Pergamon

0042-6989(95)00235-9

Vision Res., Vol. 36, No. 10, pp. 1357–1363, 1996

Printed in Great Britain
0042-6989/96 $15.00 + .00

GABA Immunopositive Axons in the Optic Nerve and Optic Tract of Macaque Monkeys

J. R. WILSON,* A. COWEY,† P. SOMOGYI‡

Received 28 March 1995; in revised form 29 August 1995

Using an antibody to gamma-aminobutyric acid (GABA), we examined the optic nerves and optic tracts from macaque monkeys at the light and electron microscopic levels to determine if there is a possible inhibitory projection from the retina to the brain. All of the monkeys ($n = 5$) had GABA immunopositive axons that were evenly distributed in their optic nerves. These immunopositive axons were slightly larger than the axons around them and comprised an average of 2.6% of the axons in the nerves. Thus, their estimated total was about 44,000 axons per nerve. In the optic tracts, the GABA immunopositive axons were not distributed evenly, but were concentrated mostly in the ventromedial part, indicating that this retinal pathway probably goes to a midbrain destination such as the superior colliculus. The present findings provide further evidence that there is a GABAergic retinal projection to the brain in primates with currently unknown physiological influences. Copyright © 1996 Elsevier Science Ltd.

GABA Retina Optic nerve Electron microscopy (EM) Primate

INTRODUCTION

The retinae of all vertebrates that have been studied send afferents into the central nervous system where most of them make excitatory connections with their postsynaptic targets (Rodieck, 1973; Sillito, 1992). However, gamma-aminobutyric acid (GABA) is a known inhibitory neurotransmitter and has been localized in the cells of the retinal ganglion cell layer of monkeys, cats, rabbits, chicks, rats, toads, salamanders and turtles (Mosinger *et al.*, 1986; Osborne *et al.*, 1986; Gläsner *et al.*, 1988; Yu *et al.*, 1988; Caruso *et al.*, 1989; Hurd & Eldred, 1989; Koontz, *et al.*, 1989; Pourcho & Owczarzak, 1989; Wässle *et al.*, 1990; Hamassaki-Britto *et al.*, 1991; Gabriel *et al.*, 1992). Although many reports have presumed all such cells to be displaced amacrine cells (e.g. Osborne *et al.*, 1986; Wässle *et al.*, 1990), several studies have used retrograde transport and specific antibodies for ganglion cells to show that some of these GABA immunopositive cells are actually retinal ganglion cells (Gabriel *et al.*, 1992; Yu *et al.*, 1988). Furthermore, Kisvarday *et al.*, (1991) reported that 20% of the remaining retinal ganglion cell axon terminals found in the degenerated dorsal lateral geniculate nucleus (dLGN) of a unilaterally destriated macaque monkey were immunopositive for GABA, as were many axons in this monkey's optic nerve.

The presence of GABA immunopositive retinal ganglion cells, which may release GABA as a transmitter at their terminals, represents an entirely new aspect of retinal influence on structures in the brain and has significant implications for sensory information processing. This would be particularly important if a higher primate, that has a visual system very similar to our own, were to display a retinal GABA immunopositive projection.

In the present study, we examined the optic nerves and optic tracts of macaque monkeys to determine if they have a significant GABA immunopositive retinal projection into the brain.

METHODS

Brain tissue from the visual system (optic tract or nerve and dLGN) from four normal (two *Macaca mulatta*, two *Macaca fascicularis*) and one unilaterally destriated macaque monkey (*Macaca fascicularis*) were used. The surgical and histological procedures for the latter monkey are described in Kisvarday *et al.* (1991). One of the normal *M. fascicularis* monkeys had a bilateral section of the uncinate fasciculus that was assumed not to affect the visual system. In all cases, the monkey was deeply anesthetized and perfused transcardially first with 0.9% saline and then with 1% paraformaldehyde, followed by a

*To whom all correspondence should be addressed at: Yerkes Research Center, Emory University, Atlanta, GA 30322, U.S.A. [*Fax* 404-727-7729; *Email* Wilson@ Rmy.emory.edu].

†University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, U.K.

‡University of Oxford, Medical Research Council, Anatomical Neuropharmacology Unit, Mansfield Road, Oxford OX1 3TH, U.K.

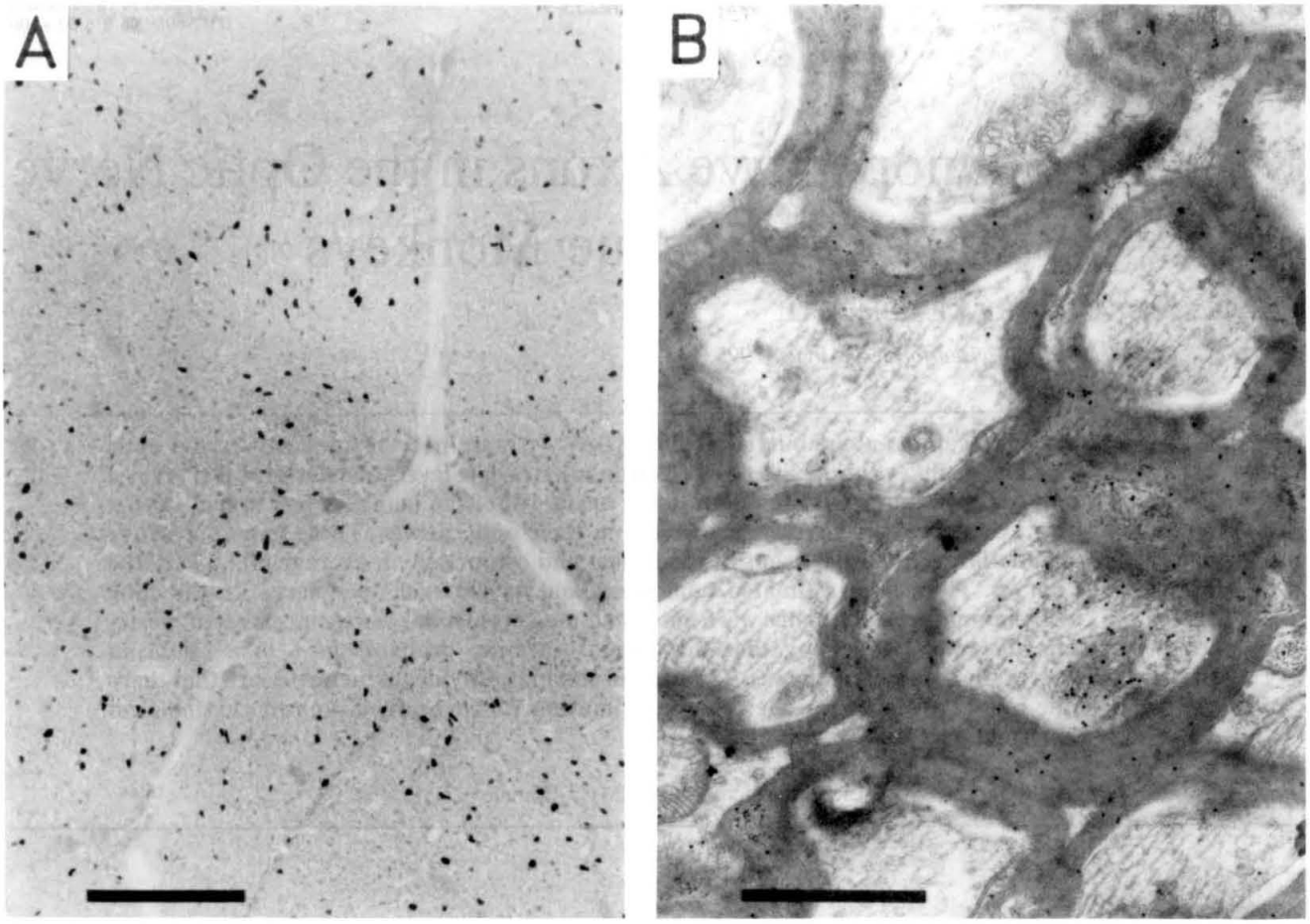

FIGURE 1. Examples of GABA immunopositive axons in the optic nerve of a macaque monkey. (A) Light photomicrograph showing distribution and relative density of GABA-labeled axons (dark spots). Scale bar = 50 μm. (B) Electron photomicrograph of optic nerve after immunogold treatment showing one GABA immunopositive axon that has many gold particles over its axoplasm. Scale bar = 1 μm.

combination of 1.25% paraformaldehyde and 2.5% glutaraldehyde fixatives in buffered solution. The optic nerves, optic tracts and brain were post-fixed in the same fixatives as used for the final perfusion, cut into small pieces or sectioned at 200 μm using a Vibratome®. They were then osmium treated (1% in phosphate buffer) and embedded in epoxy resin for thin sectioning.

Immunohistochemistry for GABA was carried out using the following procedures, which have been used in several previous studies (Kisvarday *et al.*, 1991; Somogyi, 1988). For light microscopy (LM), 0.5 μm thick sections were cut and mounted onto glass slides. The plastic was etched with saturated sodium ethanolate solution, the osmium removed with sodium periodate (1%) and the sections treated with normal swine serum (NSS, 20%) as a blocking solution. The sections were rinsed in Tris–phosphate buffered saline (TPBS) between steps and were covered with a solution of rabbit anti-GABA serum (diluted to 1:2000 or 1:4000) for 60 min followed by 50 min in horseradish peroxidase-coupled secondary antibody (swine-anti-rabbit IgG-HRP, diluted 1:50 in 1% NSS). Peroxidase activity was visualized with 3,3′-diaminobenzidine tetrahydrochloride (0.05%) and hydrogen peroxide (0.003%). Finally, the staining was intensified by a mild osmium treatment, and the sections dehydrated and cover-slipped.

The procedure for labeling immunoreactive GABA for electron microscopy (EM) has been published earlier (Somogyi, 1988). Thin sections were cut at about 70–80 nm thickness and picked up on nickel mesh or slotted grids coated with pioloform. These sections were treated with periodic acid (1% for 10 min) and sodium periodate (1% for 10 min). Some sections were treated overnight with 0.25% Triton X-100 solution containing the primary antibody (Phend *et al.*, 1992). The antiserum to GABA (Hodgson *et al.*, 1985) was used at a dilution of 1:1000–1:2000. Goat anti-rabbit IgG coupled to 15 nm gold particles (BioClinical Services Ltd, Cardiff, U.K.) was used at a dilution of 1:25 in Tris–buffer saline (pH 8.2 for 60 min), followed by a rinse in distilled water and staining with saturated uranyl acetate for 50 min.

The primary antiserum to GABA has previously been shown to recognize GABA fixed under the above conditions (Hodgson *et al.*, 1985). Control sections were run in parallel for all procedures by omitting the primary antibody or replacing it with 1% normal rabbit serum. Additionally, to exclude the possibility that the highly GABA immunoreactive optic nerve axons might contain

exceptionally high levels of glutamate, a putative transmitter of some retinal ganglion cells and possible antigen for the antibody, we also reacted serial sections of optic nerve and cerebellum with antibodies to fixed glutamate (Liu *et al.*, 1989). To further test the specificity of the methods and antibody, the antiserum to GABA (diluted 1:2000) was absorbed to fixed excess GABA or glutamate, and the antibodies to glutamate (diluted 1:4000) were absorbed to fixed glutamate. The results of these control experiments clearly ruled out the possibility that the GABA antibody was reacting with a high concentration of glutamate in the axons rather than with GABA (see also Hodgson *et al.*, 1985).

The density of gold particles was determined for axon profiles in the EM sections by using electron photomicrographs printed at 20,800 × or 27,000 × to count grains, and a computer-linked digitizing tablet was used to measure the profile areas.

To estimate the number of GABA immunopositive axons in an optic nerve, one area of each nerve was selected from three normal monkeys and the unilaterally destriated monkey. Each of these areas contained well-labeled axons without any obvious clustering. The number of GABA immunopositive axons was then counted using a camera lucida drawing of the area (63 × oil immersion objective). The area containing these counts was also measured using the same computer-linked digitizing tablet.

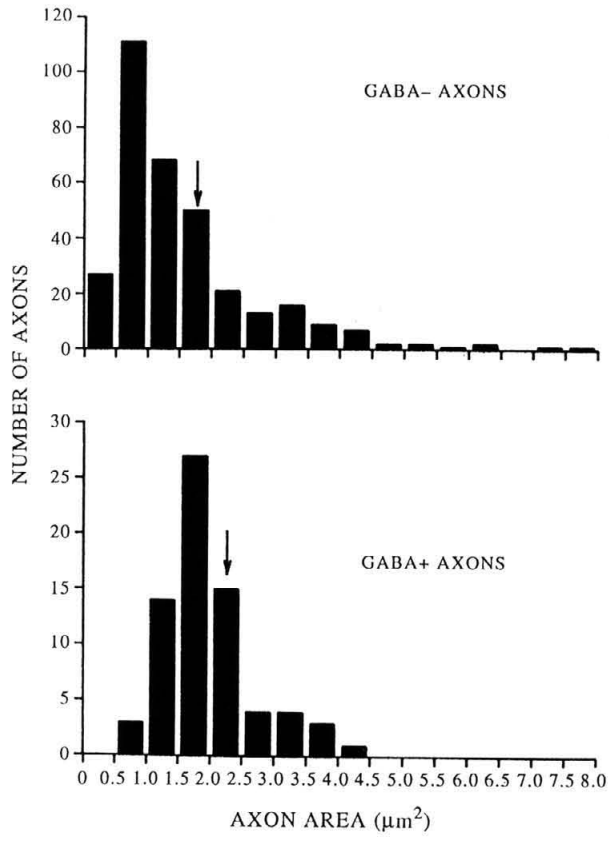

FIGURE 2. Bar graph showing the distribution of grains over optic nerve axons observed to be GABA immunopositive and immunonegative at the electron microscopic level. The arrows indicate the average grain densities for the immunonegative and immunopositive axons.

RESULTS

Optic Nerves

Semi-thin cross-sections of optic nerves showed that many axons had dense GABA immunolabeling [Fig. 1(A)]. Control sections (with no primary antibody or primary antiserum absorbed to GABA) showed no labeling. Each labeled axon had a brown reaction product that made it stand out from the surrounding, unlabeled axons. The GABA immunopositive axons were distributed evenly across the entire nerve with a few clusters occurring in an apparently non-systematic manner. In some nerves, areas that were presumably not well perfused had no immunolabeled axons at all. These latter areas were also consistently pale when toluidine blue staining was used on adjacent sections.

To ascertain that these profiles were indeed myelinated axons of the optic nerve, immunogold labeling was carried out on thin sections of the same tissue. By using semi-thin sections (0.5 μm) interleaved between thin sections (70 nm) and both reacted for GABA, an area that contained a small cluster of GABA immunopositive axons was aligned between the LM and EM sections. Upon EM examination of the sections, there was a low number of gold particles over the axoplasm of most axons and a higher density of particles over the myelin sheaths. More importantly, there were occasional axons with high densities of grains over their axoplasms [Fig. 1(B)]. All of the 18 GABA-positive axons seen at the light microscopic level were found to have high densities of gold particles over their axoplasms at the EM level (average = 33 particles/μm^2 ± 16.7 SD; n = 18; Fig. 2). The other unlabeled axons seen at the LM level had low densities of gold particles at the EM level (average = 3 particles/μm^2 ± 2.59 SD; n = 425). Thus, it was confirmed that the GABA immunopositive labeled profiles seen in the light microscope were the axoplasm of myelinated axons of the optic nerve.

The densities of GABA immunopositive axons within the optic nerves from the three normal monkeys (average of two samples each) were determined to be 6240, 7970 and 5000 labeled axons per mm^2. The total measured cross-sectional areas of these three optic nerves were 5.94, 7.28 and 7.11 mm^2. Therefore, the estimated numbers of GABA immunopositive axons in each nerve were 37,000, 58,000 and 35,600 (average = 43,600). The most recent study of axons in the macaque monkey's optic nerve estimated that there were 1.5–1.8 million axons, all of which were myelinated (Potts *et al.*, 1972). This total corresponds closely to that estimated by Perry and Cowey (1985) from counts of retinal ganglion cells. We can confirm that all the axons we saw were myelinated and estimate that about 2.6% of these were GABA immunopositive. The density of GABA immunopositive axons in the optic nerve of the cortically lesioned monkey was 5,200 labeled axons/mm^2, but its cross-

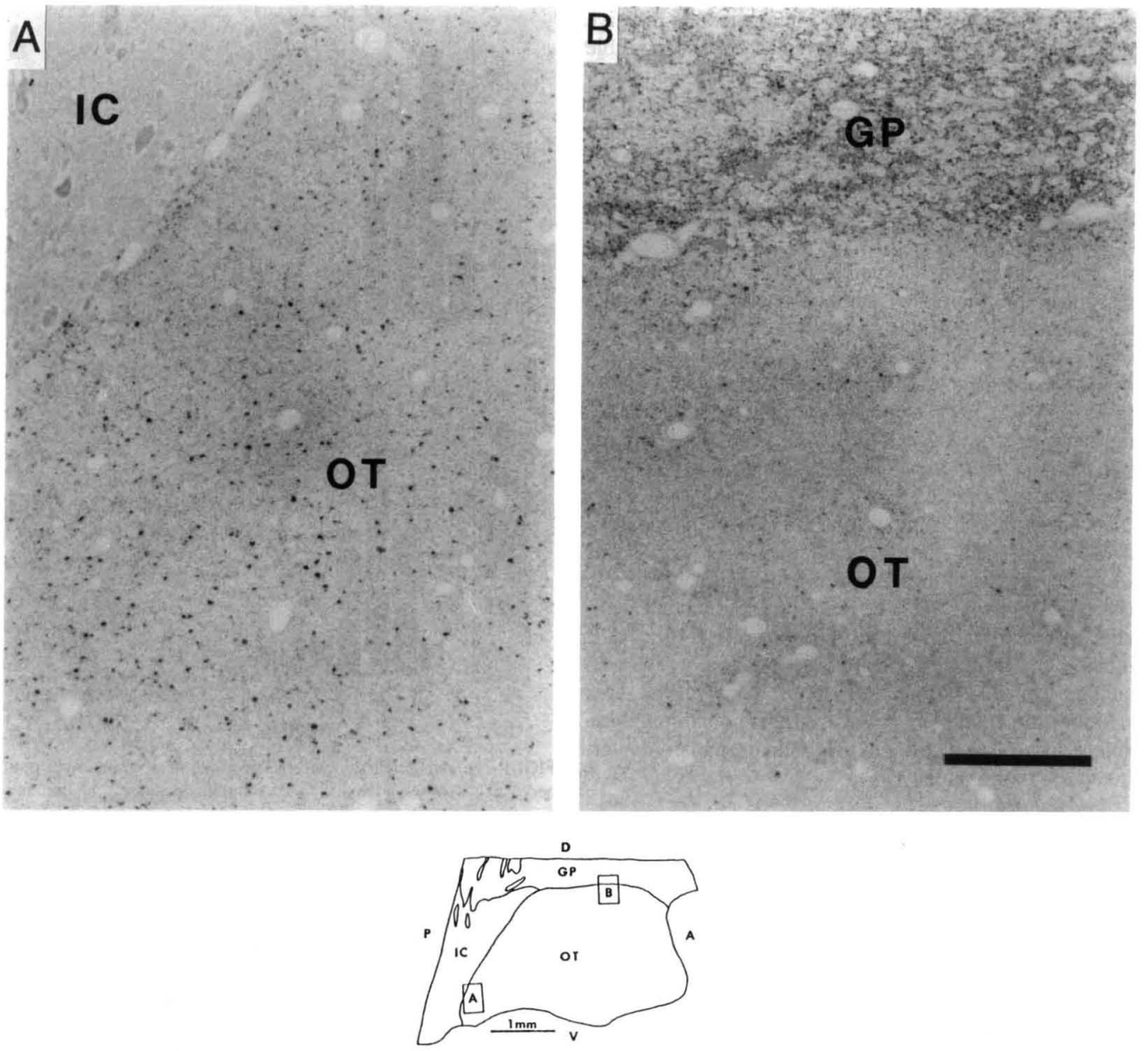

FIGURE 3. Light photomicrographs of different regions of an obliquely sectioned optic tract from a macaque monkey showing GABA immunopositive axons. (A) Ventromedial region of the optic tract (OT); IC is the internal capsule. (B) Dorsolateral region of the optic tract just below the ventral part of the globus pallidus (GP). Note the greater density of GABA immunopositive axons in the ventromedial vs the dorsolateral region. Scale bar = 100 μm.

sectional area was only 3.69 mm^2, presumably because of the loss of retinal ganglion cells resulting from transneuronal retrograde degeneration (Van Buren, 1963; Cowey *et al.*, 1989).

Cross-sectional areas of the axons were measured within the myelin sheaths of labeled and unlabeled axons in a small region of one normal optic nerve. The average area for the axons labeled for GABA was 1.98 μm^2 ± 0.73 SD (range = 0.81–4.24 μm^2, n = 71), whereas the unlabeled axons averaged 1.56 μm^2 ± 1.20 SD (range = 0.22–7.87 μm^2; n = 425). Thus, the average GABA immunopositive axon was significantly larger than the average GABA immunonegative axon (t = 2.85, $P < 0.005$), but GABA immunopositive axons were not the largest axons in the optic nerve.

Optic Tract

Two monkeys had their optic tracts examined for GABA immunopositive axons and one had both nerves and tracts examined. The optic nerve sections were located about 6 mm from the globes and the sections of optic tracts were located about 4 mm from the optic chiasm, i.e., about midway between chiasm and dLGN and 5–6 mm lateral to the midline. On one side, the sections were cut perpendicular to the tract (oblique to frontal or sagittal planes) and the other tract was cut in a sagittal plane. These were processed and embedded in plastic.

Semi-thin sections from both nerves and tracts were reacted for GABA. Figures 1 and 3 show that, although the GABA immunopositive axons were evenly spread

through the optic nerves, labeled axons in the optic tract were much more prevalent in the ventromedial part compared to the other parts, with the lowest density of labeled axons observed in the dorsolateral part. Axons of Pβ ganglion cells (also known as P-cells) project to the parvocellular dLGN (Perry *et al.*, 1984) and occupy the dorsal part of the optic tract, whereas axons of Pα cells (also called M-cells) and axons destined for the midbrain (Pγ and Pε cells) occupy predominantly the ventral parts (Reese & Cowey, 1990). The GABA immunopositive axons are therefore most likely to be from Pα, Pγ or Pε cells. Our data indicate that GABA immunopositive axons redistribute themselves once they pass through the optic chiasm and reside in the part of the optic tract that generally goes to the midbrain but, unfortunately, do not permit us to determine exactly which midbrain region receives them.

The initial impetus for studying GABA in the optic nerves resulted from observing GABAergic terminals in the dLGN that were either anterogradely labeled or had characteristics of retinal terminals (Kisvarday *et al.*, 1991). These terminals had pale mitochondria, large sizes and round synaptic vesicles (see review by Wilson, 1993). When we searched the dLGN and pregeniculate nucleus from one of the normal monkeys described here, there were very few ($< 1\%$) GABA immunopositive terminals that could possibly originate from the retina, leading to the conclusion that the latter might be collaterals of the axons terminating in the midbrain. The increased relative density of GABA positive terminals in the dLGN, following unilateral removal of striate cortex (Kisvarday *et al.*, 1991), may be simply the result of retrograde degenerative loss of other terminals and gross shrinkage of the dLGN.

DISCUSSION

Our results show that there are GABA immunopositive axons in the optic nerves and optic tracts of macaque monkeys. These axons are evenly distributed in the optic nerves, but are located mostly in the ventromedial part of the optic tract, suggesting that they may provide a direct inhibitory retinal projection to a midbrain structure.

The presence of immunoreactive GABA in axons and nerve terminals of the brain of adult mammals correlates well with (1) the presence of the enzyme glutamate decarboxylase that synthesizes GABA for the neurotransmitter pool (Ottersen & Storm-Mathisen, 1984; Mugnaini & Oertel, 1985); (2) a very low level of immunoreactive glutamate in the terminals (Somogyi *et al.*, 1986); and (3) inhibitory neurotransmission when the GABA is released. However, a notable exception is the granule cell of the hippocampal dentate gyrus, which gives rise to terminals strongly immunopositive for both GABA and glutamate (Sandler & Smith, 1991). Granule cells and their terminals lack glutamate decarboxylase and only glutamate seems to act as their neurotransmitter (e.g. Weisskopf *et al.*, 1993). In contrast to the retinal axons, all hippocampal granule cell axons are immunopositive for GABA, suggesting that GABA immunoreactivity in a select population of retinal axons, as observed here, is a different phenomenon from that in hippocampal granule cells. Although it remains to be established whether the terminals of the GABA-labeled axons in the retinal projection are enriched in GABA, the clear neurochemical difference from the majority of optic nerve axons suggests that GABA has a transmitter role in this system.

In the primate retina, some of the GABA immunopositive cells or axons in the ganglion cell layer have been thought to be retinal ganglion cells or their axons (Koontz *et al.*, 1989), but other studies have concluded that these cells are displaced amacrine cells (Wässle *et al.*, 1990). Some GABA immunopositive cells in the retinae of rabbits and toads have been shown conclusively to be retinal ganglion cells (Gabriel *et al.*, 1992; Yu *et al.*, 1988). While it cannot be ruled out from our study, the possibility that the observed GABA immunopositive axons are going to the retina as retinofugal fibers is remote because there is little evidence of such a projection in mammals (see Uchiyama, 1989). Only a few anterogradely labeled retinofugal axons were seen following large deposits of HRP in the optic nerve of macaque monkeys (Perry *et al.*, 1984). The retinae of the normal monkeys of the present study were not available for analysis, but immunohistochemical results from them would have been difficult to interpret for two reasons. First, without further retrograde label, the cells in the ganglion cell layer would not be identified easily as either ganglion cells or displaced amacrine cells. Second, any lack of GABA labeling in ganglion cells would not mean necessarily that the cells do not use GABA as a transmitter because several cell types in the brain (e.g. cerebellar Purkinje cells) show little immunolabeling for GABA in the somata despite having a high level in their axons and terminals.

Kisvarday *et al.* (1991) studied the retinae of normal monkeys and those from a monkey that had long-standing complete removal of the striate cortex of one hemisphere. The areas of the retina that projected to the lesioned cortex (via the dLGN) had extensive depletion of retinal ganglion cells (up to 80% in the macula) with many of the remaining cells showing a GABA immunopositive reaction, along with many GABA immunopositive axons passing into the optic nerve. Some retinal terminals in the dLGN of this monkey were also immunopositive for GABA using the immunogold labeling technique. However, few GABA immunopositive axons were observed in the optic nerve of the normal monkey used in that study. The present results suggest that GABA immunopositive axons are much more common in the normal optic nerve than Kisvarday *et al.* (1991) believed and that the GABA immunoreactivity observed by them in the degenerated hemiretina and in the retinal terminals in the degenerated dLGN may not reflect an increase in GABA immunopositive neurons and axons *per se*.

It was observed in the present study that the distribution of the GABA immunopositive axons was essentially random in the optic nerves. However, this was

not the case in the optic tracts, and the redistribution of the GABA immunopositive axons into the ventromedial part of the optic tract indicates that these axons were probably destined for a midbrain structure. Gabriel *et al.* (1992) labeled retinal ganglion cells retrogradely in the toad by dye injections into the optic tectum followed by immunohistochemistry for GABA. Quantitative counts of double-labeled retinal ganglion cells provided a value of 2.8% of the total retinal ganglion cells as being GABA immunopositive. This is very close to the value of 2.6% GABA immunopositive axons we have found in the macaque's optic nerve and suggests that the superior colliculus (tectum) might be the target of these axons. The fact that the GABA immunopositive axons were thicker than most of their immediate neighbors also suggests that they might belong to a sub-group of ganglion cells projecting to the midbrain, e.g. the large E-cells or Pε cells described, respectively, by Leventhal *et al.* (1981) and by Perry and Cowey (1984).

Presently, it is unclear why part of the retinal projection to the brainstem might have a direct inhibitory effect. Part of another long-distance inhibitory projection in the visual system, the pretectogeniculate pathway, terminates on interneurons, where it would presumably cause disinhibition, i.e., facilitation of retinal signals passing through projection cells (Cucchiaro *et al.*, 1993). Significantly, all the postsynaptic targets of the surviving retinogeniculate terminals in the degenerated dLGN of the decorticated monkey were also GABA immunopositive interneurons (Kisvarday *et al.*, 1991). Because the likely targets of the retinal projection to the midbrain, including the superior colliculus or tectum, also contain inhibitory interneurons, it cannot be ruled out that these neurons are the termination points of the retinal, GABAergic projection and that the resulting action might be disinhibition.

REFERENCES

Caruso, D. M., Owczarzak, M. T., Goebel, D. J., Hazlett, J. C. & Pourcho, R. G. (1989). GABA-immunoreactivity in ganglion cells of the rat retina. *Brain Research*, *476*, 129–134.

Cowey, A., Stoerig, P. & Perry, V. H. (1989). Transneuronal retrograde degeneration of retinal ganglion cells after damage to striate cortex in macaque monkeys: Selective loss of Pβ cells. *Neuroscience*, *29*, 65–80.

Cucchiaro, J. B., Uhlrich, D. J. & Sherman, S. M. (1993). Ultrastructure of synapses from the pretectum in the A-laminae of the cat's lateral geniculate nucleus. *Journal of Comparative Neurology*, *334*, 611–630.

Gabriel, R., Straznicky, C. & Wye-Dvorak, J. (1992). GABA-like immunoreactive neurons in the retina of *Bugo marinus*: Evidence for the presence of GABA-containing ganglion cells. *Brain*, *571*, 175–179.

Gläsner, G., Himstedt, W., Weiler, R. & Matute, C. (1988). Putative neurotransmitters in the retinae of three model species (*Triturus alpestris*, *Salamandra salamandra*, *Pleurodeles waltli*). *Cell Tissue Research*, *252*, 317–328.

Hamassaki-Britto, D. E., Brzozowska-Prechtl, A., Karten, H. J., Linstrom, J. M. & Keyser, K. T. (1991). GABA-like immunoreactive cells containing nicotinic acetylcholine receptors in the chick retina. *Journal of Comparative Neurology*, *313*, 394–408.

Hodgson, A. J., Penke, B., Erdei, A., Chubb, I. N. & Somogyi, P. (1985). Antisera to gamma-aminobutyric acid. I. Production and characterization using a new model system. *Journal of Histochemistry and Cytochemistry*, *33*, 229–239.

Hurd, L. B. & Eldred, W. D. (1989). Localization of GABA- and GAD-like immunoreactivity in the turtle retina. *Visual Neuroscience*, *3*, 9–20.

Kisvarday, Z. F., Cowey, A., Stoerig, P. & Somogyi, P. (1991). Direct and indirect retinal input into degenerated dorsal lateral geniculate nucleus after striate cortical removal in monkey: Implications for residual vision. *Experimental Brain Research*, *86*, 271–292.

Koontz, M. A., Hendrickson, A. E. & Ryan, M. K. (1989). GABA-immunoreactive synaptic plexus in the nerve fibre layer of primate retina. *Visual Neuroscience*, *2*, 19–25.

Leventhal, A. G., Rodieck, R. W. & Dreher, B. (1981). Retinal ganglion cell classes in old-world monkey; morphology and central projections. *Science*, *213*, 1139–1142.

Liu, C.-J., Grandes, P., Matute, C., Cuenod, M. & Streit, P. (1989). Glutamate-like immunoreactivity revealed in rat olfactory bulb, hippocampus and cerebellum by monoclonal antibody and sensitive staining method. *Histochemistry*, *90*, 427–445.

Mosinger, J. L., Yazulla, S. & Studholme, K. M. (1986). GABA-like immunoreactivity in the vertebrate retina: A species comparison. *Experimental Eye Research*, *42*, 631–644.

Mugnaini, E. & Oertel, W. H. (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In Bjorklund, A. & Hokfelt, T. (Eds), *Handbook of chemical neuroanatomy, Vol. 4: GABA and neuropeptides in the CNS* (pp. 436–595). Amsterdam: Elsevier.

Osborne, N. N., Patel, S., Beaton, D. W. & Neuhoff, V. (1986). GABA neurones in retinas of different species and their postnatal development *in situ* and in culture in the rabbit retina. *Cell Tissue Research*, *243*, 117–123.

Ottersen, O. P. & Storm-Mathisen, J. (1984). Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. *Journal of Comparative Neurology*, *229*, 374–392.

Perry, V. H. & Cowey, A. (1984). Retinal ganglion cells that project to the superior colliculus and pretectum in the macaque monkey. *Neuroscience*, *12*, 1125–1137.

Perry, V. H. & Cowey, A. (1985). The ganglion cell and cone distributions in the monkey's retina: Implications for central magnification factors. *Vision Research*, *25*, 1795–1810.

Perry, V. H., Oehler, R. & Cowey, A. (1984). Retinal ganglion cells that project to the dorsal lateral geniculate nucleus in the macaque monkey. *Neuroscience*, *12*, 1101–1123.

Phend, K. D., Weinberg, R. J. & Rustioni, A. (1992). Techniques to optimize post-embedding single and double staining for amino acid neurotransmitters. *Journal of Histochemistry and Cytochemistry*, *40*, 1011–1020.

Potts, A. M., Hodges, D., Shelman, C. B., Futz, K. J., Levy, N. S. & Mangnall, Y. (1972). Morphology of the primate optic nerve. I. Methods and total fibre count. *Investigative Ophthalmology*, *11*, 980–988.

Pourcho, R. G. & Owczarzak, M. T. (1989). Distribution of GABA immunoreactivity in the cat retina: A light and electron-microscopic study. *Visual Neuroscience*, *2*, 425–435.

Reese, B. E. & Cowey, A. (1990). The fibre organization of the monkey's optic tract. 1. Segregation of functionally distinct optic axons. *Journal of Comparative Neurology*, *295*, 385–400.

Rodieck, R. W. (1973). *The vertebrate retina. Principles of structure and function*. San Francisco, CA: W. H. Freeman and Company.

Sandler, R. & Smith, A. D. (1991). Coexistence of GABA and glutamate in mossy fiber terminals of the primate hippocampus: An ultrastructure study. *Journal of Comparative Neurology*, *303*, 177–192.

Sillito, A. M. (1992). Excitatory, inhibitory and neuromodulatory influences in central visual function. In Aertsen, A. & Braitenberg, V. (Eds), *Information processing in the cortex*, (pp. 325–365). Berlin: Springer.

Somogyi, P. (1988). Immunocytochemical demonstration of GABA in physiologically characterized, HRP-filled neurons and their postsynaptic targets. In van Leeuwen, F. W., Buijs, R. M., Pool, C. W. &

Poch, O. (Eds), *Molecular Neuroanatomy. Techniques in the Behavioral and Neural Sciences* (pp. 339–359). Amsterdam: Elsevier.

Somogyi, P., Halasy, K., Somogyi, J., Storm-Mathisen, J. & Ottersen, O. P. (1986). Quantification of immunogold labelling reveals enrichment of glutamate in mossy and parallel fibre terminals in cat cerebellum. *Neuroscience*, *19*, 1045–1050.

Uchiyama, H. (1989). Centrifugal pathways to the retina: Influence of the optic tectum. *Visual Neuroscience*, *3*, 183–206.

Van Buren, J. M. (1963). Trans-synaptic retrograde degeneration in the visual system of primates. *Journal of Neurology, Neurosurgery and Psychiatry*, *26*, 402–409.

Wässle, H., Grünert, U., Röhrenbeck, J. & Boycott, B. B. (1990). Retinal ganglion cell density and cortical magnification factor in the primate. *Vision Research*, *30*, 1897–1911.

Weisskopf, M. G., Zalutsky, R. A. & Nicoll, R. A. (1993). The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature*, *362*, 423–427.

Wilson, J. R. (1993). Circuitry of the dorsal lateral geniculate nucleus in the cat and monkey. *Acta Anatomica*, *147*, 1–13.

Yu, B. G.-Y., Watt, C. B., Lam, D. M. K. & Fry, K. R. (1988). GABAergic ganglion cells in the rabbit retina. *Brain Research*, *439*, 376–382.

Acknowledgements—We gratefully thank David Roberts and Diane Latawiec for their excellent technical support, Jean Torbit for her secretarial assistance and Frank Kiernan for his photographic work. This research was supported in part by NIH Grant RR00165 from the National Center for Research Resources to the Yerkes Regional Primate Research Center and by the United Kingdom Medical Research Council. The Yerkes Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care.