Loss of Neurons in Magnocellular and Parvocellular Layers of the Lateral Geniculate Nucleus in Glaucoma

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Objective: To determine whether there is loss of lateral geniculate nucleus relay neurons, which convey visual information to the visual cortex, in experimental glaucoma in monkeys.

Methods: Four cynomolgus monkeys with experimentally induced glaucoma in the right eye (referred to as the glaucoma group) and 5 control monkeys were studied. In both groups, the same conditions of fixation, tissue processing, staining, and measurement were used. In each monkey, the left lateral geniculate nucleus target neurons in magnocellular layer 1 and parvocellular layers 4 and 6, connected to the right glaucomatous eye, were studied. Immunocytochemistry with antibody to parvalbumin was used to specifically label relay neurons connecting to the visual cortex. The number of parvalbumin-immunoreactive neurons was estimated using an unbiased 3-dimensional counting method. The t test was used to compare the experimental and control groups.

Results: The mean (±SD) number of neurons in magnocellular layer 1 was significantly decreased in the glaucoma group compared with the control group (20 692 ± 9567 vs 37 687 ± 8017; \( P = .02 \)). The mean (±SD) number of neurons in parvocellular layers 4 and 6 was significantly decreased in the glaucoma group compared with the control group (100 141 ± 44 906 vs 174 090 ± 39 136; \( P = .03 \)). Data are given as the mean ± SD.

Conclusion: Significant loss of lateral geniculate nucleus relay neurons terminating in the primary visual cortex occurs in the magnocellular and parvocellular layers in an experimental monkey model of glaucoma.

Clinical Relevance: Knowledge of the fate of neurons in the central visual system may lead to a better understanding of the nature and progression of visual loss in glaucomatous optic neuropathy.


Following the loss of afferent fibers in the central nervous system, target neurons are known first to become atrophic and then die by the process of transneuronal degeneration.\(^1\)\(^-\)\(^3\) In neurodegenerative diseases and brain trauma, the primary injury triggers transneuronal degeneration; this causes extension of the disease process to neurons relatively spared during the primary injury.\(^4\)\(^-\)\(^6\) Little information exists regarding neuronal changes in target central visual neurons following the loss of afferent optic nerve fibers in glaucoma.

Ninety percent of optic nerve fibers that arise from retinal ganglion cells terminate in the lateral geniculate nucleus (LGN).\(^7\)\(^-\)\(^10\) Recently, studies\(^11\) of the primate LGN provide evidence for motion and color pathways organized into at least 3 distinct LGN neuronal populations occupying separate layers. Neurons in the magnocellular layers convey broadband, luminance contrast, and motion signals; neurons in parvocellular layers convey green-red color opponent signals; and koniocellular neurons located between layers convey blue-on-yellow signals.\(^11\) Understanding neuronal changes in the LGN may provide insights into affected pathways causing vision loss in glaucoma.

There are 2 populations of neurons in the LGN, namely, interneurons and relay neurons. While the projections of LGN relay neurons terminate in the primary visual cortex, the projections of interneurons are confined to the LGN.\(^12\) Parvalbumin, a calcium-binding protein, selectively identifies the population of LGN neurons terminating in the primary visual cortex.\(^13\)\(^,\)\(^14\)

This study determines whether loss of parvalbumin-immunoreactive LGN relay neurons...
neurons conveying visual information to the visual cortex occurs in glaucoma.

RESULTS

LIGHT MICROSCOPY

At low power, the overall appearance of LGNs with glaucoma (Figure 1, right) appeared atrophic compared with the control LGNs (Figure 1, left). Immunoreactivity in layers 4 and 6 was less in glaucomatous (Figure 1, right) compared with control (Figure 1, left) LGNs. Immuno-reactivity in layers 1, 2, 3, and 5 showed no apparent difference between glaucomatous and control LGNs (Figure 1, left and right).

At high power, immunoreactivity was seen in the cell body and in neuronal processes. In glaucomatous LGNs, the cell bodies of neurons appeared smaller and fewer in layers 4 and 6 (Figure 2, bottom). In addition, fewer neuronal processes were present between neurons compared with the control (Figure 2, top). In layer 1, although no difference in neuronal density was apparent, the size of the cell bodies appeared to be reduced.

IMMUNOCYTOCHEMISTRY

The primary antibody was a monoclonal antibody against parvalbumin (clones PA-235; Sigma-Aldrich Corp, St Louis, Mo). Farvalbumin, a calcium-binding protein, labels relay neurons in the LGN layers that project axons to the visual cortex.13,14 Sections were washed with Tris-buffered saline, 0.1 mol/L, and incubated with 0.2% octylphenoxypolyethoxyethanol (Triton-X; Sigma-Aldrich Corp) in Tris-buffered saline, 0.1 mol/L, for 15 minutes, followed by 3% normal goat serum for 1 hour. Sections were incubated in 1:1000 diluted antibody in phosphate-buffered saline with 3% normal goat serum overnight at 4°C. After thorough washing in repeated changes of phosphate-buffered saline and Tris-buffered saline, they were reacted with secondary immunoglobulins using avidin-biotin-peroxidase. A supersensitive detection system kit (Biogenex, San Ramon, Calif) and peroxidase were used to localize the antigen by incubation in 0.02% 3,3-diaminobenzidine and hydrogen peroxide. Sections were mounted onto slides coated with silane-based reagent (Vectabound; Vector, Burlingame, Calif), dehydrated, cleared, and cover-slipped. A negative control was obtained by omitting the primary antibody.

COUNTING METHODS

Morphometry was performed using bright-field microscopy with a color video camera (JVC, Yokohama, Japan), video and computer monitors, and a computer. Stereological procedures that provide unbiased estimates of cell numbers were used.18-22 Neurozoom software (Human Brain Project, La Jolla Calif) enabled digital superposition of the sampling grids on the tissue. Neuronal density and layer surface area measurements were performed on immunostained and cresyl...
stained sections, respectively. The 6 layers of the LGN were easily identified on stained sections. The layers were identified as layer 1 to layer 6 from ventral to dorsal. Ventral layers 1 and 2 are magnocellular layers, while the remaining dorsal layers 3 through 6 are parvocellular layers. Layers 1, 4, and 6 of the left LGN are connected to the glaucomatous right eye, while layers 2, 3, and 5 are connected to the nonglaucomatous left eye. To determine whether neurons are lost in magnocellular and/or parvocellular LGN layers connected to a glaucomatous eye, neurons in the left LGN layers 1, 4, and 6 were counted, and the counts were compared with those from the left LGN layers 1, 4, and 6 in control monkeys. Retinal ganglion cells 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 retinal ganglion cells. The difference in nerve fiber loss between the nasal and temporal quadrants of the right optic nerve in monkeys with experimental glaucoma in the right eye (n = 4) and in control monkeys (n = 5).

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**MAGNOCELLULAR LAYER 1**

Table 1 summarizes the volume, neuronal density, and number of neurons for magnocellular layer 1 in the control and glaucoma groups.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Volume (mm³)</th>
<th>Neuron Density (neurons/mm³)</th>
<th>Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.00 ± 0.25</td>
<td>16,000 ± 2,000</td>
<td>32,000</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>1.80 ± 0.20</td>
<td>12,000 ± 2,000</td>
<td>24,000</td>
</tr>
</tbody>
</table>

**Layer Volume**

The volume of layer 1 ranged from 0.70 to 2.50 mm³ in the glaucoma group, and from 2.00 to 3.04 mm³ in the control group. The mean ± SD volume of layer 1 was significantly decreased in the glaucoma group compared with the control group (P = .04).

**Neuronal Density**

Neuronal density in layer 1 ranged from 11,848 to 17,041 neurons per cubic millimeter in the glaucoma group, and...
from 13 339 to 15 466 neurons per cubic millimeter in the control group. The mean density of neurons in layer 1 did not differ significantly between the glaucoma and the control groups ($P > .05$).

The number of neurons in magnocellular layer 1 ranged from 11 929 to 33 339 in the glaucoma group, and from 13 339 to 15 466 neurons per cubic millimeter in the control group. The mean density of neurons in layer 1 did not differ significantly between the glaucoma and the control groups ($P > .05$).

**Number of Neurons**

The number of neurons in magnocellular layer 1 ranged from 11 929 to 33 339 in the glaucoma group, and from 13 339 to 15 466 neurons per cubic millimeter in the control group. The mean density of neurons in layer 1 did not differ significantly between the glaucoma and the control groups ($P > .05$).
26611 to 46941 in the control group. The mean number of neurons in magnocellular layer 1 was significantly decreased in the glaucoma group compared with the control group ($P = .02$) (Figure 3, top). The number of neurons in magnocellular layer 1 in the glaucomatous monkeys showed a tendency to decrease with increasing optic nerve fiber loss (Figure 3, bottom).

**PARVOCELLULAR LAYERS 4 AND 6**

Table 2 summarizes the volume, neuronal density, and number of neurons for parvocellular layers 4 and 6 in the control and glaucoma groups.

**Layer Volume**

The combined volume of layers 4 and 6 ranged from 3.36 to 8.06 mm$^3$ in the glaucoma group, and from 7.45 to 12.84 mm$^3$ in the control group. The mean volume of layers 4 and 6 was significantly decreased in the glaucoma group compared with the control group ($P = .02$).

**Neuronal Density**

Neuronal density in layers 4 and 6 ranged from 16 608 to 18 426 neurons per cubic millimeter in the glaucoma group, and from 15 701 to 18 857 neurons per cubic millimeter in the control group. The mean density of neurons in layers 4 and 6 did not differ significantly between the glaucoma and the control groups ($P > .05$).

**Number of Neurons**

The number of neurons in parvocellular layers 4 and 6 ranged from 56 439 to 151 822 in the glaucoma group, and from 124 971 to 227 005 in the control group. The mean number of neurons in parvocellular layers 4 and 6 was significantly decreased in the glaucoma group compared with the control group ($P = .03$) (Figure 4, top). The number of neurons in parvocellular layers 4 and 6 in the glaucomatous monkeys showed a tendency to decrease with increasing optic nerve fiber loss (Figure 4, bottom).

**COMMENT**

Previous studies$^{22,27,28}$ in monkey LGN have used the Nissl stain, which labels all neurons, including relay neurons and interneurons. In the present study, parvalbumin was used to label only relay neurons connecting to the visual cortex in the magnocellular and parvocellular layers.$^{13,14}$ Neuron density measurements for the magnocellular and parvocellular layers were similar to measurements computed by Ahmad and Spear,$^{22}$ and comparable coefficients of error for neuron number (9.5% for layer 1 and 10% for layers 4 and 6) were obtained. Since the stereological method used in this study has been shown to be unbiased by the size, orientation, or shape of the objects counted,$^{24,26}$ neuronal density measure-
ments are not biased by a possible reduction in size of neurons in magnocellular and parvocellular layers in glaucoma (qualitative observation and preliminary measurements).

Our study demonstrated neuronal loss in the magnocellular and parvocellular layers in experimental glaucoma. These results are in keeping with a descriptive study\textsuperscript{39} showing weaker immunoreactivity for synaptophysin and neurofilament in magnocellular and parvocellular layers, and with electrophysiologic studies\textsuperscript{10} reporting deficits in visually responsive cells in magnocellular and parvocellular layers in experimental glaucoma.

A previous study\textsuperscript{31} of human LGNs reported a preferential reduction of 2-dimensional estimates of neuron density in the LGN magnocellular layer in glaucoma. In our study, using 3-dimensional techniques, we found no significant decrease in neuronal density in the magnocellular and parvocellular layers. The differences in our findings might be explained by differences between human clinical and monkey experimental conditions, extent and/or duration of disease, and differences in method.

Histomorphometric studies\textsuperscript{22,23} in human clinical and monkey experimental glaucoma, including measurements of cell body size and of axon diameter of the surviving retinal ganglion cells, suggest preferential loss of large retinal ganglion cells. In addition, large retinal ganglion cells immunoreactive for neurofilament have been shown to be preferentially lost in experimental glaucoma.\textsuperscript{34} The researchers\textsuperscript{32,33} suggest that retinal ganglion cells projecting to the magnocellular layers (M) are preferentially lost in early glaucoma, based on the observation that M retinal ganglion cell bodies and axons are relatively larger compared with cell bodies and axons of retinal ganglion cells in normal retina projecting to the parvocellular layers (P). The identification of retinal ganglion cell types on size alone may not be reliable\textsuperscript{35} for several reasons: overlap in cell body size between M and P retinal ganglion cells occurs in normal monkey and human retina; S cells, a third class of retinal ganglion cells involved in the blue-on-center system, are similar in size to M cells; and atrophic changes in glaucoma include a reduction in cell body size and axon diameter of retinal ganglion cells.\textsuperscript{36} In our study, the number of neurons in the magnocellular and parvocellular layers showed a tendency to decrease with increasing optic nerve fiber loss. Further studies of the LGN with a larger sample size and with optic nerve fiber loss ranging from 30% to 60% may provide evidence for preferential loss in these pathways.

Support for neuronal damage in magnocellular and parvocellular pathways in glaucoma comes from various functional tests. Motion-automated perimeter, high-frequency temporal flicker perimeter, and frequency-doubling perimeter reveal deficits in the magnocellular pathway in glaucoma.\textsuperscript{37-41} The parvocellular pathways are known to convey red-green color information.\textsuperscript{42,43} Color pattern–electroretinogram, sweep visual-evoked potentials, and psychophysical tests for red-green sensitivity show deficits in the parvocellular pathway in glaucoma.\textsuperscript{44-46} There is evidence to suggest that the blue-sensitive pathway is conveyed through a third informa-

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{chart.png}
\caption{Figure 4. Top, Mean number of parvalbumin-immunoreactive neurons in left lateral geniculate nucleus (LGN) parvocellular layers 4 and 6 connected to the right eye of control or glaucomatous monkeys. Error bars indicate SDs. The asterisk indicates that the mean number of neurons was significantly reduced by 42% in glaucomatous monkeys (P = .03). Bottom, The number of parvalbumin-immunoreactive neurons in the left LGN parvocellular layers 4 and 6 for the 4 glaucomatous monkeys with varying percentage of optic nerve fiber loss. The horizontal solid line indicates the mean number of parvalbumin-immunoreactive neurons in the left LGN parvocellular layers 4 and 6 for the control monkeys (n = 5). The horizontal dotted line indicates the mean number of neurons minus 2 SDs for the control monkeys.}
\end{figure}

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