Selective Effects of Experimental Glaucoma on Axonal Transport by Retinal Ganglion Cells to the Dorsal Lateral Geniculate Nucleus

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Rapid-phase axonal transport to the dorsal lateral geniculate nucleus (dLGN) was determined autoradiographically in seven macaque monkey eyes with chronic intraocular pressure (IOP) elevation, in four eyes with an acute IOP elevation, and in three eyes with normal IOP. The monkeys with chronic IOP elevation showed a greater decrease in radioactive labeling of the magnocellular layers of the dLGN than the parvocellular layers by qualitative examination. Grain counts in selected specimens confirmed that transport to the magnocellular layers was less than to the parvocellular layers in monkeys with chronic IOP elevation. This selectivity was present in mildly damaged specimens and increased with greater ganglion cell loss. In monkeys with acute IOP elevation, qualitative evaluation suggested no consistent difference in transport among the dLGN layers; one animal in this group had less transport to the parvocellular than to the magnocellular layers by grain counts. Starting in early stages of the disease, chronic experimental glaucoma causes preferential damage to the ganglion cells that project to the magnocellular layers of the dLGN. Invest Ophthalmol Vis Sci 32:1593-1599, 1991

A significant minority of the retinal ganglion cells may be damaged in patients with glaucoma before visual field loss is detected with the available manual1 or automated2 perimetry techniques. Studies of human3 and monkey4 optic nerve suggest that the larger diameter axons, presumably originating from retinal ganglion cells with larger somatic size, may be lost earlier in chronic glaucoma. In human eyes with glaucoma, there are fewer remaining large ganglion cells in the damaged retinal areas.2,3 Large ganglion cells (M cells) have functional characteristics different from those of small cells (P cells).6-18 If a certain ganglion cell type is damaged preferentially, psychophysical tests that evaluate its function might detect damage in chronic glaucoma earlier than with current techniques.

Large retinal ganglion cells project to the magnocellular layers, and smaller cells predominantly to the parvocellular layers, of the monkey dorsal lateral geniculate nucleus (dLGN).7-13 We exploited this anatomic segregation of the functional pathways to investigate the selectivity of glaucomatous damage. Acute and chronic intraocular pressure (IOP) elevations were produced in monkey eyes. Rapid-phase axonal transport from the ganglion cells to the dLGN was studied by autoradiography after intravitreal injection of 3H-proline.

Materials and Methods

This investigation adhered to the principles of the ARVO Resolution on the Use of Animals in Research and was approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University School of Medicine. Elevated IOP was produced by argon laser treatment of the trabecular meshwork in one eye each of seven macaque monkeys (Macaca fascicularis).19 The IOPs of 35-48 mm Hg were sustained in the laser-treated eyes for 2-44 weeks. The optic nerve head and retinal nerve fiber layer of these eyes were photographed serially to estimate the extent of damage due to elevated IOP. This monkey model leads to changes in the optic nerve head, nerve fiber layer, and fiber diameter distribution similar to those observed in human eyes with chronic glaucoma.3,4,20-23
To study the rapid-phase axonal transport from the retinal ganglion cells to the dLGN, we injected 400 
μCi of \(^3\)H-proline in 0.1 ml of saline intravitreally through the pars plana into the seven eyes with 
chronic IOP elevation and into one normal eye each of three control monkeys with average IOP of 15 mm Hg. These animals were killed 24 hr after the injection. As a second control, one normal eye each in four 
additional monkeys had acute IOP elevations (40, 55, 60, and 100 mm Hg) produced by a fluid reservoir 
connected to a needle in the anterior chamber for 12 hr after the \(^3\)H-proline injection. Due to the need for 
general anesthesia, acute IOP elevation was not maintained for 24 hr.

The animals were killed by exsanguination under deep anesthesia, then perfused with a mixture of 
phosphate-buffered 5% glutaraldehyde and 4% paraformaldehyde retrograde through the aorta. Frozen 
coronal sections of the brain were coated with Kodak NTB2 emulsion (Rochester, NY), exposed for 4 
weeks, and developed using standard methods. A sample from each optic nerve from 1–3 mm behind the 
eye was postfixed in 1% osmium tetroxide and embedded as a cross section in epoxy resin. The total 
number of remaining optic nerve fibers was estimated by measurement of the area of remaining neural 
tissue and by random sampling of the number of fibers per unit area in this neural tissue. The 
estimated fiber complement of the glaucomatous eye was compared with that of the normal fellow eye to determine the percent of axon loss.

Initially, qualitative examinations were done on the coro
nal section of the dLGN was divided by a central me-
ridian into two halves, the lateroinferior half corre-
sponding to the superior visual field, and the medio-
superior half corresponding to the inferior visual 
field. Each half of a dLGN layer was subdivided fur-
ther into an apical and a basal part. In each of the four 
parts of the magnocellular layer and two parvocellular 
layers receiving input from the eye injected with \(^3\)H-
proline, the total number of grains was counted in ten 
randomly selected rectangular areas (each 100 \(\mu m^2\)) under a 100× oil-immersion objective. The total num-
ber of grains was also counted in ten similar areas in 
one of the layers not receiving input from the eye in-
jected with \(^3\)H-proline as a measurement of back-
ground grain density. This number was subtracted 
from the former to estimate the net axonal transport.

There could be variations in the volume of axonal 
transport between animals for the different layers of 
the dLGN and differences between the different 
dLGNs or sections due to technical variability. To 
minimize the influence of these variables, in each se-
lected section we also compared the two halves of 
each labeled layer with each other. In the animals with 
much more damage in the superior or inferior half of the 
retina, and hence with expected asymmetry in trans-
port to the dLGN, this approach would allow the rela-
tive effect on the transport to the magnocellular and 
parvocellular layers to be evaluated with the least 
number of confounding variables.

The reproducibility of the method of counting 
grains was calculated from five complete repeated 
measurements in two sections, one with high and one 
with low grain density. For both densities, these mea-
surements established that the two halves of a layer in 
the same dLGN section would have to differ by more 
than 25% for the difference to be considered signifi-
cant at the 95% confidence level. This was used as a 
statistical test for significance in these data.

**Results**

In the masked qualitative evaluation, the dLGNs of 
the normal-IOP control monkeys showed equal or 
slightly heavier labeling of the magnocellular com-
pared with the parvocellular layers. In the monkeys 
with chronic IOP elevation, no differences from nor-
mal were detected in the two animals with the least 
damage in the optic nerve (Table 1). In the remaining 
 specimens, the labeling was decreased relatively more 
for the magnocellular than for the parvocellular 
layers. With an increasing magnitude of neural loss, 
the decrease in labeling of all dLGN layers progressed 
accordingly but always with a relatively greater de-
crease for the magnocellular layers. The topographic 
distribution of the radioactive labeling in the dLGNs 
of the monkeys with chronic IOP elevation showed
that the posterior coronal sections of the dLGN, corresponding to the foveal region, always had relatively more labeling. At the same eccentricity, the decrease in labeling was relatively more for the temporal retina. The peripheral nasal retina was relatively spared even in the monkeys with advanced optic nerve damage.

The three monkeys with chronic IOP elevation that were selected for grain counts in their dLGNs had lost an estimated 20%, 50%, and 90% of their neural tissue, respectively. In the eye with 20% optic nerve damage, the neural loss was more in the superior half (25%) than in the inferior half (15%). In the eye with 50% optic nerve damage, it was more in the inferior half (60%) than in the superior half (40%).

Grain density in all labeled layers of the dLGNs receiving input from the eyes with chronic IOP elevation was less than the density in the normal IOP control specimen (Table 2). The mean grain density progressively decreased with increasing damage to the optic nerve due to chronic IOP elevation (r = 0.96, P = 0.04, by linear regression). In the normal-IOP control specimen, the mean grain density in the magnocellular layer was 105% of that in the parvocellular layers (Table 3). By contrast, all three chronic IOP-elevation specimens had lower mean grain density in the magnocellular than in the parvocellular layers (Table 3). We compared the decrease in magnocellular labeling to the parvocellular decrease by calculating a ratio of their percent declines from their respective normal values. In the earliest damage specimen, with 20% neural loss, a larger decline in magnocellular transport was evident. This selectivity increased with advancing neural loss progressing from a magnocellular/parvocellular ratio of 0.90 to a ratio of 0.62 in the animal with 90% neural loss (Table 3).

A comparison between the two halves of a layer showed further evidence for selectively greater loss of transport to the magnocellular layers in the specimen with 20% optic nerve damage. The section corresponding to the visual field between 15–25° showed significantly lower grain density (95% confidence level) in the half of the magnocellular layer corresponding to the more damaged superior half of the optic nerve (Fig. 1). The ratio of superior to inferior grain counts for the two magnocellular sampling sites was 0.62 (apical) and 0.48 (basal), averaging 0.55. This exceeded the confidence limits established by variability measurements (P < 0.05). The ratio for similar sample sites in the parvocellular layers was 0.89, 0.98, and 1.11 (average, 0.99) and was not significantly different. The other two dLGN sections corresponding to the more central regions of the retina did not show any difference between the two halves of a layer that exceeded the variability of the counting method (Fig. 1). The finding was similar for the dLGN receiving input from the eye with 50% optic nerve damage in the section corresponding to the visual field between 15–25°. In that area, the grain density in the half of the magnocellular layer corresponding to the more damaged inferior half of the optic nerve was 60% less than in its other half (110 and 272 grains per 1000 μm², respectively). In this section, a similar difference was also found between the two halves of the outer parvocellular layer (51%; 162 and 332 grains per 1000 μm², respectively), although this difference was less than that between the two halves of the magnocellular layer.

### Table 1. Qualitative axonal transport pattern in dLGN

<table>
<thead>
<tr>
<th>IOP (mm Hg)</th>
<th>Duration of IOP elevation</th>
<th>Neural loss: optic nerve*</th>
<th>dLGN labeling: † magnocellular (M) vs parvocellular (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IOP controls (3)</td>
<td>15</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Acute IOP elevation</td>
<td>40</td>
<td>12 hr</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>12 hr</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>60††</td>
<td>12 hr</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12 hr</td>
<td>None</td>
</tr>
<tr>
<td>Chronic IOP elevation</td>
<td>35††</td>
<td>20 weeks</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2 weeks</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>16 weeks</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>35††</td>
<td>20 weeks</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>16 weeks</td>
<td>55%</td>
</tr>
<tr>
<td></td>
<td>46††</td>
<td>44 weeks</td>
<td>90%</td>
</tr>
</tbody>
</table>

* Compared with the normal fellow optic nerve.
† Compared with the normal IOP controls, overall labeling was decreased in the animals with acute and chronic IOP elevation.
†† Used for grain counting. One normal IOP control was also used for grain counting.

### Table 2. Overall grain density in the dLGN layers

<table>
<thead>
<tr>
<th></th>
<th>Grain density*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IOP control</td>
<td>None</td>
</tr>
<tr>
<td>Acute IOP elevation</td>
<td>None</td>
</tr>
<tr>
<td>Chronic IOP elevation</td>
<td>20% neural loss††</td>
</tr>
<tr>
<td></td>
<td>50% neural loss</td>
</tr>
<tr>
<td></td>
<td>90% neural loss</td>
</tr>
</tbody>
</table>

* Number of grains per 1000 μm² minus background.
† Percent less than normal IOP control grain density.
†† Estimate of neural loss compared with normal fellow optic nerve.

The range of values in each experimental group does not overlap the normal range.

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In the normal-IOP control dLGN, there was no difference in the grain density (at the 95% confidence level) between the two halves of the layers in any of the three sections. Since the overall grain density was so markedly diminished in the dLGN receiving input from the eye with 90% optic nerve damage (Tables 2 and 3), a comparison between the two halves of the layers would not give any meaningful information.

Qualitative evaluation of the dLGN labeling in the four animals with acute IOP elevations indicated decreased labeling compared with the normal IOP controls. The dLGNs of two of these animals suggested some focal loss of labeling, one a decrease in both magnocellular and parvocellular layers and the other, a decrease only in one parvocellular layer. In the dLGN of one animal with an acute IOP elevation of 60 mm Hg, overall labeling was quantified and found to be decreased by 72% compared with the normal-IOP control monkey (Table 2). Contrary to the result in monkeys with chronic IOP elevation, grain density in this animal was less in the parvocellular than in the magnocellular layers (Table 3), although none of the differences between halves of layers or among layers exceeded the 95% confidence limits.

Discussion

Two parallel pathways from the retina to the central visual centers have been characterized in primates.6–18 The M-pathway uses ganglion cells with larger cell bodies and larger axon diameters projecting to the magnocellular layers of the dLGN, and the P-pathway uses ganglion cells with smaller cell bodies and smaller axon diameters projecting predominantly to the parvocellular layers of the dLGN.7–13 The M ganglion cells have faster conduction velocities, less selective responses to color, greater sensitivity to luminance contrast, and are probably distributed uniformly across the retina.14–18 The P-pathway ganglion cells have slower conduction velocities, more selective responses to chromatic stimuli, and are proportionately more concentrated in the foveal region.15–18 About 10% of the ganglion cells in the primate retina are M cells, and most of the remaining are P cells.12

This study supports other evidence that larger ganglion cells and their axons (presumably M cells) are affected earlier as a result of chronic IOP elevation.2–4 Rapid-phase axonal transport from the M-type ganglion cells to the magnocellular layers of the dLGN was decreased selectively in chronic experimental glaucoma. Even in an animal with 20% neural loss in the affected optic nerve, this selectivity was present. In human eyes with chronic glaucoma3 and in monkey eyes with chronic experimental glaucoma,4 the optic nerve fibers with larger diameters were found to be lost faster than those with smaller diameters. The larger diameter ganglion cells were also found to be

Table 3. Grain density in the magnocellular and parvocellular dLGN layers

<table>
<thead>
<tr>
<th></th>
<th>Magnocellular</th>
<th>Parvocellular</th>
<th>Ratio of magno/parvo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Loss</td>
<td>Mean (range)</td>
<td>% Loss</td>
</tr>
<tr>
<td>Control</td>
<td>69%</td>
<td>922 (681–1156)</td>
<td>73%</td>
</tr>
<tr>
<td>Acute IOP elevation</td>
<td>44%</td>
<td>512 (297–622)</td>
<td>38%</td>
</tr>
<tr>
<td>Chronic IOP elevation</td>
<td>79%</td>
<td>190 (19–287)</td>
<td>71%</td>
</tr>
<tr>
<td>20% neural loss</td>
<td>95%</td>
<td>44 (7–147)</td>
<td>92%</td>
</tr>
</tbody>
</table>

Magno = Magnocellular; Parvo = parvocellular; % loss = 100 minus mean in acute elevation or chronic glaucoma divided by mean control; all percent loss figures are statistically significant and exceed the 95% confidence limit for variability; grain density = number of grains per 1000 μm², minus background counts; Ratio of magno/parvo = magnocellular mean density [glaucoma]/magnocellular mean density [control] divided by parvocellular mean density [glaucoma]/parvocellular mean density [control]; in normal IOP control, this ratio-magnocellular mean density/parvocellular mean density.
lost more rapidly in chronic human glaucoma.\textsuperscript{2} We just published data\textsuperscript{28} from four monkey retinas with chronic experimental glaucoma that show a strong preference for a selective large cell loss in midperipheral retina using a published method.\textsuperscript{2} Zambere and co-workers\textsuperscript{29} presented data from two monkeys with a different method and found no selective effect. The preponderance of evidence supports the conclusion that chronic IOP elevation causes selectively greater loss of large ganglion cells.

We found no significant differential effect of chronic IOP elevation on the magnocellular and parvocellular layers in the sections corresponding to the foveal region. We cannot determine whether this derives from a difference in the response of the M cells in this region or from our inability to detect a true difference. Our study of ganglion cell body diameter in human glaucomatous eyes also did not detect a selective cell loss in the near-foveal region.\textsuperscript{2}

Studies of functional damage have confirmed that the properties attributable to the M cells are measurably affected early in chronic human and experimental glaucoma. These include effects on the pattern electroretinogram,\textsuperscript{30-32} scotopic threshold perimetry,\textsuperscript{33,34} and temporal contrast sensitivity.\textsuperscript{35} It is hoped that this selective loss of function in the M-pathway will provide means to develop practical new tests for the detection and evaluation of early glaucomatous damage.

The superior and inferior parts of the optic nerve show more rapid atrophy than the nasal and temporal parts in chronic human glaucoma.\textsuperscript{1,3} The site of damage to the retinal ganglion cell axons in glaucoma is the lamina cribrosa of the optic nerve head at the level of the sclera.\textsuperscript{24,36} Because the superior and inferior parts of the lamina cribrosa have less connective tissue, these regions are less able to withstand the effects of elevated IOP.\textsuperscript{37-39} The superior and inferior parts of the lamina cribrosa contain axons of the ganglion cells located in the midperipheral retina\textsuperscript{40,41} and hence have a higher proportion of large axons.\textsuperscript{42} Thus, the midperipheral M ganglion cells may be lost more rapidly in chronic glaucoma because a higher proportion of their axons pass through the more susceptible parts of the lamina cribrosa. However, because larger axons are lost more rapidly even when they pass through the temporal part of the optic nerve head,\textsuperscript{3} the preferential loss of M cells and/or their axons in chronic glaucoma may be a result of both their inherent intolerance to injury and the distribution pattern of their axons in the optic nerve head.

In the animals with chronic IOP elevation, the decrease in transported radioactivity could have occurred for two reasons: either the cells were alive, but their axonal transport was blocked at the nerve head, or the cells were dead. Our acute IOP elevation controls showed no tendency to a greater decrease in magnocellular labeling. In one eye with very high IOP, grain counts suggested that the axonal transport was decreased more to the parvocellular than to the magnocellular layers. Hence, if there were a component of acute blockade of transport in our animals with chronic IOP elevation, it would have tended to negate the finding of greater decrease of transport to the magnocellular layers. Either the acute blockade was a negligible factor at the modest IOP levels of the chronic IOP-elevation animals, or selective M cell death was even greater than suggested by these transport data.

There is other evidence that acute, severe (60 mm Hg) IOP elevation selectively disrupts the function of P cells.\textsuperscript{43,44} It is possible that the P cells suffer more profound acute transport blockade but can recover, while the M cells die more rapidly from the same or less degree of block. Alternatively, features of cell function other than axonal transport obstruction may determine which cells die first. Thus, the relevance of experiments in which IOP elevation is set substantially above the levels normally seen in chronic human glaucoma or the chronic monkey model should be questioned, at least in producing information on the fine details of damage selectivity in this disorder.

Key words: glaucoma, macaque monkey, retinal ganglion cells, axonal transport, dorsal lateral geniculate nucleus, pathology, optic nerve

References


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