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Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma

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Abstract

The vulnerability of subpopulations of retinal neurons delineated by their content of cytoskeletal or calcium-binding proteins was evaluated in the retinas of cynomolgus monkeys in which glaucoma was produced with an argon laser. We quantitatively compared the number of neurons containing either neurofilament (NF) protein, parvalbumin, calbindin or calretinin immunoreactivity in central and peripheral portions of the nasal and temporal quadrants of the retina from glaucomatous and fellow non-glaucomatous eyes. There was no significant difference between the proportion of amacrine, horizontal and bipolar cells labeled with antibodies to the calcium-binding proteins comparing the two eyes. NF triplet immunoreactivity was present in a subpopulation of retinal ganglion cells, many of which, but not all, likely correspond to large ganglion cells that subserve the magnocellular visual pathway. Loss of NF protein-containing retinal ganglion cells was widespread throughout the central (59–77% loss) and peripheral (96–97%) nasal and temporal quadrants and was associated with the loss of NF-immunoreactive optic nerve fibers in the glaucomatous eyes. Comparison of counts of NF-immunoreactive neurons with total cell loss evaluated by Nissl staining indicated that NF protein-immunoreactive cells represent a large proportion of the cells that degenerate in the glaucomatous eyes, particularly in the peripheral regions of the retina. Such data may be useful in determining the cellular basis for sensitivity to this pathologic process and may also be helpful in the design of diagnostic tests that may be sensitive to the loss of the subset of NF-immunoreactive ganglion cells.

Keywords: Glaucoma; Retina; Neurofilament; Calcium-binding protein

1. Introduction

Anatomical and physiological studies of glaucoma in humans [33,34] and experimental glaucoma induced in monkeys [12,13,32] suggest that retinal ganglion cells with large somata and large diameter axons, which correspond to the magnocellular visual pathway, show an increased degree of vulnerability to degeneration. Glaucoma is classically associated with increased intraocular pressure, although it is unclear whether pressure or other factors are the immediate cause of damage to the retina and optic nerve. Mechanical damage to axons in the optic nerve at the level of the lamina cribrosa followed by perturbations in axoplasmic transport has been proposed as a mechanism leading to axonal degeneration [2,10,31,32,35], although other pathogenic mechanisms that lead to the vulnerability of particular groups of retinal neurons are possible [39]. For example, detection of elevated levels of excitatory amino acids in the vitreous of glaucomatous patients [6] and of monkeys with glaucoma [40] suggests that excitotoxicity might also play a role in the retinal neuron degeneration.

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Elucidation of the precise subgroup(s) of retinal neurons preferentially affected by the disease process is important for two main reasons. (1) Such studies may indicate predictive features of the vulnerable subset of neurons which may in turn suggest rationales for therapeutic intervention. (2) Identification of particular groups of neurons comprising physiological pathways that are preferentially affected could lead to the design of diagnostic tests that are sensitive to the earliest pathologic changes which may in turn allow for earlier clinical intervention. Neurochemical markers for neurons can be utilized to help distinguish different subpopulations of neurons and may, thus, also be useful in the assessment of the vulnerability of subclasses of retinal neurons in diseases, such as glaucoma.

Calcium-binding proteins and cytoskeletal proteins have been used to distinguish subgroups of neurons in the retina. Specifically, members of the EF-hand family of calcium-binding proteins, such as parvalbumin, calbindin and calretinin, have been shown to label certain subgroups of neurons throughout the vertebrate retina [7,14,27,36,38]. Additionally, a class of three interrelated cytoskeletal protein subunits known as the neurofilament (NF) 'triplet' appears to be consistently present in certain subpopulations of retinal ganglion neurons and variably present in other retinal neurons across mammalian species [4,5,11,19,43,44,48,53]. Large α-type ganglion cells have been shown to be immunoreactive for the NF triplet across many mammalian species [11,48,53] and the same group of neurons are also revealed by certain silver impregnation methods [28,46] which are likely to be chemically specific for a conserved region of all three NF triplet subunits [1,53]. In addition to these large neurons, another group of smaller ganglion cell neurons has been shown to contain a relatively lower degree of immunoreactivity for these cytoskeletal protein subunits [48,53]. To identify more precisely the retinal neurons affected in glaucoma, we have now investigated the vulnerability of these calcium-binding protein-containing and NF-containing neurons in the ganglion and inner nuclear cell layers of the retina in an established monkey model of glaucoma [21].

2. Materials and methods

2.1. Animals and experimental manipulation

A total of seven cynomolgus monkeys *Macaca fascicularis* were utilized for this study. Three monkeys (3–4 kg) of normal gestation and development were selected for creation of glaucoma in one eye (experimental animals). The other four animals were examined for the normal localization of the neuronal markers of interest (control animals). All experimental animals had undergone baseline eye examination and had normal anterior and posterior segments, open corneoscleral angles, normal optic nerve heads and normal intraocular pressures, the latter recorded for a 12-h diurnal cycle using a calibrated pneumatonometer. To produce the glaucoma, animals were sedated with intramuscular ketamine hydrochloride (5 mg/kg) and received 0.5% topical proparacaine. The mid-portion of the trabecular meshwork of one eye of each animal was treated with argon laser burns using a single mirror goniolens specifically made for monkey eyes. Initial treatment consisted of ~100 burns, each 50 μm in diameter, delivered over 360°, with 1.5 W of power for 0.5 s exposure time. Animals were treated with prednisolone acetate 1% 1 gtt to the treated eye every 6 h for the first 4 days after laser surgery. Frequent intraocular pressure measurements in the immediate postoperative days were made and eyes that did not show sustained elevation of intraocular pressure (to at least the high 20 mm Hg range or greater) on diurnal testing were retreated by the 3rd week. Pressures were monitored at least weekly, thereafter, and if at any point during the survival period intraocular pressure was noted to decline on two successive readings to the normal range, retreatment was undertaken [21]. Optic nerve head photography was performed periodically. Table 1 shows the summary of baseline clinical characteristics of experimental monkeys and treatment parameters. Refractive error was determined by cycloplegic retinoscopy and all animals were within 0.5 diopters of emmetropia. The cup/disc ratio presented in Table 1 refers to the ratio of the diameter of central excavation ('cup') to that of total optic nerve head ('disc'). Normal cup/disc ratios are ~0.2–0.3. Normal intraocular pressures are usually 12–20 mm Hg.

Table 1
Baseline characteristics and treatment parameters of experimental animals

Animal	1	2	3
Baseline cup/disc ratio	0.1	0.3	0.2
Pretreatment peak IOP (right/left) (mm Hg)	16/17	16/16	15/15
Treated eye	Right	Left	Right
Treatment 1 (burns)	107	21	92
Peak IOP (right/left)	16/17	15/10	13/13
Treatment 2 (days)	41	19	19
(burns)	83	75	109
Peak IOP (right/left)	30/13	13/20	35/15
Treatment 3 (days)	–	125	–
(burns)		86	
Peak IOP (right/left)	34/16	14/43	35/13
Total survival time after first treatment (days)	126	226	226
Final peak IOP (right/left)	40/16	13/30	34/16
Final cup/disc ratio (right/left)	0.9/0.1	0.3/0.9	1.0/0.2

IOP, intraocular pressure.

Animals were observed periodically for the development of typical glaucomatous optic nerve head excava-

tion as assessed by clinical ophthalmoscopy. In this respect, two of the animals developed severe cupping of the optic nerve head and one developed moderate cupping. All treatments and animal maintenance were conducted in accord with the Association for Research in Vision and Ophthalmoscopy (ARVO) resolutions concerning animal welfare.

2.2. Tissue processing and immunohistochemistry

All animals were deeply anesthetized with ketamine (25 mg/kg) and Nembutal (30 mg/kg) and perfused transcardially through the left ventricle initially with ice-cold 0.1 M phosphate-buffered 1% paraformaldehyde for 60 s followed by ice-cold 0.1 M phosphate-buffered 4% paraformaldehyde for 8–10 min. The eyes were enucleated and postfixed with the optic nerve in the 4% paraformaldehyde solution for ~ 24 h followed by washes in a 10- mM phosphate-buffered, 100-mM saline solution (PBS) and cryoprotection through a series of graded sucrose solutions (18–30%).

Incisions through the wall of the globe and retina were made to divide the eye into four quadrants (nasal, temporal, superior and inferior). Rectangular segments of retina centered 3–5 and 8–10 mm from the optic disc were dissected from both the nasal and temporal quadrants of the glaucomatous and non-glaucomatous eyes of the experimental animals (i.e., total of four segments/eye). These tissue segments were rapidly frozen in OCT-embedding medium and 35-μm transverse cryostat sections were cut. Tissue sections were collected in sequence across eight wells into PBS. Transverse sections of the retina from the optic disc to the periphery were also cut from the nasal and temporal quadrants of eyes from four control animals. Wholemount material consisting of retina between the segments taken for cryostat sectioning were processed for NF immunohistochemistry as previously described [48,53].

The antibodies to cytoskeletal and calcium-binding proteins used in the current study are presented in Table 2. For immunohistochemistry, tissue sections from a well were mounted on chrome-alum/gelatin-subbed slides using a single-hair brush. Incubations with primary antibodies were done on slides placed overnight in a humid chamber. Antibodies were diluted in PBS containing 0.3% Triton X-100. For single labeling preparations, sections were washed in PBS and incubated in either a horse anti-mouse IgG antibody conjugated to fluorescein isothiocyanate (FITC) (Vector Labs, Burlingame, CA; 1:200 dilution) for visualizing the anti-calbindin, anti-parvalbumin and anti-NF monoclonal antibodies or with a biotinylated anti-rabbit IgG raised in goat (Vector Labs; 1:200 dilution) for 2 h followed by washes and then FITC-conjugated avidin (Vector Labs; 1:200 dilution) for 1 h in the case of the anti-calretinin antibody. Wholemounts of segments of the retina as well as sections of the optic nerve from glaucomatous and non-glaucomatous eyes were also immunolabeled with M14, the antibody to the NF-L subunit.

Tissue sections from control animals were processed for single labeling with the antibodies presented in Table 2. As these antibodies were raised in different host species (e.g., mouse or rabbit), double-labeling immunofluorescence was conducted to determine the extent of overlap in the distribution of these markers in the retina. The only combination that was not assessable was between the NF antibodies and the antibody to calbindin as these were both mouse monoclonal antibodies. For double-labeling preparations, mouse monoclonal antibodies were visualized as described above and rabbit polyclonal antibodies were incubated with the biotinylated anti-rabbit antibody described above and followed by streptavidin Texas red (Amersham; 1:200 dilution). The secondary antibodies were tested for undue reactivity with sections lacking primary antibody incubation and for cross-reactivity to either the inappropriate primary or to each other. All fluorescence slides were cover-slipped using Permafluor (Immunon, Pittsburgh, PA) mounting media and viewed with a Zeiss Axiophot fluorescence microscope equipped with appropriate filter blocks for the selective visualization of either FITC or Texas red.

For Nissl staining, sections were mounted on subbed slides, stained with Cresyl violet, dehydrated through a series of graded alcohols and coverslipped with DPX mounting media (Fluka).

Table 2
Immunohistochemical markers

Code	Type	Immunoreactivity	Source
SMI32	Mouse IgG	NF-M/NF-H	Sternberger Immunochemicals
M14	Mouse IgG	NF-L	R.A. Marugg and B. Riederer
300	Mouse IgG	Calbindin	Swant
7696	Rabbit IgG	Calretinin	Swant
235	Mouse IgG	Parvalbumin	Swant
9064	Rabbit IgG	Parvalbumin	Swant

2.3. Quantitative analysis

Using a 40 × objective, the total number of neurons labeled with a particular neuronal marker using fluorescence optics was counted from experimental retina sections horizontally aligned through the center of the microscope field. Four fields from four different sections were counted for each marker in a given retinal location. Similar analysis was performed on Nissl-stained sections, counting cells with large nuclei and stained cytoplasm which are, thus, likely to correspond to nerve cells [12]. Only cells with sharp boundaries in a focal field were counted for immunolabeled and Nissl-stained preparations. The counts for each marker across samples and animals were statistically compared by ANOVA using Statview 4.0.

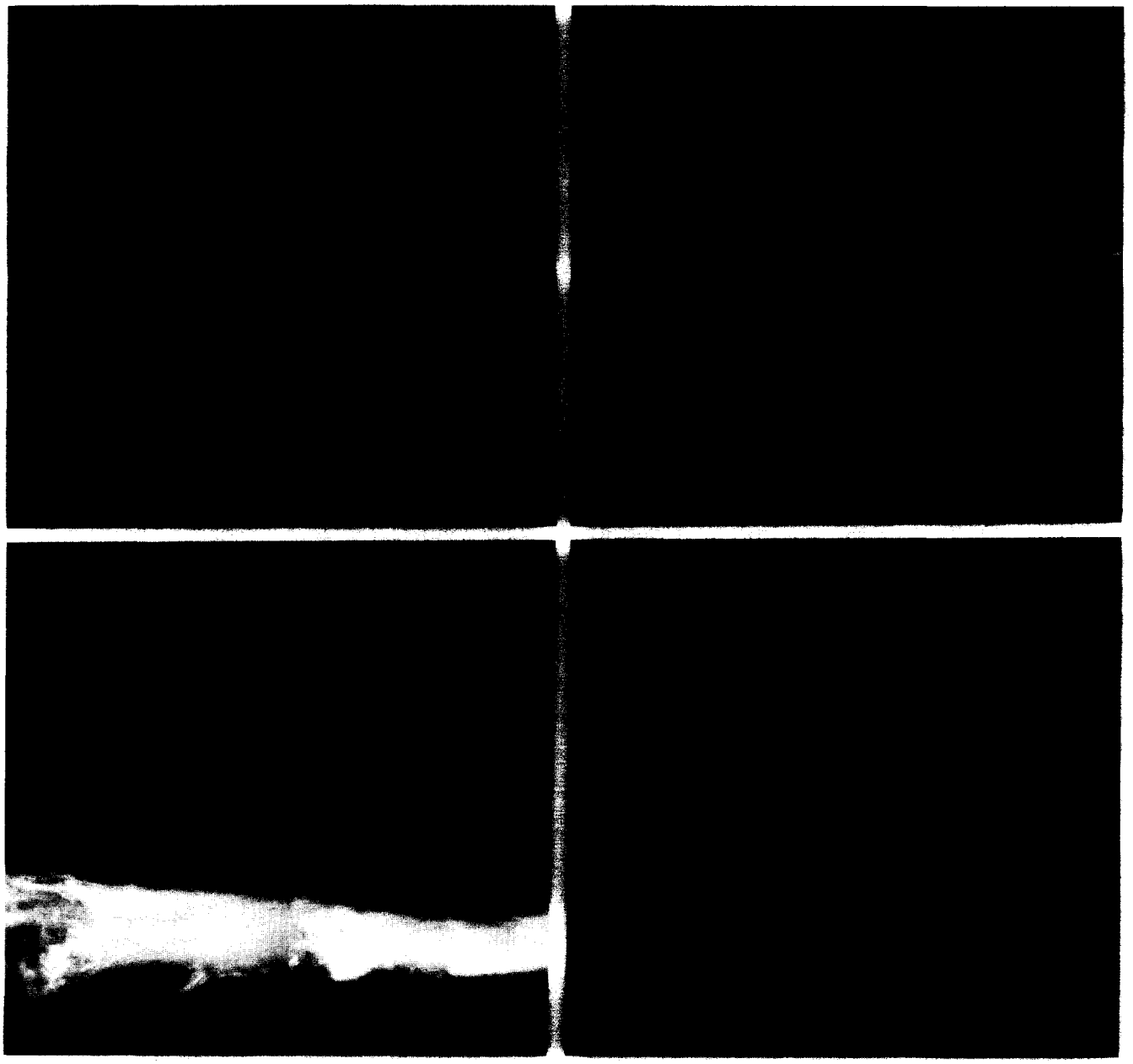

Fig. 1. Double-labeling for either parvalbumin (A) and calbindin (B) or NF protein (anti-NF-L) (C) and calretinin (D) in transverse sections of a control monkey eye. Intense parvalbumin immunoreactivity is present within horizontal cells in close proximity to the outer plexiform layer and many of these cells also show faint calbindin immunoreactivity (e.g., arrows). In contrast, cells in the inner nuclear layer showing intense calbindin immunoreactivity and a bipolar cell-like morphology are unlabeled for parvalbumin (e.g., open arrows). C shows the normal distribution of neurofilament immunoreactivity in the retina. A varying degree of immunoreactivity is present in cell bodies in the ganglion cell layer (e.g., arrows) and many processes in the underlying optic nerve fiber layer as well as some processes in the overlying inner plexiform layer are also labeled. In the same section, calretinin immunoreactivity is present in many cells in the inner nuclear layer adjacent to the inner plexiform layer (open arrows). In addition, many calretinin-immunoreactive processes are present in the inner plexiform layer. Many of the NF-immunoreactive cells in the ganglion cell layer also show faint immunoreactivity for calretinin (e.g., curved arrows). Scale bars, 50 (A,B) and 25 μm (C,D).

3. Results

3.1. Distribution of immunolabeling for NF triplet and calcium-binding proteins in non-glaucomatous eyes

The antibodies to NF-L (M14) (Fig. 1) and NF-M/NF-H (SMI32) preferentially labeled cell bodies in the ganglion cell layer. Towards the central regions of the retina, these antibodies labeled many cell bodies with a low degree of intensity of immunoreactivity whereas at progressive distances from the center, larger cells with a relatively high degree of immunoreactivity were detected in addition to smaller, less intensely immunoreactive cells. The dendritic tree of these larger cells could often be discerned, with the primary dendrites sometimes extending to the outer part of the inner plexiform layer. Both M14 and SMI32 also labeled bundles of optic nerve fibers. Labeling of fine processes of regular caliber, that may correspond to axons, was also detected in the inner plexiform layer. Very rarely, small cells in the inner nuclear layer adjacent to the inner plexiform layer were observed to contain immunoreactivity for NF-L or NF-M/NF-H. Otherwise, no other cells in the inner or outer nuclear layers were labeled with either of these anti-NF antibodies.

The antibody to calretinin intensely labeled many cells in the inner nuclear layer that were located immediately adjacent to the inner plexiform layer (Fig. 1). A very dense plexus of calretinin-immunoreactive processes was located in the inner plexiform layer and appeared to be stratified into at least two, and sometimes three, layers. Many cells in the ganglion cell layer also showed a low-to-moderate degree of calretinin immunoreactivity as did some processes in the optic nerve fiber layer. In addition, some other smaller cells in the ganglion cell layer as well as some cells in the inner nuclear layer that appeared to correspond morphologically to bipolar cells showed a relatively low degree of immunoreactivity for calretinin.

Parvalbumin immunoreactivity in the retina (Fig. 1) was very similar to that described by Röhrenbeck et al. [36]. Intense parvalbumin immunoreactivity was localized to cells located in the inner nuclear layer, adjacent to the outer plexiform layer, and immediately above these cells was a dense plexus of immunolabeled fibers, indicating that these may correspond to horizontal cells as suggested by Röhrenbeck et al. [36]. Parvalbumin immunoreactivity was also present in other cells in the inner nuclear layer but most notably in a group of cell bodies adjacent to the inner plexiform layer that may be amacrine cells. A few parvalbumin-immunoreactive processes were also present in the inner plexiform layer. Many small to large cells in the ganglion cell layer as well as processes in the nerve fiber layer showed a relatively low degree of parvalbumin immunoreactivity. In contrast, a group of small neurons in the ganglion cell layer, usually in close proximity to the inner plexiform layer, were intensely labeled for parvalbumin and were observed to give rise to short dendrite-like processes as well as longer processes extending into the inner plexiform layer.

The distribution of calbindin immunoreactivity (Fig. 1) was also closely similar to that described by Röhrenbeck et al. [36]. Calbindin immunoreactivity was localized to cone-like photoreceptors in the outer nuclear layer and their processes in the outer plexiform layer. A high degree of calbindin immunoreactivity was also present in cells located near the inner nuclear layer that were observed to give rise to short, thick processes that extended to the outer plexiform layer as well as a thinner process that extended to the inner plexiform layer, indicating that these cells may correspond to bipolar cells. Other calbindin-immunoreactive cells were located adjacent to the outer plexiform layer and most of these cells showed a relatively low degree of calbindin immunoreactivity. Calbindin immunoreactivity was also present in processes within the outer plexiform layer, indicating that the adjacent faintly labeled cells may correspond to horizontal cells. Other, typically faintly, immunoreactive cells were located throughout the inner nuclear layer, and a small number of moderately labeled cells were present in the ganglion cell layer adjacent to the inner plexiform layer. These latter cells often gave rise to processes that extended throughout the inner plexiform layer. Retinal ganglion cells and the optic nerve fiber layer showed a low degree of calbindin immunoreactivity.

Double-labeling demonstrated that the neurons in the inner nuclear layer that were labeled for parvalbumin were not immunoreactive for either NF-L or calretinin. Calbindin and parvalbumin immunoreactivity did overlap in the great majority of neurons located adjacent to the outer plexiform layer but the parvalbumin-immunoreactive cells typically showed a relatively low degree of calbindin immunoreactivity (filled arrows in Fig. 1A,B). In contrast, the bipolar-like cells showing a high degree of calbindin immunoreactivity were not parvalbumin-immunoreactive (open arrows in Fig. 1A,B). In the ganglion cell layer, many of the neurons faintly labeled for parvalbumin were also immunoreactive for NF-L.

Many of the cells in the retinal ganglion cell layer labeled for NF-L also showed a low degree of immunoreactivity for calretinin, and the infrequent, large cells in this layer that showed a high degree of immunoreactivity for calretinin were also labeled for NF-L and parvalbumin. ~90% of the small neurons in the ganglion cell layer adjacent to the inner plexiform layer that were intensely labeled for parvalbumin and which may correspond to displaced amacrine cells, were also immunoreactive for calbindin and a very small propor-

tion of these parvalbumin-immunoreactive cells showed faint labeling for NF-L.

3.2. Glaucomatous vs. non-glaucomatous eyes

The number of cells immunolabeled for these markers was quantitatively assessed in the retinas of the glaucomatous and non-glaucomatous eyes from the three experimental animals. Specifically, the neurons assessed included NF-immunoreactive cells in the ganglion cell layer, calbindin-immunoreactive cells of the inner nuclear layer within two cell body lengths of the outer plexiform layer that show moderate to high levels of immunoreactivity and give rise to a process that extended to the inner plexiform layer (bipolar cells), calretinin-immunoreactive cells in the inner nuclear layer adjacent to the inner plexiform layer (amacrine cells) and parvalbumin-immunoreactive cells of the inner nuclear layer within two cell body lengths of the outer plexiform layer (horizontal cells).

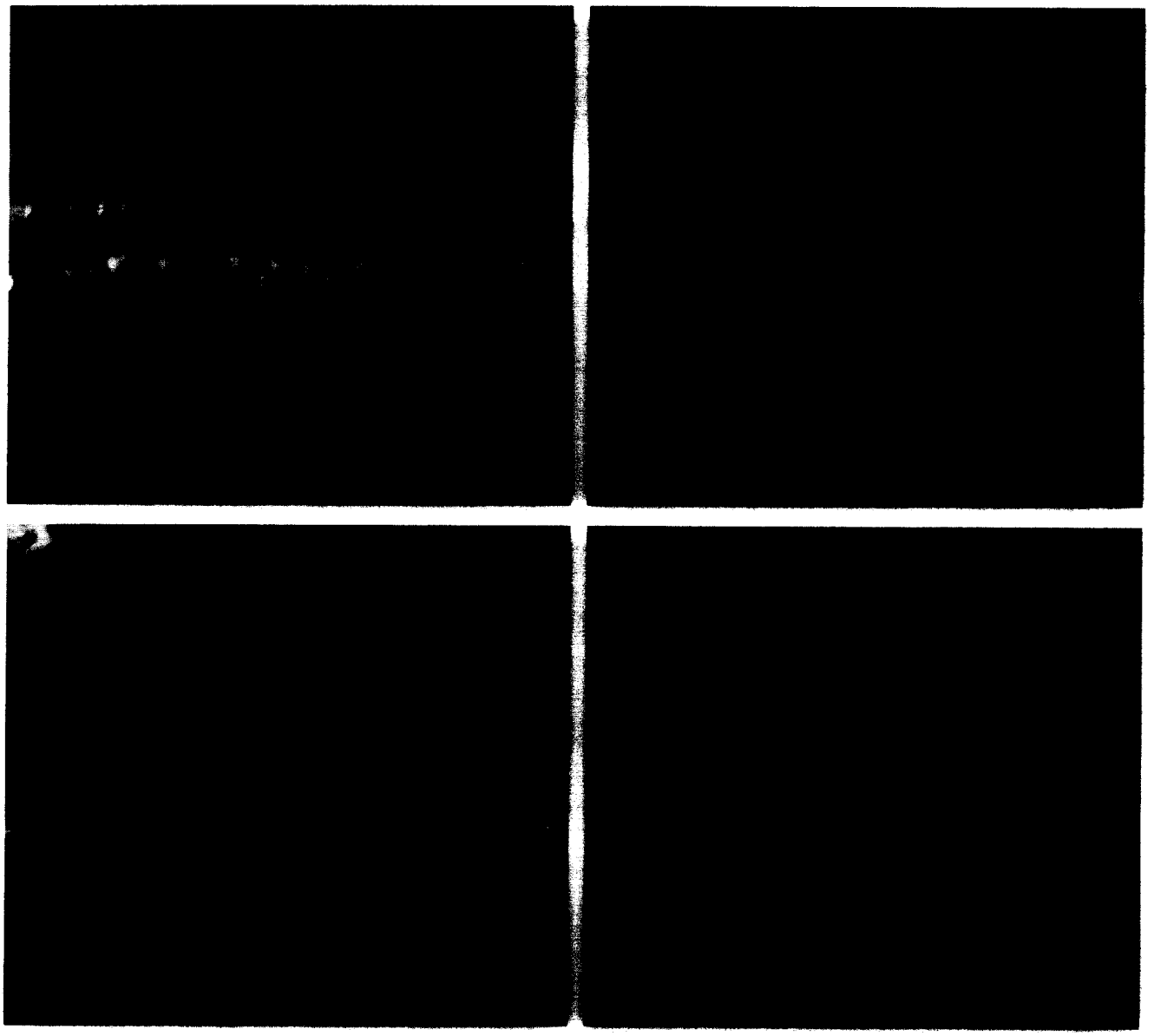

Fig. 2. Calcium-binding protein immunoreactivity in transverse sections of retina 3–5 mm from the optic disc in the nasal quadrant from non-glaucomatous (A,C) and glaucomatous (B,D) fellow eyes. A and B show calbindin immunoreactivity. Many calbindin-immunoreactive bipolar-like cells are labeled in both the glaucomatous and non-glaucomatous eyes (e.g., arrows). In addition, small intensely immunoreactive cells are located in the ganglion cell layer in both the glaucomatous and non-glaucomatous eyes (e.g., open arrows) and these may correspond to displaced amacrine cells. Calretinin immunoreactivity is shown in C and D. High calretinin immunoreactivity is present in a neurons adjacent to the inner plexiform layer that are likely to correspond to amacrine cells (e.g., curved arrows). An equivalent proportion of these calretinin-immunoreactive neurons are present in the glaucomatous eye relative to the non-glaucomatous eye. In addition, laminated calretinin immunoreactive processes are present in the inner plexiform layer in both the non-glaucomatous and glaucomatous eyes. Scale bar for A–D, 50 μm.

ANOVA demonstrated that the number of cells labeled with the calcium-binding proteins in the inner nuclear layer was not statistically different between the glaucomatous and non-glaucomatous eyes (Table 3, Fig. 2). In addition, the fiber plexuses labeled by these different neuronal markers in the inner and outer plexiform layers appeared to be very similar in glaucomatous and non-glaucomatous eyes. Although not quantitatively assessed, no gross differences between these retinas were apparent in the number of calbindin-immunoreactive cones or parvalbumin-immunoreactive cells in the inner nuclear layer adjacent to the inner plexiform layer. There was a general depletion of the parvalbumin-immunoreactive cells in the ganglion cell layer from glaucomatous eyes as well as a reduction of parvalbumin-labeled fibers in the nerve fiber layer. However, in the ganglion cell layer, many of the small cells adjacent to the inner plexiform layer that were intensely immunoreactive for parvalbumin or calbindin were still present in the glaucomatous eyes relative to the non-glaucomatous fellow eyes.

The number of NF-immunoreactive neurons was significantly depleted ($P < 0.001$) in all areas sampled of retinas from the eyes with glaucoma relative to those

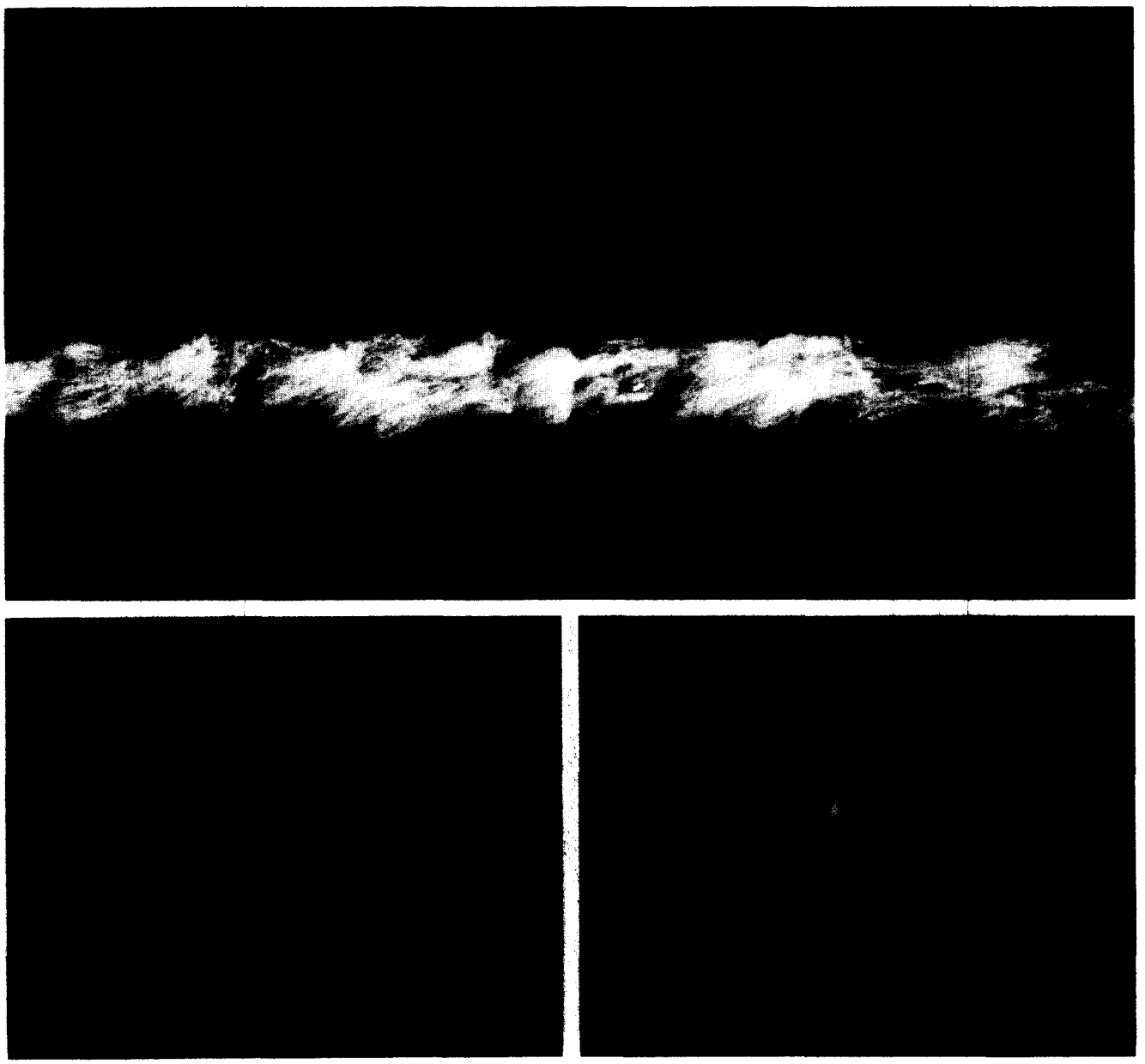

Fig. 3. Fig. A shows NF (anti-NF-L) immunoreactivity in a transverse section from a non-glaucomatous eye corresponding to a segment 3–5 mm from the optic disc in the nasal quadrant of the retina. Many cell bodies in the ganglion cell layer show neurofilament immunoreactivity (e.g., arrows) although some smaller cells show a relatively low degree of immunolabeling (e.g., curved arrows). B and C show the corresponding region in the glaucomatous eye from the same animal showing a loss of NF-immunoreactive cells and fibers and where remaining cells contain a perinuclear accumulation of NF immunoreactivity (arrows). Scale bar for A–C, 25 μm.

from the non-glaucomatous fellow eyes, with the magnitude and areal proportion of this loss being very similar between animals (Table 3, Fig. 3). The proportional loss of NF-immunoreactive neurons was most dramatic in peripheral regions of the nasal and temporal retina (96–97%) as compared with more central regions (59–77%). Notably, whereas NF-immunoreactive cell density was highest in the central temporal sample of the normal retina (Table 3), the proportion of the NF-immunoreactive cell loss in the glaucomatous retina in this region was the least of the areas examined (59%). This region also had the highest degree of variability in NF-immunoreactive cell counts as indicated by the measures of variance (Table 3), which is likely to reflect this segment's proximity to the ganglion cell dense region adjacent to the fovea. Despite the high SD values of these temporal samples, a statistically detectable difference existed in counts from glaucomatous eyes compared with non-glaucomatous eyes.

There was also a relative depletion of NF-L-labeled axons in the nerve fiber layer of the glaucomatous eyes throughout these samples (Fig. 4). Similarly, there was widespread loss of NF-immunoreactive axons, both large and small in size, in sections of the optic nerve from the glaucomatous eyes as compared with the non-glaucomatous eyes. However, in most sections from glaucomatous eyes, there were still many fine NF-L-labeled processes present in the inner plexiform layer. Surviving NF-immunoreactive neurons in glaucomatous retinas varied in size and some of these were shown to contain perinuclear accumulations of immunolabeled material that were intensely immunoreactive for NF-L (Fig. 3).

Quantitative assessment of Nissl-stained material demonstrated that there were statistically significantly fewer stained cells in the ganglion cell layer of glaucomatous eyes relative to the non-glaucomatous eyes ($P < 0.001$, able 3). Notably, ~ 50% of cells were lost in central samples and ~ 44 and 32% of cells were lost in the peripheral samples of nasal and temporal retina, respectively.

4. Discussion

4.1. Chemical coding of retinal neurons

In the current study, the distribution of parvalbumin and calbindin immunoreactivity in the retina was similar to single labeling data previously reported in cynomolgus monkeys [36]. In contrast to the serial section co-localization results of Röhrenbeck et al. [36], we did not observe any parvalbumin immunoreactivity

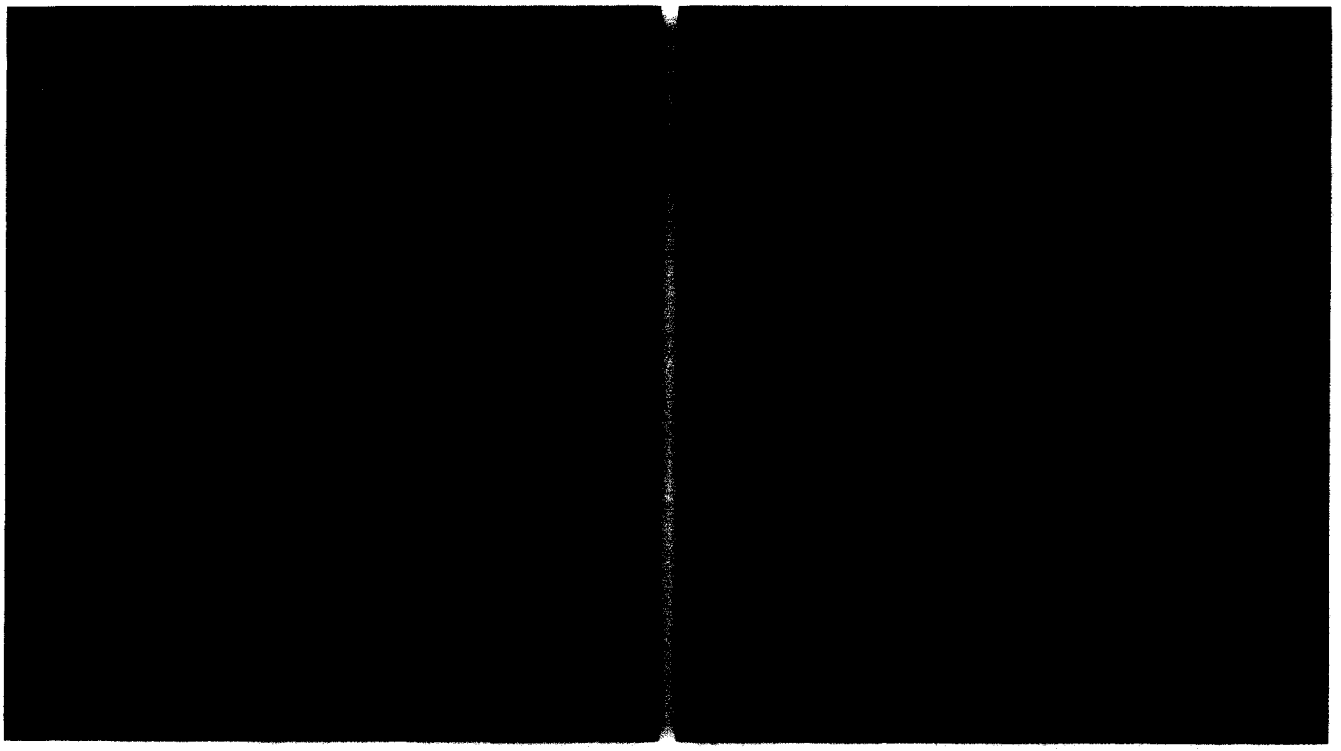

Fig. 4. Wholemount preparations of the retina immunolabeled for NF protein (anti-NF-L) in a segment corresponding to 5–8 mm from the optic disc in the nasal quadrant. Fewer immunoreactive processes are present in the optic nerve fiber layer of the retina from the eye with glaucoma (B) as compared with the retina of the non-glaucomatous eye relative (A). Some NF-immunoreactive cells are present in the glaucomatous retina in this region (arrows in B). Scale bar for A and B, 25 μm.

Table 3
Quantitative assessment of number of neurons labeled with neuronal markers or stained with Cresyl violet in glaucomatous and non-glaucomatous fellow eyes

	Non-glaucomatous	Glaucomatous	% difference
NF-L			
Nasal 3–5	47.5±7.2	11.2±8.7	−76.4 [a]
Nasal 8–10	16.8±4.5	0.5±0.8	−97.0 [a]
Temporal 3–5	68.0±20.5	27.7±22.5	−59.3 [a]
Temporal 8–10	9.3±2.5	0.3±1.0	−96.8 [a]
Calretinin (amacrine cells)			
Nasal 3–5	45.8±6.0	43.3±5.8	−5.5
Nasal 8–10	28.8±4.3	26.3±4.5	−8.7
Temporal 3–5	56.8±9.5	57.7±7.8	+1.6
Temporal 8−10	23.0±3.0	20.5±2.7	−10.9
Calbindin (bipolar cells)			
Nasal 3–5	46.5±7.3	44.8±6.7	−3.7
Nasal 8–10	27.2±3.3	25.0±4.0	−8.1
Temporal 3–5	39.8±4.8	39.0±4.0	−2.0
Temporal 8–10	23.5±3.3	24.0±4.5	+2.1
Parvalbumin (horizontal cells)			
Nasal 3–5	64.7±31.2	63.8±29.2	−1.4
Nasal 8–10	31.7±9.0	31.2±5.7	−1.6
Temporal 3–5	81.2±11.8	77.3±16.7	−4.8
Temporal 8–10	23.2±8.0	24.2±6.2	+4.3
Nissl (ganglion cell layer)			
Nasal 3–5	119.5±12	67.2±11.7	−43.8 [a]
Nasal 8–10	38.8±10.5	19.5±7.8	−49.7 [a]
Temporal 3–5	181.0±24.2	91.3±26.0	−49.6 [a]
Temporal 8–10	37.7±5.3	25.5±5.0	−32.4 [a]

Figures correspond to mean number of labeled cells/1000 μm of transversely sectioned retina ± SD.
[a] ANOVA indicates significant difference between values for glaucomatous and non-glaucomatous eyes ($P < 0.01$).

in the bipolar cells that were well-labeled for calbindin in double-labeled preparations. A high degree of calretinin labeling was present in amacrine cells in the inner nuclear layer and their processes, organized in a laminated fashion, in the inner plexiform layer of these cynomolgus monkeys. Double-labeling verified that the calretinin-immunoreactive amacrine cells were a different subpopulation of neurons than the subpopulation of amacrine cells labeled for parvalbumin and that these latter cells were located deep to the calretinin-immunoreactive cells. The distribution of calretinin immunolabeling in cynomolgus retinas was similar to that noted for other monkey species, such as *Cercopithecus aethiops sabaeus* [27], although in this latter species calretinin immunoreactivity was also localized to many horizontal cells and cones.

NF triplet subunit immunoreactivity, determined with antibodies to NF-L and NF-M/NF-H, appeared to be localized principally to ganglion cells in the monkey retina, although a low degree of immunoreactivity was present in some of the displaced amacrine cells labeled for parvalbumin as well as in a very small subpopulation of neurons located in the inner nuclear layer adjacent to the inner plexiform layer. The distribution of ganglion neurons showing differential levels of immunoreactivity for these NF triplet subunits was very similar to that observed in this layer in other mammalian species, including humans [48,53]. Close to the center of the retina, many ganglion cells showed a low degree of immunoreactivity for these NF subunits but, with progressive distance from the center, many more cells showed elevated immunoreactivity for these NF subunits and many of these corresponded to relatively large cells. Concerning this latter subgroup of cells, NF immunoreactivity was observed to extend into the dendritic processes and the primary dendrites of these neurons could extend to the outermost part of the inner plexiform layer before branching. NF-immunoreactive processes were observed throughout the inner plexiform layer. Most of these are likely to correspond to the more distal segments of the dendrites of the larger NF-labeled ganglion cells. However, some of these processes possibly originate from the small subpopulation of putative NF-immunoreactive amacrine cells on either side of the inner plexiform layer or may even correspond to collateral axons from ganglion cells [3].

By comparing cell counts of NF-labeled ganglion cells with Nissl counts of cells from non-glaucomatous eyes, NF-labeled cells in the central nasal and temporal samples may account for ~37–40% of the total number of nerve cells and ~43 and 25% of the neurons in the peripheral samples of nasal and temporal retina, respectively. However, these figures cannot be considered as definitive as they are comparing immunofluorescent material with sections that have been alcohol-treated and, thus, probably shrunken, for Cresyl violet staining. In addition, while the cells counted in Nissl-stained sections are likely to correspond to nerve cells (see also 12), this stain would not distinguish between ganglion cells and other nerve cell types in this layer, such as displaced amacrine cells. Furthermore, it appears that the larger ganglion cells with a high degree of NF triplet immunoreactivity constitute a small proportion of all NF-immunoreactive cells, although their proportion is relatively larger in the peripheral samples. Also indicated by Nissl-stained and NF-labeled counts, there is a relatively larger number of neurons and NF-immunoreactive cells in the central samples from the temporal quadrant as compared with the nasal quadrant, which is likely to reflect the closer proximity of the temporal segment to the fovea.

4.2. Selective vulnerability of retinal ganglion cells in glaucoma

The quantitative assessment of immunoreactive cells in parvalbumin-, calbindin- and calretinin-labeled retinal sections from glaucomatous and non-glaucomatous fellow eyes clearly demonstrated that there is no significant difference in the number of neurons in the inner

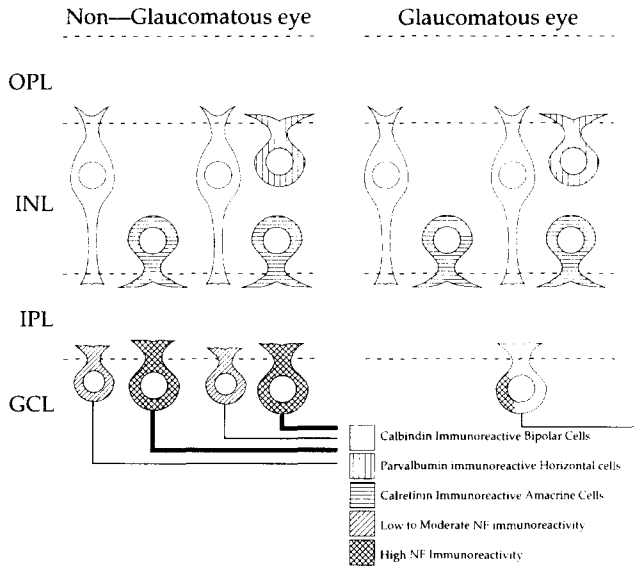

Fig. 5. Schematic summarizing distribution of neuronal markers in the retina of the glaucomatous eye relative to the non-glaucomatous eye. The data in the current study shows that neurons containing calcium-binding protein immunoreactivity in the inner nuclear layer are unaffected by the degenerative process associated with experimental glaucoma. In contrast, NF-immunoreactive cells in the ganglion cell layer demonstrate a high degree of vulnerability and neurons that remain in this layer may show an intraperikaryal accumulation of NF immunoreactivity. OPL, outer plexiform layer; INL, inner nuclear layer, IPL, inner plexiform layer; GCL, ganglion cell layer.

nuclear layer showing immunoreactivity for these calcium-binding proteins between these eyes (Fig. 5). The number of displaced amacrine cells in the ganglion cell layer, identified by their content of parvalbumin and calbindin immunoreactivity, was also relatively unaffected in the glaucomatous eyes, which was consistent with previous observations on the low degree of vulnerability of such cells in other experimental glaucoma studies [12]. In contrast, there was a significant depletion of NF-immunoreactive cells in the ganglion cell layer of the glaucomatous eyes relative to the non-glaucomatous eyes that is concomitant with a dramatic decrease in NF- immunoreactive fibers in the optic nerve fiber layer of the retina as well as in the optic nerve itself (Fig. 5). In central samples of retina, this loss resulted in ~59 and 77% fewer NF-immunoreactive cells in the nasal and temporal quadrants, respectively, of glaucomatous eyes as compared with the non-glaucomatous fellow eyes. The loss of NF-immunoreactive cells was even more pronounced in peripheral samples where there were ~96–97% fewer NF-labeled cells in the glaucomatous eyes than the non-glaucomatous eyes. Nissl counts indicated a fairly uniform loss of neurons across most of the samples (44–50%) with proportionately fewer cells lost in the peripheral temporal sample (32%). By comparing Nissl counts relative to the NF data, it would seem that the loss of NF-immunoreactive cells accounts for ~70 and 45% of the total number of neurons lost in the central nasal and temporal samples, respectively, and ~85 and 74% of cells in the peripheral nasal and temporal samples, respectively. Hence, these data show that there is a relatively large loss of cells within the subpopulation of ganglion cells containing NF triplet immunoreactivity and that, particularly for peripheral regions, the loss of NF-labeled cells accounts for a large proportion of the total number of ganglion cell layer neurons lost in these glaucomatous primate eyes.

The NF-containing neurons in the ganglion cell layer, therefore, represent a subgroup of retinal neurons particularly vulnerable to glaucoma-induced damage in these experimental animals. The larger ganglion cells containing a relatively high degree of NF immunoreactivity in the peripheral retina appear to be particularly vulnerable whereas the subgroup of smaller neurons closer to the fovea that contain a relatively low degree of NF immunoreactivity are proportionately less affected. These data correspond closely to the evidence presented by Quigley and associates [12,13,32–34] that the larger ganglion neurons of the retina are preferentially affected in glaucoma in humans as well as in experimentally induced glaucoma in monkeys. The mean loss of neurons throughout the ganglion cell layer of the animals investigated in the current study correlates well with the level of cell loss observed in experimental animals with 'moderate' damage reported in Glovinsky et al. [12]. In this latter study, the peak of selective loss of large cells is noted at this moderate stage.

The large retinal ganglion cells that were intensely labeled for NF antibodies may be identical with the α-like cells stained with silver methods that are present in many vertebrate species [28,46] and may, thus, correspond to A, Pa or parasol cells described in morphological studies of the primate retina [23,29,30,57]. Indeed, Silveira and Perry [46] have shown that the neurons heavily stained with silver impregnation, methods that have a chemical avidity for the NF triplet proteins [1] and a histochemical selectivity for NF triplet-containing neurons [53], are likely to correspond to the 10% of neurons in the monkey retina that comprise the magnocellular (M) pathway. This pathway is thought to be principally involved in encoding motion-, luminance- and depth-related visual information [25,42] and projects preferentially to the magnocellular layers of the lateral geniculate nucleus [23,30]. In a similar fashion to the subpopulation of peripheral sensory neurons that contain intense NF triplet immunoreactivity [20,52], these neurons are also likely to give rise to the larger optic nerve fibers that have a faster speed of conductance than smaller axons, underlying the importance of the neurons that contain a high degree of NF immunoreactivity as a main contributor to the M pathway. The relatively large proportion of

NF-immunoreactive cell bodies in peripheral nasal retina as compared with peripheral temporal retina can be considered equivalent to the findings on the distribution of silver-stained neurons [46] and may be correlated with a relatively larger dendritic field of such peripheral M cells [46,57].

It is also important to consider which subpopulation of retinal ganglion cells corresponds to those that contain faint immunoreactivity for the NF triplet, which were also shown to be vulnerable to glaucomatous damage, although to a lower degree than the larger neurons showing high NF immunoreactivity. If NF content in axons is correlated generally with axon diameter and conduction velocity [8,17,20,58], then it may be expected that the differential amount of NF immunoreactivity in ganglion cells may be correlated with axonal size. Thus, the faintly immunoreactive cells may correspond to a cell type that gives rise to axons intermediate in size to those neurons with the largest or smallest axons. These neurons may, therefore, be considered as the larger neurons comprising a subcomponent of the P pathway, corresponding morphologically to B, [23] Pβ [30] or midget [57] cells, and projecting preferentially to the parvocellular layers of the lateral geniculate nucleus [23,30]. The exact cell type showing this kind of NF immunoreactivity requires further morphological analysis, although it is consistent with the proposed heightened vulnerability of larger P cells with particular chromatic response properties as discussed by Glovinsky et al. [12] and with studies indicating a physiological deficit in parvocellular layers of the dorsal lateral geniculate nucleus in experimental glaucoma [47] and also with widespread alterations in metabolic and neuronal markers in both magnocellular as well as parvocellular layers of the dorsal lateral geniculate nucleus of the monkeys examined in the current study (J.C. Vickers, R.A. Schumer, S.M. Podos and J.H. Morrison, unpublished data).

It remains to be established to what extent a neuron's content of a cytoskeletal protein class, such as the NF triplet, is primarily or secondarily linked to its vulnerability to degenerate in glaucoma. Alterations in NF have been clearly linked with neuronal degeneration with a number of other degenerative diseases affecting the brain, including Alzheimer's disease [16,26,54], Parkinson's disease [50] and amyotrophic lateral sclerosis [22]. The parallels with Alzheimer's disease are particularly intriguing as selective vulnerability to degeneration in the cortex in Alzheimer's disease has been linked to the subpopulation of projection neurons containing the NF triplet whereas locally projecting cortical interneurons containing calbindin, parvalbumin and calretinin have been shown to be less affected [16]. Furthermore, there are pathologic alterations in NF that occur in cortical neurons that may be antecedent to the formation of the hallmark neurofibrillary tangle in Alzheimer's disease [26,54] and perikaryal accumulations of NF are also observed in some of the surviving neurons in the glaucomatous eyes of the current study. Interestingly, studies of optic nerve degeneration in Alzheimer's disease indicate a preferential vulnerability of larger axons [37] and preliminary studies indicate an alteration in NF in peripherally located large ganglion cells [24]. Susceptibility to perikaryal alterations and/or accumulations of NF have also been observed within peripherally located large ganglion cells in aluminum-treated rabbits [9] and in relatively old transgenic mice containing the human NF-M subunit [55]. Hence, there may be important parallels between the age-related disease process underlying Alzheimer's disease and that which leads to glaucoma.

Damage to optic nerve fibers at the level of the lamina cribrosa may be expected to cause an alteration in cytoskeletal proteins as part of a neurons general chromatolytic response, although experimental studies of optic nerve transection [41] would suggest that optic nerve damage may not entirely account for the large depletion of NF-labeled cell bodies and fibers seen in the current study. Ischemia and/or excitatory amino acid toxicity have been linked to alterations in the cytoskeleton (reviewed in [56]) and selective degeneration of retinal neurons [45,49,51], which may in turn be due to the specific distribution of particular excitatory amino acid receptor subtypes to subclasses of neurons [15,18]. Evidence of an increase in levels of glutamate in the posterior vitreous in the glaucomatous eyes of the monkeys used in the current report [40] as well as in human glaucoma [6] may further support the proposal that excitatory amino acid toxicity may have a role to play in the degeneration of retinal neurons in glaucoma. Further studies examining the distribution of excitatory amino acid receptor subunits in primate retina, in combination with experimental studies of neuroprotective agents, may indicate that therapeutic strategies that seek to attenuate excitatory amino acid toxicity may be helpful in preventing retinal damage in glaucoma.

4.3. Conclusions

These studies suggest that much of the intrinsic circuitry of the retina remains relatively intact in the eyes of monkeys in which elevated intraocular pressure and glaucoma have been experimentally induced. In contrast, retinal ganglion cells die in this condition and many of the susceptible ganglion cells are identified by their content of a cytoskeletal protein class, the NF triplet. An equivalent subpopulation of retinal ganglion cells in the human retina is selectively labeled with NF antibodies [48] and further studies may verify whether this neuronal subpopulation is also vulnerable in hu-

man glaucoma and whether there is a similar relative preservation of other retinal cell types. The identification of large ganglion cells that contain NF immunoreactivity as ostensibly the primary vulnerable neuronal cell type in peripheral retina lends support to the possible usefulness of clinical tests that address the M-pathway functions of peripheral retina as a possible way of revealing the earliest damage associated with glaucoma. Studies using other specific neuronal markers may also be helpful in determining the particular features of these neurons that contribute directly towards their vulnerability and may be suggestive of interventions that may protect these cells in glaucoma. Finally, the molecular profile of vulnerability that emerges from these studies of the glaucomatous retina bears important similarities to that of cortical neurons in Alzheimer's disease, suggesting that there may be commonalities in the mechanisms of neuron death across these two age-related neurodegenerative disorders.

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References

[1] Autilio-Gambetti, L., Crane, R. and Gambetti, P., Binding of Bodian's silver and monoclonal antibodies to defined regions of human neurofilament subunits: Bodians's silver reacts with a highly charged unique domain of neurofilaments, *J. Neurochem.*, 46 (1986) 327–332.

[2] Coleman, A.L., Quigley, H.A., Vitale, S. and Dunkelberger, G., Displacement of the optic nerve head by acute changes in intraocular pressure in monkey eyes, *Ophthalmology*, 98 (1991) 35–40.

[3] Dacey, D.M., Wide-spreading terminal axons in the inner plexiform layer of the cat's retina: evidence for intrinsic axon collaterals of ganglion cells, *J. Comp. Neurol.*, 242 (1985) 247–262.

[4] Dräger, U.C., Coexistence of neurofilaments and vimentin in a neurone of adult mouse retina, *Nature (London)*, 303 (1983) 169–172.

[5] Dräger, U.C., Edwards, D.L. and Barnstable, C.J., Antibodies against filamentous components in discrete cell types of the mouse retina, *J. Neurosci.*, 4 (1984) 1025–1042.

[6] Dreyer, E.B. and Lipton, S.A., A proposed role for excitatory amino acids in glaucoma visual loss, *Invest. Ophthalmol. Vis. Sci.*, 34 (Suppl.) (1993) 1504.

[7] Endo, T., Kobayashi, M., Kobayashi, S. and Onaya, T., Immunocytochemical and biochemical localization of parvalbumin in the retina, *Cell Tiss. Res.*, 243 (1986) 213–217.

[8] Friede, R.L. and Samorajski, T., Axonal calibre related to neurofilaments and microtubules in sciatic nerve fibres of rats and mice, *Anat. Rec.*, 167 (1970) 379–386.

[9] Fry, K.R., Edwards, D.M., Shaw, K.A. and Watt, C.B., The rabbit retina: a long-term model system for aluminum-induced neurofibrillary degeneration, *Neurosci. Lett.*, 124 (1991) 216–220.

[10] Gaasterland, D., Tanishima, T. and Kuwabara, T., Axoplasmic flow during chronic experimental glaucoma, *Invest. Ophthalmol. Vis. Sci.*, 17 (1978) 838–846.

[11] Gábriel, R. and Straznicky, C., Immunocytochemical localization of parvalbumin- and neurofilament triplet protein immunoreactivity in the cat retina: colocalization in a subpopulation of AII amacrine cells, *Brain Res.*, 595 (1992) 133–136.

[12] Glovinsky, Y., Quigley, H.A. and Dunkelberger, G.R. Retinal ganglion cell loss is size dependent in experimental glaucoma, *Invest. Ophthalmol. Vis. Sci.*, 32 (1991) 484–491.

[13] Glovinsky, Y., Quigley, H.A. and Pease, M.E., Foveal ganglion cell loss is size dependent in experimental glaucoma, *Invest. Ophthalmol. Vis. Sci.*, 34 (1993) 395–400.

[14] Hamano, K., Kiyama, H., Emson, P.C., Manabe, R., Nakauchi, M. and Tohyama, M., Localization of two calcium binding proteins, calbindin (28 kD) and parvalbumin (12 kD), in the vertebrate retina, *J. Comp. Neurol.*, 302 (1990) 417–424.

[15] Hamassaki-Britto, D.E., Hermans-Borgmeyer, I., Heinemann, S. and Hughes, T., Expression of glutamate receptor genes in the mammalian retina: the localization of GluR1 through GluR7 mRNAs, *J. Neurosci.*, 13 (1993) 1888–1898.

[16] Hof, P.R. and Morrison, J.H., The cellular basis of cortical disconnection in Alzheimer disease and related dementing conditions. In R.A. Terry et al. (Eds.), *Alzheimer Disease*, Raven Press, New York, NY, 1994, pp. 197–229.

[17] Hoffman, P.N., Thompson, G.W., Griffin, J.W. Landes, P.W. Cowan, N.J. and Price, D.L., Neurofilament gene expression: a major determinant of axonal calibre, *Proc. Natl. Acad. Sci. USA*, 84 (1987) 3472–3476.

[18] Hughes, T.E., Hermans-Borgmeyer, I. and Heinemann, S., Differential expression of glutamate receptor genes (GluR1-5) in the rat retina, *Vis. Neurosci.*, 8 (1992) 49–55.

[19] Kivelä, T., Tarkkanen, A. and. Virtanen, I., Intermediate filaments in the human retina and retinoblastoma, *Invest. Ophthalmol. Vis. Sci.*, 27 (1986) 1075–1084.

[20] Lawson, S.N. and Waddell, P.J., Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons, *J. Physiol. (London)*, 435 (1991) 41–63.

[21] Lee, P.-Y., Podos, S.M., Howard-Williams, J.R., Severin, C.H., Rose, A.D. and Siegel, M.J., Pharmacological testing in the laser-induced monkey glaucoma model, *Curr. Eye Res.*, 4 (1985) 775–781.

[22] Leigh, P.N. and Swash, M., Cytoskeletal pathology in motor neuron diseases. In L.P. Rowland (Ed.), *Advances in Neurology, Vol. 56, Amyotrophic Lateral Sclerosis and Other Motor Neuron Diseases*, Raven Press, New York, NY, 1991 pp. 115–124.

[23] Leventhal, A.G., Rodiek, R.W. and Dreher, B., Retinal ganglion cell classes in the old world monkey: morphology and central projections, *Science*, 213 (1981) 1139–1142.

[24] Martin, L.J., Pardo, C.A., Price, D.L. and Troncoso, J.C., Neuritic pathology in the retina in Alzheimer's disease, *J. Neuropathol. Exp. Neurol.*, 50 (1991) 302.

[25] Merigan, W.H. and Maunsell, J.H.R., How parallel are the primate visual pathways? *Annu. Rev. Neurosci.*, 16 (1993) 369–402.

[26] Metuzals, J., Robitaille, Y., Houghton, S., Gauthier, S., Kang, C.Y. and Leblanc, R., Neuronal transformations in Alzheimer's disease, *Cell Tiss. Res.*, 252 (1988) 239–248.
[27] Pasteels, B., Rogers, J. Blachier, F. and Pochet, R., Calbindin and calretinin localization in retina from different species, *Vis. Neurosci.*, 5 (1990) 1–16.
[28] Peichl, L., Ott, H. and Boycott, B.B., Alpha ganglion cells in mammalian retinae, *Proc. R. Soc. London*, 231 (1987) 169–197.
[29] Perry, V.H. and Cowey, A., The morphological correlates of X- and Y-like retinal ganglion cells in the retina of monkeys, *Exp. Brain Res.*, 43 (1981) 226–228.
[30] Perry, V.H., Oehler, R. and Cowey, A., Retinal ganglion cells that project to the dorsal lateral geniculate nucleus in the macaque monkey, *Neuroscience*, 12 (1984) 1101–1123.
[31] Quigley, H.A., Addicks, E.M., Green, W.R. and Maumenee, A.E., Optic nerve damage in human glaucoma. II. The site of injury and susceptibility to damage, *Arch. Ophthalmol.*, 99 (1981) 635–649.
[32] Quigley, H.A., Sanchez, R.M., Dunkelberger, G.R., L'Hernault, N.L. and Baginski, T.A., Chronic glaucoma selectively damages large optic nerve fibers, *Invest. Ophthalmol. Vis. Sci.*, 28 (1987) 913–920.
[33] Quigley, H.A., Dunkelberger, G.R. and Green, W.R., Chronic human glaucoma causing selectively greater loss of large optic nerve fibers, *Ophthalmology*, 95 (1988) 357–363.
[34] Quigley, H.A., Dunkelberger, G.R. and Green, W.R., Retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma, *Am. J. Ophthalmol.*, 107 (1989) 453–464.
[35] Radius, R. and Pederson, J.E., Laser-induced primate glaucoma, *Arch. Ophthalmol.*, 102 (1984) 1693–1698.
[36] Röhrenbeck, J., Wässle, H. and Boycott, B.B., Horizontal cells in the monkey retina: immunocytochemical staining with antibodies against calcium binding proteins, *Eur. J. Neurosci.*, 1 (1989) 407–420.
[37] Sadun A.A. and Bassi, C.J., Optic nerve damage in Alzheimer's disease, *Ophthalmology*, 97 (1990) 9–17.
[38] Sanna, P.P., Keyser, K.T., Celio, M.R., Karten, H.J. and Bloom, F.E., Distribution of parvalbumin immunoreactivity in the vertebrate retina, *Brain Res.*, 600 (1993) 141–150.
[39] Schumer, R.A. and Podos, S.M., The nerve of glaucoma! *Arch. Ophthalmol.*, 112 (1994) 37–44.
[40] Schumer, R.A., Podos, S.M., Lipton, S.A. and Dreyer, E.B., Increased glutamate in the vitreous of monkeys with induced glaucoma, *Invest. Ophthalmol. Vis. Sci.*, 35 (Suppl.) (1994) 1484.
[41] Scherer, J. and Schnitzer, J., Intraorbital transection of the rabbit optic nerve: consequences for ganglion cells and neuroglia in the retina, *J. Comp. Neurol.*, 312 (1991) 175–192.
[42] Shapley, R., Visual sensitivity and parallel retinocortical channels, *Annu. Rev. Psychol.*, 41 (1990) 635–658.
[43] Shaw, G. and Weber, K., The structure and development of rat retina: an immunofluorescence microscopical study using antibodies specific for intermediate filament proteins, *Eur. J. Cell Biol.*, 30 (1983) 219–232.
[44] Shaw, G. and Weber, K., The intermediate filament component of the retina: a comparison between different mammalian species, *Eur. J. Cell Biol.*, 33 (1984) 95–104.
[45] Siliprandi, R., Canella, R., Carmignoto, G., Schiavo, N., Zanellato, A., Zanoni, R. and Vantini, C., *N*-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. *Visual Neurosci.*, 8 (1992) 567–573.
[46] Silveira, L.C.L. and Perry, V.H., The topography of magnocellular projecting ganglion cells (M-ganglion cells) in the primate retina, *Neuroscience*, 40 (1991) 217–237.
[47] Smith, III, E.L., Chino, Y.M., Harwerth, R.S., Ridder, W.H., Crawford, M.L.J. and DeSantis, L., Retinal inputs to the monkey lateral geniculate nucleus in experimental glaucoma, *Clin. Vis. Sci.*, 8 (1993) 113–139.
[48] Straznicky, C., Vickers, J.C., Gábriel, R. and Costa, M., A neurofilament protein antibody selectively labels a large ganglion cell type in the human retina, *Brain Res.*, 582 (1992) 123–128.
[49] Sucher, N.J., Aizenman, E. and Lipton, S.A., *N*-methyl-D-aspartate antagonists prevent kainate neurotoxicity in rat ganglion cells in vitro, *J. Neurosci.*, 11 (1991) 966–971.
[50] Trojanowski, J.Q., Schmidt, M.L., Shin, R.-W., Bramblett, G.T., Rao, D. and Lee, V.M.-Y., Altered tau and neurofilament proteins in neurodegenerative diseases: diagnostic implications for Alzheimer's disease and Lewy body dementias, *Brain Pathol.*, 3 (1993) 45–54.
[51] Tung, N.N., Morgan, I.G. and Ehrlich, D., A quantitative analysis of the effects of excitatory neurotoxins on retinal ganglion cells in the chick, *Vis. Neurosci.*, 4 (1990) 217–223.
[52] Vickers, J.C. and Costa, M., Neurofilament protein triplet immunoreactivity in the dorsal root ganglia of the guinea-pig, *Cell Tiss. Res.*, 265 (1991) 159–167.
[53] Vickers, J.C. and Costa, M., The neurofilament triplet is present in distinct subpopulations of neurons in the central nervous system of the guinea-pig, *Neuroscience*, 49 (1992) 73–100.
[54] Vickers, J.C., Delacourte, A. and Morrison, J.H., Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease, *Brain Res.*, 594 (1992) 273–278.
[55] Vickers, J.C., Lazzarini, R.A., Friedrich, V.L., Janssen, W.G., Elder, G.A. and Morrison, J.H., Cell-type specific neurofilamentous accumulations in transgenic mice expressing the human mid-sized neurofilament subunit, *Soc. Neurosci. Abstr.*, 19 (1993) 1689.
[56] Vickers, J.C., Huntley, G.W., Edwards, A.M., Moran, T., Rogers, S.W., Heinemann, S.F. and Morrison, J.H., Quantitative localization of AMPA/kainate and kainate glutamate receptor subunit immunoreactivity in neurochemically identified subpopulations of neurons in the prefrontal cortex of the macaque monkey, *J. Neurosci.*, 13 (1993) 2982–2992.
[57] Watanabe, M. and Rodiek, R.W., Parasol and midget ganglion cells of the primate retina, *J. Comp. Neurol.*, 289 (1989) 434–554.
[58] Weiss, P.A. and Mayer, R., Organelles in neuroplasmic ('axonal') flow. *Proc. Natl. Acad. Sci. USA*, 68 (1971) 846–850.