects over a certain area.

Most retinal ganglion cells exhibit a circular centre surround organisation of the receptive field.[6] For example, ON-centre cells respond optimally when light falls on the centre of the receptive field and are inhibited when stimulated by light in the surround. Similarly, OFF-centre cells are stimulated by light in the surround and inhibited by light in the centre. Therefore, the receptive fields of RGCs are organised to respond to differences between illumination of the centre and surround—that is, contrast.[7] M and P cells comprise both ON and OFF-centre cells. P cells have smaller receptive fields than M cells, and whereas M cells are not selective for wavelength, P cells exhibit colour opponency. RGC morphological and functional diversity has been described thoroughly in the literature.[8]

Despite the different characteristics of M and P cells, it has not been possible to isolate fully M cells from P cells using psychophysical tests. For example, the role of P cells in spatial vision, form perception, and acuity has been questioned,[9] but the general consensus is that P cells are responsible for processing chromatic information.[10] Furthermore, investigators have found little or no difference in the spatial or resolving power of M and P cells regardless of retinal eccentricity.[11] In human peripheral vision, when isolating neural subpopulations of the retina by motion aliasing (the false representation of the stimulus by undersampling), it is the P cell system which is isolated, confirmation that P cells have an important role in motion perception.[12] Similarly, P cells convey information about the *motion* of moderate and high spatial frequency targets.[13 14] This imperfect functional dichotomy has obvious implications for the conclusions we make regarding the results of psychophysical tests, which are described as being exclusively selective for a particular pathway.

Abbreviations: HRP, horseradish peroxidase; IOP, intraocular pressure; LGN, lateral geniculate nucleus; OHT, ocular hypertension; POAG, primary open angle glaucoma; RGC, retinal ganglion cell; SAP, standard automated perimetry

SELECTIVE CELL LOSS

The hypothesis that selective damage may occur in glaucoma is important. If one of these pathways is damaged preferentially in the early stages of the disease, psychophysical tests could be used to isolate selectively the function of that pathway and provide the basis for earlier detection of disease. Selective cell loss in glaucoma has been supported by histological, electrophysiological, and psychophysical studies.

There has been particular interest in isolating M cell function in early glaucoma diagnosis since there has been histological evidence for earlier damage to large optic nerve fibres, most of which are axons of M cells.[15 16] However, this finding has been challenged. It is not the purpose of this article to discuss every test which has been studied, rather we wish to point out that a number of studies isolating M and P cell function suggest that both cell types are damaged in early glaucoma.

PSYCHOPHYSICAL TESTS OF M AND P CELL CHANNELS

Histological evidence of preferential damage to large optic nerve fibres provided the impetus for the evaluation of psychophysical tests aimed at detecting M cell dysfunction.[17–31] Although these tests demonstrated deficits in motion perception in POAG and ocular hypertension (OHT) patients, deficits were also found using tests that are designed to isolate P cell function.[32–38]

A more rational approach would be to evaluate comparable M and P cell tests in the same patient group. Several investigators have compared a variety of M cell and P cell tests on the same group of patients.[39–45] These studies have demonstrated equivalence between M and P cell tests in detecting early glaucoma damage. Hence, the results of these studies are not consistent with selective cell damage. Therefore, although histological evidence has been interpreted as providing support for selective loss of magnocellular RGCs, this has not been the consistent result with a range of psychophysical tests.[39 40]

Awareness of the complexity of the retinogeniculate pathway and a greater understanding of RGC functional properties are essential for the development and interpretation of psychophysical tests. For example, the parietal cortex receives most of its input from M cells and is involved in redirecting visual attention, object localisation, and the control of pursuit eye movements.[46–48] However, the use of these psychophysical stimuli for isolating M cell function remains highly speculative.

CELL SHRINKAGE

The concept that the relation between cell size and cell type is preserved in glaucoma has been questioned by recent studies. Detailed histological analysis of retinal ganglion cell morphology following intracellular injection of fluorescent dyes has shown that the cell soma, dendritic tree, and axon in both M and P cells can shrink before the onset of cell death.[49] A subsequent study, analysing a large population of retinal ganglion cells labelled by retrograde transport of neuronal tracer, horseradish peroxidase (HRP), in the primate glaucoma model has shown a similar level of cell shrinkage (16–20%) for these cell types,[50] which would be sufficient to generate apparent selective cell death of larger retinal ganglion cells.[51] The same study was also unable to demonstrate a significant reduction in the ratio of M to P cell types in retinal areas with cell loss that would be expected if the magnocellular pathway were selectively damaged. Therefore, these studies did not provide clear evidence for selective cell loss in glaucoma. The idea of cell shrinkage and functional "predeath" is novel to glaucoma because it may represent a window of opportunity for intervention, possibly with neuroprotective therapies. However, the basis for this hypothesis remains tentative since studies performed using the primate ocular hypertension model might have limited relevance to chronic human glaucoma.

The notion of non-selective cell damage does not undermine selective testing of those pathways with reduced redundancy.[40] For example, bistratified RGCs comprise 1% of RGCs in the central retina and mediate the blue-yellow signal.[52] Reduced redundancy in this population of cells may explain the ability of blue on yellow perimetry to detect visual field loss earlier than conventional perimetry.[39 42] Even if there is cell shrinkage, if M cells are affected disproportionately, then functional deficits in that population of cells may be detected earlier using appropriate stimuli.

THE LATERAL GENICULATE NUCLEUS IN GLAUCOMA

There have been few studies of cellular changes in the primate lateral geniculate nucleus (LGN) with glaucoma. Using human necropsy material, M cell density was significantly reduced compared to P cell density and interpreted as preferential loss of M cells.[53] In addition to some technical limitations, this study did not address the relation between LGN volume, and cell number in these chronic glaucoma cases.[54] In a previous enucleation study, there was substantial neuronal loss in both the parvocellular and magnocellular laminae that received input from the enucleated eye. However, this was represented by a loss of *volume* in the parvocellular laminae—that is, the laminae became thinner, without a change in cell density.[55] Both cell density and the volume of the parvocellular laminae should be determined when comparing cell loss between the different laminae. This was demonstrated in a more acute model in rhesus monkeys whereby cell density was increased in M and P cell layers (31% versus 59%). The increased P cell density was most likely the result of a greater reduction in laminar volume compared to cell loss. Conversely, the more modest M cell density increase was the result of a finer balance between reduction in laminar volume and cell loss.[56]

In Weber's study of primate LGN, M cell loss was significantly greater than P cell loss (38% versus 10%). Using parvalbumin to exclusively stain LGN relay neurons, other investigators have not found a differential reduction in laminar density in the primate glaucoma model.[57] There is also recent evidence for an equivalent reduction in metabolism in P and M cell layers of the LGN and primary visual cortex in glaucoma.[58]

Before the useful interpretation of all these studies, it is important to appreciate the inputs and local circuitry of the LGN. RGCs represent only 5–10% of the input to the LGN[59]; other inputs from the brainstem and cortex can help preserve LGN function. Therefore, the changes in the M and P cell layers may reflect this as much as the result of any selective damage to axons. In the cat, LGN interneurons are directly innervated by X cell axons and are firmly embedded in the X pathway (X cells are the smaller cells of the retinogeniculate pathway and exhibit linear spatial summation).[60] In the same study there was no evidence for interneurons in the Y cell (the larger cell type exhibiting non-linear spatial summation) pathway. This close association might aid the survival of the smaller X cells, either by providing some sort of local neuroprotection, or by preventing the cells from becoming excitotoxic in the degenerating nucleus. It is also possible that the smaller cells have lower thresholds to activation than Y cells, and therefore can maintain some basal level of activity longer than Y cells. This might be reflected in differences in the distributions of synaptic inputs to the two classes of cells. The more compact nature of the P cell dendritic fields might also mean that more synapses remain capable of influencing the

soma as distal dendritic processes degenerate (A J Weber, personal communication).

FUTURE APPROACHES

All the psychophysical tests that have been used are actually testing a *pathway* from retina to cortex. For example, the familiar contrast sensitivity function is representative of the spatial resolving power of the visual system as a whole. If we want to study the actual RGC modulation transfer function in isolation we would have to devise a way to eliminate noise from the other higher components of the retinocortical pathway (J Rovamo, personal communication). This is a difficult task, but highlights the problems involved if testing is to be truly accurate and if the pure response of RGCs is to be recorded.

Recent studies have demonstrated shrunken, distorted, and "sick looking" RGCs in the primate glaucoma model.[49 50] Therefore, we have to consider the following points:

- What would be the physiological behaviour of a dysfunctional cell?
- What receptive field properties could be tested to identify such cells?

Rather than attempting to detect the loss of cells, we should be trying to detect novel physiological responses of altered cells. If changes are occurring in cell soma and dendritic field size then subtle deficits in the contrast sensitivity function should also be apparent in areas without defects in the retinal nerve fibre layer. The reversibility of such deficits should be examined, after the application of IOP lowering drops or neuroprotective agents,[61–63] in cases of early POAG or in glaucoma suspects. If the changes were reversible, it would imply that these represent the signals from altered cells, which are still amenable to neuroprotective rescue.

CONCLUDING REMARKS

Recent detailed histological studies have paved the way for a better understanding of cellular changes in early glaucoma. Rather than designing psychophysical tests that are sensitive to changes in cell population, perhaps we should be investigating the physiological changes that occur in surviving and dysfunctional cells. It is possible that some of the recent psychophysical techniques are responding to dysfunction in cells rather than the total loss of function. The test-retest variability found with all perimetry may reflect this fluctuating physiological state.

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