Atrophy of Relay Neurons in Magno- and Parvocellular Layers in the Lateral Geniculate Nucleus in Experimental Glaucoma

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PURPOSE. To determine whether in glaucoma there is atrophy of relay neurons in magnocellular and/or parvocellular lateral geniculate nucleus (LGN) layers projecting to the visual cortex and to compare the degree of neuronal atrophy in magnocellular layers with that in parvocellular layers.

METHODS. Seven cynomolgus monkeys with unilateral experimentally induced glaucoma and five control monkeys were studied. The left LGN neurons in magnocellular layer 1 and parvocellular layers 4 and 6, connected to the right glaucomatous eye were examined. Immunocytochemistry with antibody to parvalbumin was used to specifically label relay neurons connecting to the visual cortex. Neuronal cell body cross-sectional area was estimated using unbiased point-counting methodology. Experimental and control groups were compared using t-tests. Analysis of covariance (ANCOVA) tests were used to compare the percentage of decrease in mean neuronal area between layers 1, 4, and 6, as a function of percentage of optic nerve fiber loss or mean IOP. There was significant correlation between percentage of optic nerve fiber loss and mean IOP.

RESULTS. The mean cross-sectional area of relay neurons in magnocellular layer 1 and parvocellular layers 4 and 6 were significantly decreased in glaucoma compared with controls by 28%, 37%, and 45%, respectively. Neuronal area decreased in a linear fashion, with increasing optic nerve fiber loss or increasing mean IOP for layers 1, 4, and 6. The percentage of neuronal shrinkage in each of parvocellular layers 4 and 6, as a function of optic nerve fiber loss (P = 0.05; P = 0.001, respectively) or mean IOP (P = 0.046; P = 0.0008, respectively), was greater than that seen in magnocellular layer 1.

CONCLUSIONS. Relay neurons in the LGN, which project to the visual cortex, undergo significant shrinkage in glaucoma, and neurons in parvocellular layers undergo significantly more shrinkage than neurons in magnocellular layers. (Invest Ophthal Vis Sci. 2001;42:3216–3222)

Neurons in the central nervous system may undergo atrophy or cell shrinkage in response to a given insult, and persistence of the pathologic state may lead to neuronal death. Decrease in cell body size in atrophic neurons correlates with a decrease in function and is of particular interest, since it may be reversible.

When disconnected from their major afferent pathways, neurons shrink and may subsequently die. Transneuronal degeneration is a well-known process in the extension of neurodegenerative diseases. In glaucoma, severe optic nerve fiber loss has been shown to be accompanied by loss of neurons in the lateral geniculate nucleus (LGN), in keeping with the concept of transneuronal degeneration. Studies of the optic nerve in glaucoma have shown evidence of preferential loss of larger fibers. In the central visual pathways, damage to both magnocellular and parvocellular pathways has been shown in the experimental monkey model of glaucoma; however, it is controversial whether one of these pathways is preferentially affected. Whereas a recent study showed preferential loss of neurons in the LGN in magnocellular layers, another study described evidence of damage to both magnocellular and parvocellular LGN layers.

Within the LGN, there are two types of neurons: inhibitory interneurons confined to the LGN and relay neurons that proceed to synapse in the visual cortex. Most of the degenerative and compensatory changes in the LGN occur in the relay neurons rather than interneurons after total deafferentation. We have previously shown loss of relay LGN neurons in both magnocellular and parvocellular layers in glaucoma. Using parvalbumin, a selective marker for relay neurons of the LGN, the purpose of this study was to determine whether there is atrophy of relay neurons in magnocellular and/or parvocellular LGN layers projecting to the visual cortex and to compare the degree of atrophy in magnocellular relay neurons with that in parvocellular relay neurons.

METHODS

Subjects

All studies were performed according to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Seven young adult cynomolgus monkeys (Macaca fascicularis) with experimental glaucoma induced in the right eye by argon laser scarification of the trabecular meshwork (ALT) and five normal control cynomolgus monkeys were studied. Experimental glaucoma was induced in the right eye of each of seven monkeys according to the protocol described by Gaasterland and Kupfer. The survival time after laser ALT was 14 months.

In experimental glaucoma and control monkeys, intraocular pressure (IOP) measurements were performed under light sedation with a
From 0% to 100% as indicated in Table 1. In the ALT-treated right eyes, the interval between IOP measurements ranged from 5 to 39 days (mean, 26 days) over a 14-month period after laser trabeculoplasty. Table 1 summarizes the IOP values in the ALT-treated right eyes and in control monkeys. The mean IOP in the ALT-treated right eyes was significantly increased and ranged from 28.6 to 54.5 mm Hg. In all ALT-treated eyes, IOP measurements were above 21 mm Hg for at least 13 months. In control monkeys, mean IOP ranged from 14 to 21 mm Hg.

The method by which optic nerve fibers were counted in the glaucomatous right optic nerve and the control optic nerve in monkeys with experimental glaucoma has been previously described.20 Optic nerve fiber loss compared with the fellow control optic nerve ranged from 0% to 100% as indicated in Table 1.

**Tissue Processing**

Under deep general anesthesia as previously described,10 perfusion through the heart in experimental and control animals was performed with 4% paraformaldehyde, 0.1% glutaraldehyde, and 4% paraformaldehyde, respectively, in 0.1 M phosphate buffer (pH 7.4). For tissue processing and serial coronal 50-μm sectioning of the LGN, care was taken to ensure the same procedures for all monkey brains.10

**Immunocytochemistry**

The primary antibody was a monoclonal antibody against parvalbumin (clones PA-235; Sigma, St. Louis, MO). Parvalbumin, a calcium-binding protein, labels relay neurons in the LGN layers that project axons to the visual cortex.23,24 Tissue sections were immunostained, according to a previously described protocol.10

**Morphometry**

The tissue was viewed using a bright-field microscope (Reichert Jung, Vienna, Austria) with a color video camera (JVC, Yokohama, Japan), video, and computer monitors. The six layers of the LGN were easily identified on stained sections. The ventral layers 1 and 2 are magnocellular layers, whereas the remaining dorsal layers three to six are parvocellular layers. Layers 1, 4, and 6 of the left LGN are connected to the right eye (glaucomatous, in this study), whereas layers 2, 3, and 5 are connected to the left eye (nonglaucomatous). To determine whether neurons in magnocellular and/or parvocellular LGN layers connected to a glaucomatous eye are shrunken, the cross-sectional area of neurons in the left LGN layers 1, 4, and 6 were measured, and the measurements were compared with those from the left LGN layers 1, 4, and 6 in control monkeys. Retinal ganglion cells (RGCs) of the right nasal hemiretina and fovea project to the left LGN layers 1, 4, and 6 and compose approximately 50% of the right eye RGCs.27 The difference in number of surviving nerve fibers between the nasal and temporal quadrants of the right optic nerves was not statistically significant in experimental glaucoma (P > 0.05) in the monkeys examined in this study; therefore, changes observed in the left LGN layers 1, 4, and 6 are representative of changes observed in target LGN neurons. Left LGN layers 1, 4, and 6 of monkeys with a normal visual system were used as controls rather than the left LGN layers adjacent to examined layers 2, 3, and 5 or the right LGN layers 1, 4, and 6 of the laser-treated monkeys, because a significant decrease in cell size has been observed in deprived layers under monocular experimental conditions.28

Measurements were performed on three sections representative of the anterior, middle, and posterior parts of each LGN. Cross-sectional area measurements were made on parvalbumin-immunostained sections at high power using an oil immersion objective (×100, numeric aperture, 1.32), bright-field microscope, and color video camera. Immunostained neurons were visualized on the computer and video monitors. Cell body cross-sectional area measurements were made at locations determined by a random and systematic sampling procedure using a superimposed grid method.29 Computer software (Neurozoom; Human Brain Project, La Jolla, CA) enabled digital superposition of the sampling grids on the tissue. Only sample locations within the LGN layers were used for cross-sectional area measurements. The size of the sampling grid was adjusted for each layer so that there were at least 65 samples for that layer through the nucleus. To measure cross-sectional areas in an unbiased fashion, optical dissector methodology was used to assess the maximum cross-sectional area. A three-dimensional optical box was composed of x, y, and z axes of 50 × 50 × 10 μm, respectively. A 50 × 50-μm counting frame was projected onto the video monitor. By measuring only neurons completely within the frame and intersecting the upper or righthand borders, sampling bias was minimized.30 The cross-sectional area of new neurons that came into focus as the operator focused through the optical box was measured. The excursion along the focusing axis (10 μm) and the thickness of the section were measured with a micrometer (MTI2; Heidenhain, Traunreut, Germany) mounted on the microscope stage. A point-counting grid generated by the software was visualized on the computer monitor. Using the mouse, the operator marked the points located on a cell body. Each point corresponded to an area of 4 μm² for the grid used for all layers. The cross-sectional area of the cell body was calculated by multiplying the number of points on the cell body with the area corresponding to a grid point by the software. Neuronal cell body cross-sectional area per layer was estimated by calculating the average cross-sectional area for at least 67 neurons. Neuronal radius was calculated as the square root of the cross-sectional area divided by π.

**Statistical Analysis**

Student’s t test was used to compare the mean cross-sectional area means of neurons in the LGN of glaucomatous versus control monkeys. To assess atrophic changes, neuronal area percentage decrease (percentage of neuronal shrinkage) was used as the dependent variable. Neuronal area percentage decrease for each layer of each animal subject was calculated as the mean of neuronal area means in a given layer of the control group minus the mean neuronal area divided by the mean of neuronal area means for the control group. The relationships between percentage of neuronal shrinkage and percentage of optic nerve fiber loss and between percentage of neuronal shrinkage and mean IOP were assessed separately, using linear regression models.

To compare percentage of neuronal shrinkage among magnocellular layer 1 and parvocellular layers 4 and 6, two analyses of covariance (ANCOVA) were performed (GLM procedure of Statistical Analysis Software; SAS, Cary, NC) one for percentage of optic nerve fiber loss and one for mean IOP. The dependent variable was always percentage of neuronal shrinkage, and the layer was the grouping factor. Using the interaction term between percentage of neuronal shrinkage and layer, we determined whether the slopes were significantly different. The model, without the interaction term enabled assessment of whether the means differed among the three layers, and of which combinations of means, if any, were significantly different.

**RESULTS**

**Magnocellular Layer 1**

**Light Microscopy.** Parvalbumin immunoreactivity was seen in the neuronal cell body and processes in glaucoma and control magnocellular layer 1. Compared with the control (Fig. 1A), in glaucoma, neuronal cell bodies were smaller, and cell body shrinkage appeared to increase with increasing optic nerve fiber loss of 29%, 61%, and 100% (Figs. 1B, 1C, 1D, respectively).

**Cross-sectional Area Measurements.** Morphometric studies were performed in left LGN magnocellular layer 1 connected to the right optic nerve in monkeys with experimental glaucoma in the right eye (n = 7) and in control monkeys (n = 5). The percentage frequency distribution of neuronal radius in the glaucoma group shifted to the left,
compared with the control group (Fig. 2). An increase in the frequency of the smaller neurons was seen along with a corresponding decrease in the frequency of the larger neurons. Table 1 summarizes the cross-sectional area of neurons for parvocellular layer 4 in control and glaucoma groups. The mean cross-sectional area of neurons for parvocellular layer 4 in the glaucoma group compared with the control group (Fig. 2). An increase in the frequency of the smaller neurons was seen along with a corresponding decrease in the frequency of the larger neurons.

Table 1: Cross-sectional Area of LGN Parvalbumin Immunoreactive Neurons in Layers 1, 4, and 6 in Control and Glaucoma Groups

<table>
<thead>
<tr>
<th>Mean IOP (mm Hg)</th>
<th>Maximum IOP (mm Hg)</th>
<th>Cross-sectional Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Layer 1</td>
</tr>
<tr>
<td>Control</td>
<td>227.3 ± 86.5</td>
<td>250.8 ± 91.3</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>91* (61)†</td>
<td>147.8 ± 63.2</td>
</tr>
<tr>
<td></td>
<td>92 (100)</td>
<td>137.4 ± 50.5</td>
</tr>
<tr>
<td></td>
<td>93 (100)</td>
<td>135.1 ± 63.1</td>
</tr>
<tr>
<td></td>
<td>96 (29)</td>
<td>174.1 ± 63.4</td>
</tr>
<tr>
<td></td>
<td>98 (0)</td>
<td>179.6 ± 70.6</td>
</tr>
<tr>
<td></td>
<td>105 (1)</td>
<td>255.7 ± 71.1</td>
</tr>
<tr>
<td></td>
<td>106 (17)</td>
<td>169.4 ± 54.5</td>
</tr>
</tbody>
</table>

Cross-sectional area data are expressed as mean square micrometers ± SD.

*Arbitrary ID number.
†Percentage of optic nerve fiber loss.

There was a significant correlation between optic nerve fiber loss and IOP ($P < 0.05$), whereas those for layers 1 and 4 were significant at $P = 0.01$. These relationships were linear and statistically significant; those for layers 1 and 4 were significant at $P < 0.05$, whereas those for layer 6 were significant at $P < 0.01$.

**Neuronal Shrinkage as a Function of Optic Nerve Fiber Loss.** Figure 7A shows the plot of percentage decrease in mean neuronal area as a function of percentage of optic nerve fiber loss for layers 1, 4, and 6. The figure shows that for each of the three layers, percentage decrease in mean neuronal area increased with increasing optic nerve fiber loss. The linear regression lines are superimposed on the plots. The estimated regression equations for layers 1, 4, and 6 of percentage decrease in mean neuronal area were $14.6 + (0.31 \times$ percentage optic nerve fiber loss), $26.6 + (0.24 \times$ percentage optic nerve fiber loss), and $31.1 + (0.32 \times$ percentage optic nerve fiber loss), respectively. These relationships were linear and statistically significant; those for layers 1 and 4 were significant at $P > 0.05$, whereas those for layer 6 were significant at $P < 0.01$. There was a significant correlation between optic nerve fiber loss and IOP ($r = 0.94, P = 0.002$). The interaction term of the ANCOVA model indicates that the difference in slopes was not statistically significant ($P = 0.74$). However, mean neuronal shrinkage appeared to increase with increasing optic nerve fiber loss of 29%, 61%, and 100% (Figs. 5B, 5C, 5D, respectively).
**FIGURE 1.** Microphotographs of magnocellular layer 1 parvalbumin immunoreactive neurons of the left LGN from control (A) and glaucomatous monkeys with 29%, 61%, and 100% optic nerve fiber loss (B, C, and D, respectively). Note overall shrinkage of cell body in glaucomatous monkeys compared with the control and that shrinkage increases with optic nerve fiber loss. Bar, 10 μm.

**FIGURE 2.** Percentage frequency distribution histograms for layer 1 to compare the cross-sectional area measurements (radius scale, in micrometers) of the seven glaucomatous monkeys (n = 706) to the five control monkeys (n = 500). The heights of the bars indicate the frequency of the intervals.

**FIGURE 3.** Microphotographs of parvocellular layer 4 parvalbumin immunoreactive neurons of the left LGN from control (A) and glaucomatous monkeys with 29%, 61%, and 100% optic nerve fiber loss (B, C, and D, respectively). Note overall shrinkage of cell body in glaucomatous monkeys compared with the control and that shrinkage increases with optic nerve fiber loss. Bar, 10 μm.

**FIGURE 4.** Percentage frequency distribution histograms for layer 4 to compare the cross-sectional area measurements (radius scale in micrometers) of the seven glaucomatous monkeys (n = 670) to the five control monkeys (n = 613). The heights of the bars indicate the frequency of the intervals.

**FIGURE 5.** Microphotographs of parvocellular layer 6 neurons of the left LGN from control (A) and glaucomatous monkeys with 29%, 61%, and 100% optic nerve fiber loss (B, C, and D, respectively), immunostained for parvalbumin. Note overall shrinkage of cell body in glaucomatous monkeys compared with the control and that shrinkage increases with optic nerve fiber loss. Bar, 10 μm.

**FIGURE 6.** Percentage frequency distribution histograms for layer 6 to compare the cross-sectional area measurements (radius scale in micrometers) of the seven glaucomatous monkeys (n = 795) to the five control monkeys (n = 604). The heights of the bars indicate the frequency of the intervals.
shrinkage in parvocellular layer 6 was significantly greater than that observed in magnocellular layer 1 ($P = 0.001$). Mean neuronal shrinkage in parvocellular layer 4 was significantly greater than that observed in magnocellular layer 1 ($P = 0.05$), whereas the means of parvocellular layers 4 and 6 did not differ significantly ($P = 0.08$).

**Neuronal Shrinkage as a Function of Mean IOP.** Figure 7B shows the plot of percentage decrease in mean neuronal area as a function of mean IOP for layers 1, 4, and 6. The figure shows that in all three layers neuronal shrinkage increased as mean IOP increased. The linear regression lines are superimposed on the plots. The estimated regression equations for percentage decrease in mean neuronal area in layers 1, 4, and 6 were $-32.0 + (1.46 \times \text{mean IOP})$, $-5.9 + (1.04 \times \text{mean IOP})$ and $-12.1 + (1.39 \times \text{mean IOP})$, respectively. These relationships are linear and statistically significant: $P < 0.05$ for layers 1 and 4 and $P < 0.01$ for layer 6. There was a significant correlation between optic nerve fiber loss and IOP ($r = 0.94, P = 0.002$). The interaction term of the ANCOVA model indicates that the difference in slope was not statistically significant ($P = 0.658$). However, mean neuronal shrinkage in parvocellular layer 6 was significantly greater than that observed in magnocellular layer 1 ($P = 0.0008$). Mean neuronal shrinkage in parvocellular layer 4 was also significantly greater than that observed in magnocellular layer 1 ($P = 0.046$). The mean neuronal shrinkage in parvocellular layer 6 showed a trend to be greater than that observed in parvocellular layer 4 ($P = 0.075$).

**DISCUSSION**

Previous studies of normal monkey LGN have used Nissl stain that labels all neurons including relay neurons and interneurons. In the present study, parvalbumin was used for specific labeling of relay LGN neurons connecting to the visual cortex. Our cross-sectional neuronal area measurements for magno- and parvocellular layers were similar to measurements by Headon et al. 51

According to our results, relay neurons in magno- and parvocellular layers undergo atrophy in experimental glaucoma. The mean neuronal shrinkage observed in the two glaucomatous animals with complete optic nerve fiber loss in this study was similar to that observed by Matthews 6 months after enucleation: 43% vs. 54% in magnocellular layer 1, 49% vs. 66% in parvocellular layer 4, and 62% vs. 66% in parvocellular layer 6.

The present study demonstrates for the first time in the central nervous system that cell size decrease relates to the degree of deafferentation, previously suggested by findings in studies of the olfactory system and somatosensory system. The linear relationship between the degree of optic nerve damage and degree of atrophy in relay LGN neurons suggests a link between loss of retinal ganglion cells and shrinkage of their target neurons. Although significant input to the LGN from the cortex and several subcortical structures has been described, atrophic changes observed in target relay neurons appears to be directly related to the loss of connections with RGCs, the major afferent input to the LGN. In addition, the linear relationship found in this study between shrinkage of relay neurons and mean IOP is in keeping with the correlation previously noted between atrophy of Nissl-stained neurons and mean IOP. We also observed significant neuronal shrinkage in monkeys with ocular hypertension and no optic nerve fiber loss, suggesting that neuronal atrophy in the LGN may be an early event, at least partially related to elevated IOP. Statistical determination of which of these parameters (i.e., optic nerve fiber loss or IOP) is more important in atrophy was not possible in this study, because of the large sample size needed for this type of analysis.

Our results also suggest that atrophy of relay neurons in parvocellular layers is greater than that observed in magnocellular layers in glaucoma. In two monkeys with complete optic nerve fiber loss, neuronal atrophy was noted in both magnocellular and parvocellular layers, as may be expected. In addition, neuronal atrophy appeared to be more severe in parvocellular layers than in magnocellular layers, in keeping with previous studies showing that neuronal atrophy is more severe in parvocellular layers than in magnocellular layers after enucleation. Investigators in a recent study were unable to detect a differential cell size effect between magnocellular and parvocellular neurons in experimental glaucoma. In addition to overall shorter survival times compared with that of the present study, the difference may be due to the criterion used to identify neurons—namely, the presence of a distinct nucleolus. Because this organelle is known to shrink during transneuronal degeneration, this criterion may have introduced a bias in the selection of neurons that show less atrophy for area measurement. Finally, Nissl stain labels relay neurons in addition to interneurons. The interneurons, confined to the LGN, are relatively resistant to transneuronal degeneration, and interneurons demonstrate less atrophic change compared with relay neurons after enucleation. This, combined with the fact that magnocellular layers are known to have a greater percentage of interneurons than parvocellular layers, may also explain why no relative difference in atrophy between magnocellular and parvocellular layers was detected in the previous study. In addition, the seven monkeys in our study each had a longer survival time of 14
months compared with survival times ranging from 0.5 to 6 months with similar mean IOP.17
Neuronal atrophy is believed to precede neuronal loss. Mathews et al.4,6 showed that whereas neuronal atrophy occurred within 6 months after ocular enucleation, loss of neurons was not seen until 12 months after ocular enucleation. In the present study, the shrinkage of LGN neurons in experimental glaucoma was found at both early and advanced stages of glaucomatous damage, and increased in a linear fashion with optic nerve fiber loss. However, significant loss of LGN magnocellular neurons (68% and 61%) and parvocellular neurons (60% and 68%) was restricted to monkeys with optic nerve fiber loss of 61% and 100%, respectively.19 That neuronal atrophy precedes neuronal loss is not supported by the results of a recent study in which significant neuronal loss in the magnocellular layer was reported as early as 2.5 weeks after IOP elevation and before the detection of significant atrophy.17 This discrepancy is probably explained by the difficulty in detecting the nucleolus (the criterion used by the investigators to identify neurons), particularly in atrophic neurons with shrunken nuclei.20

In this study, optic nerve fiber loss ranged from 0% to 100% and mean optic nerve fiber loss was 45%. Although this range of optic nerve fiber loss may reflect the full spectrum of glaucomatous optic nerve damage in humans, further studies are needed to assess the degree of neuronal atrophy in LGN and its relationship to the degree of optic nerve fiber loss in human glaucoma.

The presence of degeneration in LGN neurons has several implications regarding progressive glaucomatous damage. The target neurons may provide trophic support for RGCs and in fact, damage to LGN neurons has been shown to cause RGC atrophy and degeneration.99 The degeneration of LGN neurons in experimental glaucoma may increase the susceptibility of surviving RGCs to ongoing damage. Additionally, the changes in relay LGN neurons that project to visual cortex, may explain in part the metabolic and neurochemical changes seen in the primary visual cortex in glaucoma.13,18,40,41 We propose that in addition to therapies to rescue RGCs directly, neuroprotective strategies to rescue LGN neurons in glaucoma may further enhance RGC survival. Indeed, a neurotrophic factor has been shown to prevent the atrophy in LGN neurons induced by monocular visual deprivation.42 Although the mechanisms underlying neuronal atrophy are not yet known, understanding the atrophic process in glaucoma may provide insights into glaucomatous progression and its prevention.

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References


