Laboratory Science

Magnocellular and parvocellular visual pathways are both affected in a macaque monkey model of glaucoma

JC Vickers, PhD,[1] PR Hof, MD,[2,3,4] RA Schumer, MD, PhD,[4] RF Wang, MD,[4] SM Podos, MD[4] and JH Morrison, PhD[2,3]

[1]Division of Pathology, Clinical School, University of Tasmania, Australia and [2]Fishberg Research Center for Neurobiology, [3]Department of Geriatrics and Adult Development and [4]Department of Ophthalmology, Mount Sinai School of Medicine, New York, USA

Abstract

Purpose: Neurochemical changes in nerve cells were investigated in the lateral geniculate nucleus (LGN) and primary visual cortex of macaque monkeys with experimentally induced glaucoma.

Methods: Glaucomatous damage was induced in one eye of experimental animals by elevation of intraocular pressure following laser burns to the trabecular meshwork. Staining for the metabolic marker cytochrome oxidase, as well as immunolabelling for the neuronal markers synaptophysin and neurofilament proteins, was conducted on sections of the LGN and primary visual cortex.

Results: In the LGN, staining for cytochrome oxidase and immunolabelling for synaptophysin were reduced in the parvocellular and magnocellular layers that received input from the glaucomatous eye and neurofilament protein labelling was reduced in the parvocellular layers. Cytochrome oxidase staining demonstrated the presence of denervated ocular dominance columns in layer IVC of the primary visual cortex of experimental animals.

Conclusions: Pre- and post-synaptic neurochemical alterations in the magnocellular and parvocellular visual pathways of the brain are associated with experimentally induced glaucoma in macaque monkeys.

Key words: cytochrome oxidase, glaucoma, lateral geniculate nucleus, magnocellular, neurofilaments, parvocellular, retina, synaptophysin, visual cortex.

Primary open angle glaucoma in humans and experimentally induced glaucoma in monkeys results in the selective degeneration of specific retinal neuron subtypes.[1] In particular, larger ganglion cells that are likely to correspond to the magnocellular (M) visual pathway are particularly vulnerable, although smaller ganglion cells, such as parvocellular (P) cells, can also be affected.[1–6] In contrast, retinal interneurons do not degenerate.[1]

How this disease may affect other visual centres of the brain is of particular interest with respect to the progressive nature of cellular degeneration in the retina, as well as the segregation of the M and P pathway in the lateral geniculate nucleus (LGN) and visual cortex. Autopsy studies of human patients with glaucoma are indicative of transynaptic cell damage to the M layers of the LGN but not P layers.[7] Similarly, radioactive transport studies from glaucomatous eyes in experimental monkeys indicate preferential damage to the M pathway relative to the P pathway.[8] However, Smith *et al.*[9] have demonstrated deficits in visually responsive cells in both P and M layers post-synaptic to the glaucomatous eye in experimental monkeys.

Following on from our report on selective neuronal vulnerability in the retina of glaucomatous monkeys,[1] we have now investigated the glaucoma-induced cytochemical changes that occur in the LGN and visual cortex of these animals. The production of glaucomatous damage to one eye only, as well as the controlled tissue processing conditions for immunohistochemistry, may make these experimental animals an ideal model system for unravelling the involvement of M and P pathways in glaucoma. We have used the metabolic marker cytochrome oxidase, as well as immunohistochemical markers for synaptic terminals (synaptophysin) and cell bodies (neurofilament proteins), to elaborate possible glaucoma-induced changes in pre- and post-synaptic neuronal components in the M and P layers of the LGN. The distribution of neurofilament protein

■ *Correspondence:* Dr J Vickers, Division of Pathology, Clinical School, University of Tasmania, 43 Collins Street, Hobart, Tasmania 7000, Australia. Email: <James.Vickers@path.utas.edu.au>

labelling is of particular interest, as we have observed that the M cells of the monkey retina preferentially contain this protein.[1] In addition, we have also examined area V1 of the cortex for possible transneuronal cellular changes in higher visual pathways as a result of this retinal damage.

METHODS

A total of seven Cynomolgus monkeys (*Macaca fascicularis*) were used in the present study, four of which had undergone experimentally induced glaucoma[10,11] in one eye following the procedures outlined in our previous report.[1] Briefly, experimental animals were sedated with ketamine hydrochloride (5 mg/kg, i.m.) and received 0.5% topical proparacaine followed by multiple argon laser burns to the mid portion of the trabecular meshwork of one eye. Damage to the trabecular meshwork disrupts drainage of aqueous humour from the eye, thus resulting in the increased intraocular pressure that is typically associated with glaucoma. Animals were monitored for sustained increased intraocular pressure and were retreated with laser burns as required. Animals were also ophthalmologically monitored for the development of glaucoma-related changes (excavation) of the optic nerve head. Treatment parameters and animal maintenance were conducted at the Mount Sinai School of Medicine (NY, USA) in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolutions concerning animal welfare, as well as the Institutional Animal Care and Use Committee (IACUC) of the Mount Sinai School of Medicine. Three of the animals included in the present study had been used in previous analyses.[1,12] These monkeys had high intraocular pressure (range 30–40 mmHg; normal 12–20 mmHg), after 126–226 days and increased cup/disc ratios (defined as the ratio of the horizontal diameter of the central excavation of the optic nerve head to that of the total nerve head; range 0.9–1.0; normal 0.1–0.3[1]).

To optimally prepare the tissue for immunohistochemical techniques, animals were deeply anaesthetized with ketamine (25 mg/kg, i.m.) and Nembutal (30 mg/kg, i.v.; Boehringer Inglehein, Artamon, NSW, Australia) and were perfused transcardially through the left ventricle initially with phosphate-buffered 1% paraformaldehyde for 1 min followed by phosphate-buffered 4% paraformaldehyde for 8–10 min. Brains were then removed and post-fixed in the 4% paraformaldehyde solution for a total of 6 h followed by cryoprotection through a series of graded sucrose solutions (12, 16 and 18%). The LGN was frozen and sectioned at 40 µm in the coronal plane in two experimental animals and in three control animals and in the sagittal plane in two further experimental animals. Tissue sections were processed for cytochrome oxidase (CO) histochemistry[13] and immunohistochemical markers for neurofilament proteins (SMI-32, recognizes a non-phosphorylated epitope on the middle and high molecular weight neurofilament triplet subunits, dilution 1:5000; Sternberger Monoclonals Inc., Baltimore, MD, USA) and synaptophysin (dilution 1:200; Boehringer-Mannheim, Mannheim, West Germany). These antibodies were visualized using standard immunoperoxidase techniques (Vectastain ABC kit; Vector Labs, Burlingame, CA, USA) and 3,3'-diaminobenzidine in the presence of hydrogen peroxide as the reactive chromogen. Blocks of the primary visual cortex (area V1) were sectioned at 40 µm in the coronal plane as well as tangentially to the pial surface and were stained with CO histochemistry.

RESULTS

Examination of CO staining of LGN sections in control animals demonstrated high cell body and neuropil labelling in the M and P layers, with the interlaminar regions showing a comparatively low degree of staining (Fig. 1). In the experimental animals, M and P layers that received input from the glaucomatous eye showed a lower level of CO staining than layers corresponding to the non-glaucomatous eye (Fig. 1). Synaptophysin immunoreactivity was principally localized

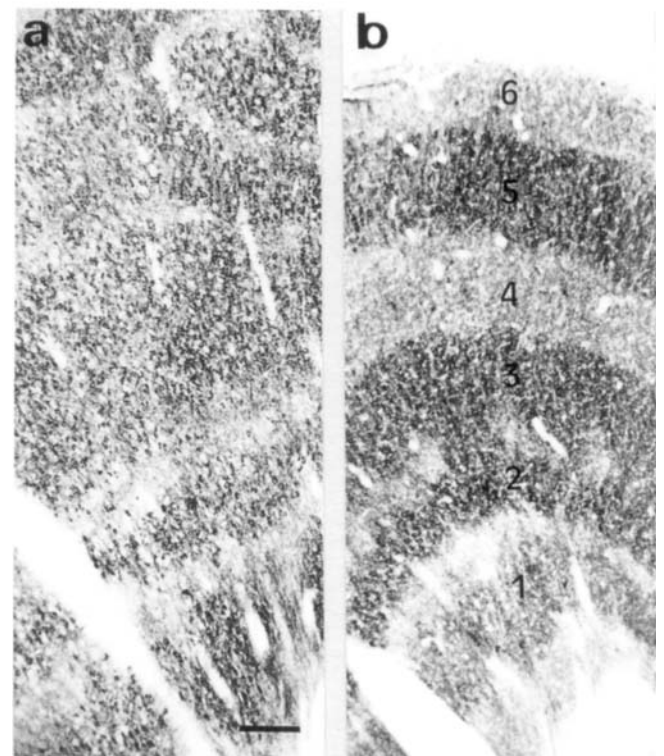

Figure 1. Lateral geniculate nucleus sections stained with cytochrome oxidase from (a) a control, non-glaucomatous animal and (b) a glaucomatous animal showing decreased cytochrome oxidase staining in layers 1, 4 and 6, corresponding to damage to the contralateral eye. Bar, 200 µm.

to the neuropil throughout the LGN (Fig. 2). In experimental animals, synaptophysin immunolabelling was markedly reduced in layers corresponding to input from the glaucomatous eye (Fig. 2).

Neurofilament (SMI-32) immunoreactivity was localized to nerve cells located in all six layers of the LGN, with M cells showing a relatively high degree of labelling (Fig. 3). Decreased neurofilament immunoreactivity was present in the P layers that received input from the glaucomatous eye relative to the intact layer, but no decrement was observed in the M layers (Fig. 3).

In area V1 of the cerebral cortex, CO staining was very similar to that previously described.[15] Periodic decreased CO staining was localized to layer IVC α and β in experimental animals (Fig. 4).

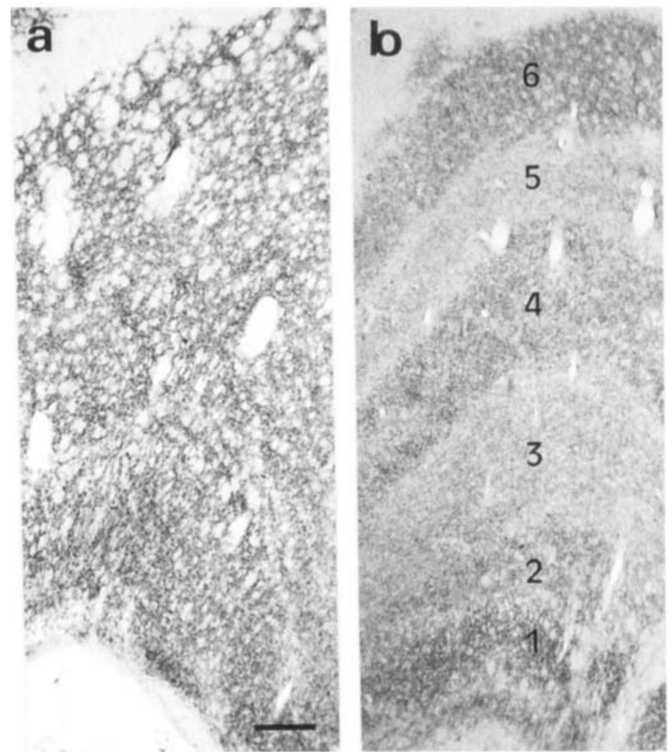

Figure 2. Lateral geniculate nucleus sections immunolabelled for the synaptic marker synaptophysin from (a) a control, non-glaucomatous animal and (b) an experimental animal showing decreased synaptophysin immunoreactivity in layers 2, 3 and 5 (ipsilateral glaucomatous eye). Bar, 200 μm.

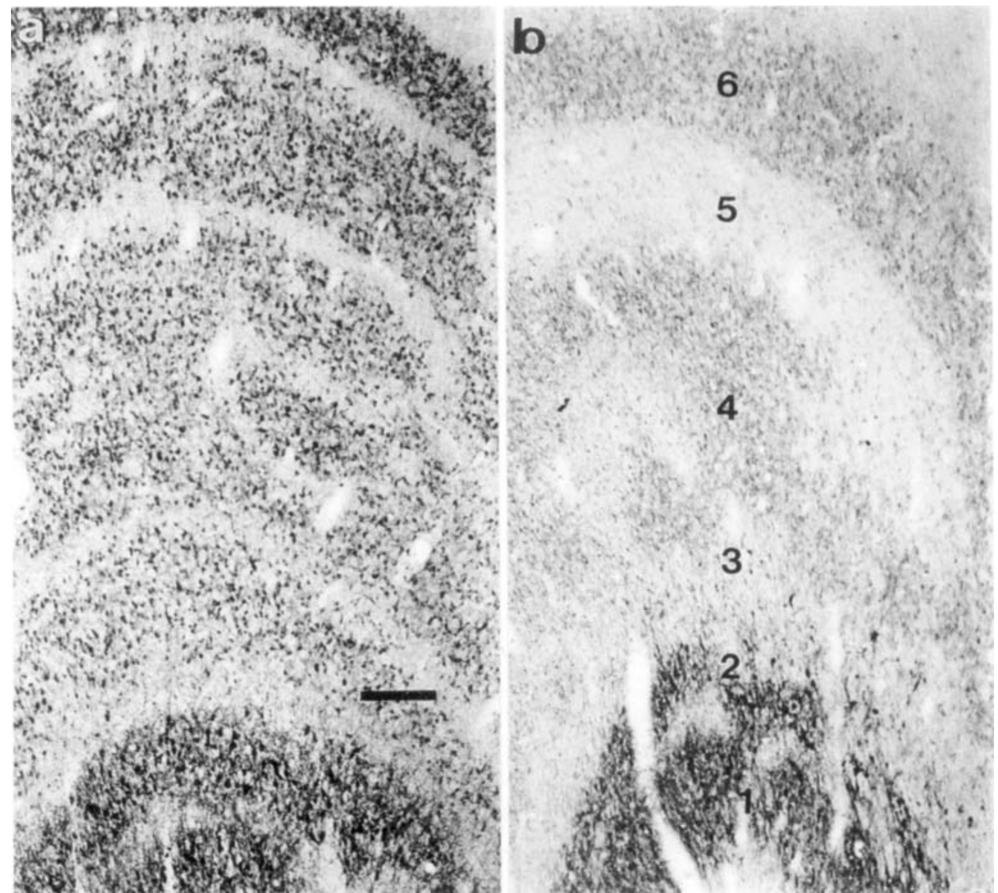

Figure 3. Lateral geniculate nucleus (LGN) sections immunolabelled for neurofilament proteins from a control, non-glaucomatous animal (a). Magnocellular layers show relatively high neurofilament immunoreactivity. (b) Sections of LGN from an experimental animal showing decreased neurofilament immunoreactivity in parvocellular layers 3 and 5, which receive input from the ipsilateral damaged eye. Bar, 200 μm.

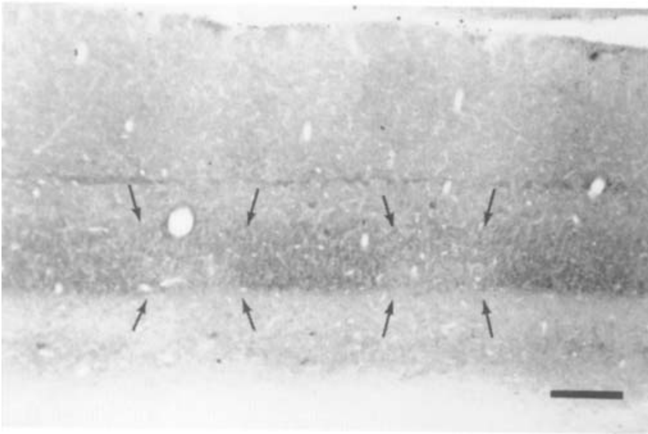

Figure 4. Section of the primary visual cortex from a glaucomatous animal with the pial surface at the top. Cytochrome oxidase staining shows a pronounced periodic decrease of labelling IVC (indicated by small arrows). Bar, 200 μm.

Discussion

These results demonstrate that experimental glaucoma can lead to changes in neurochemical features of both the M and P pathways in the LGN. These data are consistent with the notion that while glaucoma may preferentially cause damage to M cells in the retina, P cells are also affected.[1,2] Presynaptic alterations in the M and P retinal projections are indicated by decreased synaptophysin immunoreactivity in the corresponding layers of the LGN, although ultrastructural studies would be required to further characterize the precise extent of presynaptic axon terminal loss. Furthermore, specific neurochemical alterations appear to occur in LGN post-synaptic neurons, including decreased CO activity in all layers corresponding to the damaged eye and, interestingly, a pronounced decrease in immunoreactivity for neurofilaments in the P layers. It is not certain whether the decreased neurofilament immunoreactivity in LGN neurons is due to down-regulation of neurofilament expression or a change in the morphological features of neurons (e.g. transneuronal atrophy[14]) that secondarily leads to decreased amounts of these proteins.

In addition, it is interesting that the M layers of the LGN contain cells showing relatively high levels of immunoreactivity for neurofilaments. In a similar fashion, M retinal ganglion cells of the retina contain high neurofilament immunoreactivity, with some parvocellular cells showing more moderate levels of labelling for this protein class.[1] These data indicate that the M visual pathway may be characterized by a specific neurochemical phenotype and that while changes in these proteins occur in the M cells in the retina,[1] they do not necessarily occur in the neurons that they project to in the thalamus.

Our studies also demonstrate decreased CO staining in layer IVC α and β of area V1, in a pattern reminiscent of ocular dominance columns,[13,15] which is likely to be reflective of degeneration of both M and P pathways from the single glaucomatous eye.

Smith *et al.*[9] have shown there are deficits in the number of visually responsive cells in both the M and P layers in experimentally induced glaucoma. However, in human glaucoma, previous reports[7] have indicated that there is a decreased density of M layer cells but little or no change in the density of P layer cells. It would be instructive to ascertain whether neurochemical alterations similar to those we find in experimental monkeys also occur in human cases of glaucoma. Clearly, the animal model is a vital tool to unravelling associated brain changes, as there is the advantage of controlling the extent of glaucomatous damage and restricting it to one eye. Further quantitative data using unbiased stereological methods, taking into consideration both changes in neuronal and neuropil density as well as overall tissue shrinkage, are required to definitively ascertain the extent to which nerve cell loss occurs in LGN M and P layers in this experimental model. In addition, the macaque monkey model may be used to define whether a particular time course of the disease is associated with reversible cellular changes in the LGN as opposed to cell death. The extent of plasticity in these neurochemical changes in the adult brain[16] and the possibility of permanent cellular dysfunction in brain neurons that comprise pathways linked to damaged eyes are important considerations for therapeutic intervention in human glaucoma.

Acknowledgements

A-M Edwards, WGM Janssen and RS Woolley provided expert technical assistance. This work was supported by NIH grant AG06647 (JHM), CORE NIH grant EY01867 (SMP), an unrestricted grant to the Department of Ophthalmology (SMP) from Research to Prevent Blindness, the Rudin Foundation (SMP), an AMRAD Postdoctoral Award (JCV), the MSD Foundation (JCV), the Ian Potter Foundation (JCV), the National Glaucoma Research, a programme from the American Health Assistance Foundation (PRH) and the American Foundation for Aging Research (PRH). JCV is the recipient of an RD Wright Research Fellowship from the National Health and Medical Research Council of Australia.

References

1. Vickers JC, Schumer RA, Podos SM, Wang RF, Riederer BM, Morrison JH. Differential vulnerability of neurochemically identified subpopulations of retinal neurons in a monkey model of glaucoma. *Brain Res.* 1995; **680**: 23–35.
2. Glovinsky Y, Quigley HA, Dunkelberger GR. Retinal ganglion

cell loss is size dependent in experimental glaucoma. *Invest. Ophthalmol. Vis. Sci.* 1991; **32**: 484–91.

3. Glovinsky Y, Quigley HA, Pease ME. Foveal ganglion cell loss is size dependent in experimental glaucoma. *Invest. Ophthalmol. Vis. Sci.* 1993; **34**: 395–400.
4. Quigley HA, Sanchez RM, Dunkelberger GR, L'Hernault NL, Baginski TA. Chronic glaucoma selectively damages large optic nerve fibers. *Invest. Ophthalmol. Vis. Sci.* 1987; **28**: 913–20.
5. Quigley HA, Dunkelberger GR, Green WR. Chronic human glaucoma causing selectively greater loss of large optic nerve fibers. *Ophthalmology* 1988; **95**: 357–63.
6. Quigley HA, Dunkelberger GR, Green WR. Studies of retinal ganglion cell atrophy correlated with automated perimetry in human eyes with glaucoma. *Am. J. Ophthalmol.* 1989; **107**: 453–64.
7. Chaturvedi N, Hedley-White ET, Dreyer EB. Lateral geniculate nucleus in glaucoma. *Am. J. Ophthalmol.* 1993; **116**: 182–8.
8. Dandona L, Hendrickson A, Quigley HA. Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. *Invest. Ophthalmol. Vis. Sci.* 1991; **32**: 1593–9.
9. Smith EL III, Chino YM, Harwerth RS, Ridder WH, Crawford MLJ, DeSantis L. Retinal inputs to the monkey lateral geniculate nucleus in experimental glaucoma. *Clin. Vis. Sci.* 1993; **8**: 113–39.
10. Radius R, Pederson JE. Laser-induced primate glaucoma. II. Histopathology. *Arch. Ophthalmol.* 1984; **102**: 1693–8.
11. Lee P-Y, Podos SM, Howard-Williams JR, Severin CH, Rose AD, Siegel MJ. Pharmacological testing in the laser-induced monkey glaucoma model. *Curr. Eye Res.* 1985; **4**: 775–81.
12. Nimchinsky EA, Vickers JC, Young WG *et al.* An optical fractionator analysis of neuronal changes in the lateral geniculate nucleus of monkeys with experimental glaucoma. *Soc. Neurosci. Abstr.* 1996; **22**: 1607.
13. Wong-Riley MTT. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Res.* 1979; **171**: 11–28.
14. Guillery RW. Quantitative studies of transneuronal atrophy in the dorsal lateral geniculate nucleus of cats and kittens. *J. Comp. Neurol.* 1973; **149**: 423–38.
15. Wong-Riley M, Carroll EW. The effect of impulse blockage on cytochrome oxidase activity in monkey visual cortex. *Nature* 1984; **307**: 262–4.
16. Hendry S, Carder RK. Organization and plasticity of GABA neurons and receptors in monkey visual cortex. *Prog. Brain Res.* 1992; **90**: 477–502.