Neuroscience Vol. 41, No. 1, pp. 213–226, 1991
Printed in Great Britain

0306-4522/91 $3.00 + 0.00
Pergamon Press plc
IBRO

A GABAERGIC PROJECTION FROM THE PRETECTUM TO THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT

J. B. CUCCHIARO, M. E. BICKFORD and S. M. SHERMAN*

Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794-5230, U.S.A.

Abstract—To study the projection from the pretectum to the lateral geniculate nucleus, we placed wheat-germ agglutinin conjugated to horseradish peroxidase into the lateral geniculate nuclei of six cats, allowed this marker to be retrogradely transported by afferent axons to their parent somata in the pretectum, and revealed the label in these cells with stabilized tetramethylbenzidine histochemistry. In three cases we made large pressure injections that completely infiltrated the lateral geniculate nucleus and extended into neighboring thalamic nuclei; in the other three we made smaller iontophoretic injections largely confined to the A- and C-laminae of the lateral geniculate nucleus. In both types of injection we found labeled pretectal cells mainly in the nucleus of the optic tract but also found some cells labeled in the olivary pretectal nucleus and the posterior pretectal nucleus. After one of the larger injections we analysed both sides of the pretectum and found that 11% of the labeled cells were located contralaterally and were distributed in the same three nuclei. We analysed only the ipsilateral side in the remaining five cats. In those five experiments we also immunohistochemically stained the pretectal sections with an antibody directed against the neurotransmitter, GABA. Of the retrogradely labeled pretectal cells, 40% were also labeled for GABA, and those were similar in soma size (350 μm^2 in cross-sectional area) to those labeled only with the retrograde marker (331 μm^2). GABA-positive cells not labeled by retrograde transport were smaller (246 μm^2) than either of these other cell populations.

These results indicate that at least 40% of the cells involved in the projection from the pretectum to the lateral geniculate nucleus are GABAergic. We suggest that this extrathalamic projection may serve to inhibit thalamic GABAergic cells. This, in turn, would disinhibit geniculate relay cells, thereby facilitating the geniculate relay of retinal information to cortex.

The lateral geniculate nucleus is the most peripheral location at which nonretinal inputs, particularly those descending from visual cortex and ascending from the midbrain, can influence the relay of visual information to cortex. The nature of the modifications imposed upon the transmission of retinal signals to cortex, or retinogeniculate transmission, is poorly understood. However, in cats, where this has been most thoroughly studied, activation of the brainstem pathways is generally associated with shifts in attention and increased arousal.[27,45,46]

One mechanism underlying these changes is the control by brainstem axons of thalamic neuronal circuits that inhibit geniculate relay cells. These circuits involve two types of inhibitory cells that use the neurotransmitter GABA: intrinsic interneurons and extrinsic cells of the thalamic reticular nucleus or perigeniculate nucleus, which, in cats, may be part of the thalamic reticular nucleus. Brainstem axons generally inhibit these thalamic GABAergic neurons, thereby releasing relay cells from tonic inhibition and enhancing retinogeniculate transmission. This has been best documented for the cholinergic pathway that originates in the parabrachial region of the midbrain. Acetylcholine, the neurotransmitter used by the majority of parabrachial axons, both directly excites relay cells and directly inhibits both populations of GABAergic inhibitory cells.[8,28–30,47]

One brainstem pathway that has until now received relatively little attention is that from the pretectum to the lateral geniculate nucleus. In cats this projection emanates from three of the six pretectal nuclei: the nucleus of the optic tract, the posterior pretectal nucleus, and the olivary pretectal nucleus.[2,25,26] These three nuclei contain a high proportion of cells that may use GABA as a neurotransmitter, since they stain with antibodies directed against either GABA or glutamate decarboxylase, the rate-limiting enzyme for GABA synthesis.[11,39,40,50] Two observations suggest the possibility that some of these GABAergic cells in the pretectum are projection cells. Firstly, some of these cells, particularly in the nucleus of the optic tract, are quite large, which is not a general feature of interneurons.[11,50] Secondly, in our recent electron microscopic study of axons in the lateral geniculate nucleus labeled by the anterograde transport of *Phaseolus vulgaris* leucoagglutinin from the nucleus of the optic tract,[4] we found many labeled synaptic terminals with morphological features characteristic of GABAergic terminals.[9,32–34] These pretectal axons form terminal arbors in both the

*To whom correspondence should be addressed.
Abbreviations: HRP, horseradish peroxidase.

perigeniculate and lateral geniculate nuclei, and within the lateral geniculate nucleus, form synaptic contacts with cells having morphological features of GABAergic interneurons.[4] This raises the possibility that pretectal input, like that from the parabrachial region, serves to enhance retinogeniculate transmission by disinhibiting relay cells.

To test whether the pretectum provides a non-thalamic source of GABAergic input to the lateral geniculate nucleus, we combined the retrograde transport of wheat-germ agglutinin conjugated to horseradish peroxidase (HRP) from the lateral geniculate nucleus with immunocytochemical localization of GABA in cells of the pretectum. If pretectal cells contain both retrogradely transported HRP and GABA-positive immunocytochemical staining, this would suggest that those pretectal projection cells use GABA as a neurotransmitter. Not only would this furnish evidence for a relatively rare type of projection (given that the majority of GABAergic neurons are local circuit interneurons), but it would also provide an important step in determining the nature of the pretectal influence on retinogeniculate transmission.

EXPERIMENTAL PROCEDURES

We used six adult cats for these experiments. Each was prepared for physiological recording as described previously from this laboratory.[3] The cats were anesthetized initially with 4% halothane in a 1:1 mixture of N_2O/O_2, and during the experiment the animal was maintained on 1–2% halothane in a 7:3 mixture of N_2O/O_2 plus a continuous infusion of sodium pentobarbital (1 mg/kg per h). The animal was also paralysed with 5 mg of gallamine triethiodide followed by a continuous infusion of 3.6 mg/h gallamine triethiodide and 0.7 mg/h of D-tubocurarine. We artificially respired the cat and monitored end-tidal CO_2, electroencephalogram, and electrocardiogram. Body temperature was maintained at 38°C with a feedback heating blanket. We located the lateral geniculate nucleus by electrophysiological recordings of visually evoked activity guided by stereotaxic coordinates. We then used a micropipette (20–30 μm tip diameter) to inject a solution of 5% wheat-germ agglutinin conjugated to HRP in 0.2 M KCl and 0.1 M $NaPO_4$, pH 7.4. We made the injections with pressure (1.5–3.0 μl) in three experiments (designated in Results as cases 1–3) or iontophoresis in three other experiments (cases 4–6); for iontophoresis, we used 5-μA current pulses at 0.14 Hz for 40–60 min.

Following a survival time of 18–36 h, each cat was deeply anesthetized with sodium pentobarbital and transcardially perfused with a solution of 2–4% paraformaldehyde and 0.05–0.5% glutaraldehyde in phosphate buffer. We then removed the brain and placed it in phosphate buffer overnight; on the next day, we used a Vibratome to cut 40–50-μm-thick frontal sections. To reveal cells retrogradely labeled with HRP, the sections were reacted with tetramethylbenzidine in 0.2 M sodium acetate buffer at pH 3.3 for 20 min at room temperature.[31] We stabilized the tetramethylbenzidine crystals, first with 5% ammonium heptamolybdate in acetate buffer at pH 3.3 for 20 min at 4°C, and then with a solution containing 0.05% diaminobenzidine, 0.025% $CoCl_2$, and 0.01% H_2O_2 in phosphate buffer at pH 7.4 for 5–8 min at room temperature.[20]

We subsequently treated sections containing HRP-labeled cells in five of the six cats (cases 2–6) for the immunocytochemical localization of GABA. We put the sections into 2% bovine serum albumin in phosphate buffer at pH 7.4 for 20 min at room temperature. We then incubated them for 16 h at 4°C, using gentle agitation, in a solution containing the anti-GABA antibody (Incstar, Inc.; raised in rabbit) diluted 1:3000 in 1% bovine serum albumin and 0.3% Triton X-100 in phosphate buffer. All subsequent steps were carried out at room temperature. We washed the sections in three changes (10 min each) of 1% bovine serum albumin in phosphate buffer, and we then incubated them with gentle agitation in a solution of biotinylated anti-rabbit IgG (Vector Labs; raised in goat) diluted 1:200 in 1% bovine serum albumin in phosphate buffer for 1 h. The sections were then washed in three changes (10 min each) of 1% bovine serum albumin in phosphate buffer and incubated with gentle agitation in a solution of avidin and biotinylated HRP diluted 1:100 in 1% bovine serum albumin in phosphate buffer for 1 h. Finally, we washed the sections in three changes (10 min each) of phosphate buffer and reacted them with cobalt-intensified diaminobenzidine for 2–3 min. Control sections were treated similarly except that the primary antibody solution was either left out or replaced by a solution containing anti-GABA antibody that had been preincubated (24 h) with GABA conjugated to bovine serum albumin with glutaraldehyde (1 mg of GABA per ml of undiluted antibody). The GABA anti-GABA control solution was diluted 1:3000 in 1% bovine serum albumin and 0.3% Triton X-100.

We analysed the material with the aid of a camera lucida attachment on a microscope with both normal bright-field and interference-contrast illumination. In some instances, since we did not counterstain the tissue for Nissl substance, the interference-contrast illumination enabled us to visualize unlabeled cells and to verify that HRP and anti-GABA staining essentially filled the somata. We plotted the locations of pretecal cells containing retrogradely transported HRP using a × 16 objective with a Eutectics neuron tracing system. For each section analysed we plotted every cell labeled with HRP, and we subdivided these into those labeled only with HRP and those also labeled with the anti-GABA antibody; we refer to the latter as "double-labeled cells". With the use of a × 100 oil immersion lens, we traced soma outlines of labeled pretectal cells, whether double or single labeled with HRP or the anti-GABA antibody. Soma areas were calculated from the drawings using a digitizing tablet. Every cell retrogradely labeled with HRP was included in our analysis. Cells labeled only with the anti-GABA antibody were selected and drawn from the same fields as the HRP-labeled cells, but we made no attempt to draw every such cell; instead an effort was made only to select a representative population of single, anti-GABA labeled cells from matched regions. To minimize biases in plotting and measuring labeled cells, including the determination of single- and double-labeled cells, two separate observers plotted, scored, and measured cells for cases 4 and 5.

RESULTS

Injection sites

Line drawings of coronal sections through the HRP injection sites are shown in Fig. 1. Figure 1A–C shows the distribution of HRP when the injection was made by pressure into the lateral geniculate nucleus for cases 1–3. This produced a relatively large injection in which the label spread to include all regions of the lateral geniculate nucleus (including the A- and C-laminae, the medial interlaminar nucleus, and the geniculate wing), the ventral lateral geniculate nucleus, the perigeniculate nucleus, the thalamic reticular nucleus, the lateral posterior–pulvinar complex

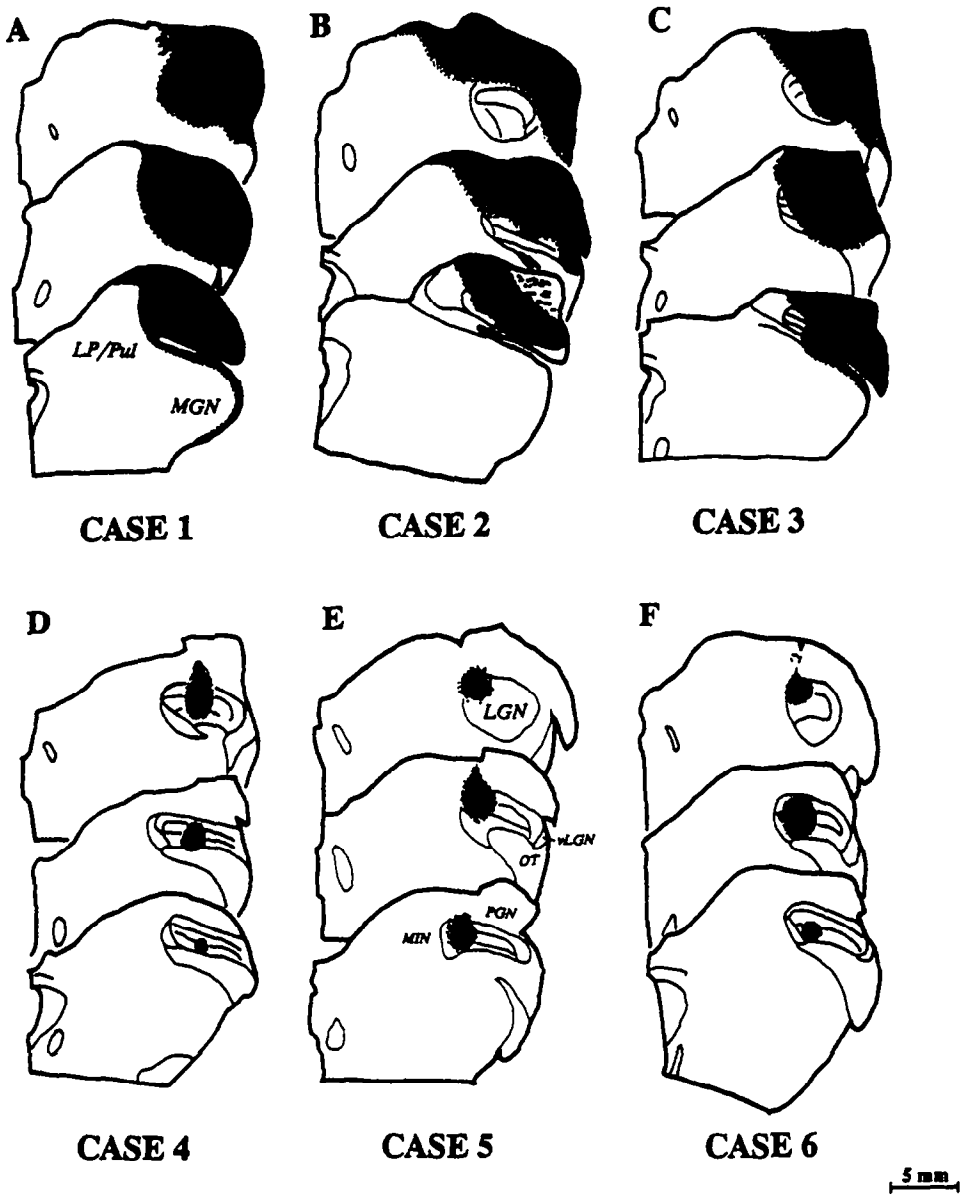

Fig. 1. Line drawings of the HRP injection sites in three sections from each of the six cases included in this study. We used pressure for the injections illustrated in A (case 1), B (case 2), and C (case 3), and the reaction product is present in regions including and adjacent to the lateral geniculate nucleus. We used iontophoresis for the injections illustrated in D (case 4), E (case 5), and F (case 6), and the reaction product is mostly limited to the A- and C-laminae of the lateral geniculate nucleus. For each section, dorsal is up and medial is to the left; the most caudal section is at the bottom of each series. LGN, lateral geniculate nucleus; LP/Pul, lateral posterior–pulvinar complex; MGN, medial geniculate nucleus; MIN, medial interlaminar nucleus; PGN, perigeniculate nucleus; vLGN, ventral lateral geniculate nucleus.

and the medial geniculate complex. Figure 1D–F illustrates injections that were made iontophoretically into the lateral geniculate nucleus for cases 4–6. Here, the spread of HRP was largely limited to the A- and C-laminae, lateral portions of the medial interlaminar nucleus, and part of the perigeniculate nucleus where the electrode passed through.

Labeling of pretectal neurons

Horseradish peroxidase labeling. Figure 2 illustrates several cells labeled with HRP by retrograde transport from the lateral geniculate nucleus in case 5 (see Fig. 1E). The stabilized tetramethylbenzidine reaction product appeared as large black intracellular granules in the somata and proximal dendrites of labeled cells. In cells labeled only with HRP, the intracellular space between labeled granules was completely clear because we did not counterstain this material for Nissl substance (see Fig. 2A,B). The cell's nucleus could usually be identified as an area devoid of granules, except when overlying granules were very dense. When interference microscopy was used (see

Experimental Procedures), we were able to visualize the surrounding membranes, many intracellular organelles, and neighboring cells that remained unlabeled.

GABA labeling. Staining with the anti-GABA antibody appeared as a homogeneous, dark lavender, cytoplasmic staining of the somata and some proximal dendrites (see Fig. 2E,F). Because of limited penetration of the anti-GABA antibody, only cells near the top and bottom surfaces of the sections were stained. We thus assume in our quantitative estimates below that we have underestimated the number of GABAergic neurons in the pretectum. We also found many puncta stained for GABA, and these presumably represent GABAergic terminals (see Fig. 2F).

Double labeling with horseradish peroxidase and GABA. Double-labeled cells contained both the

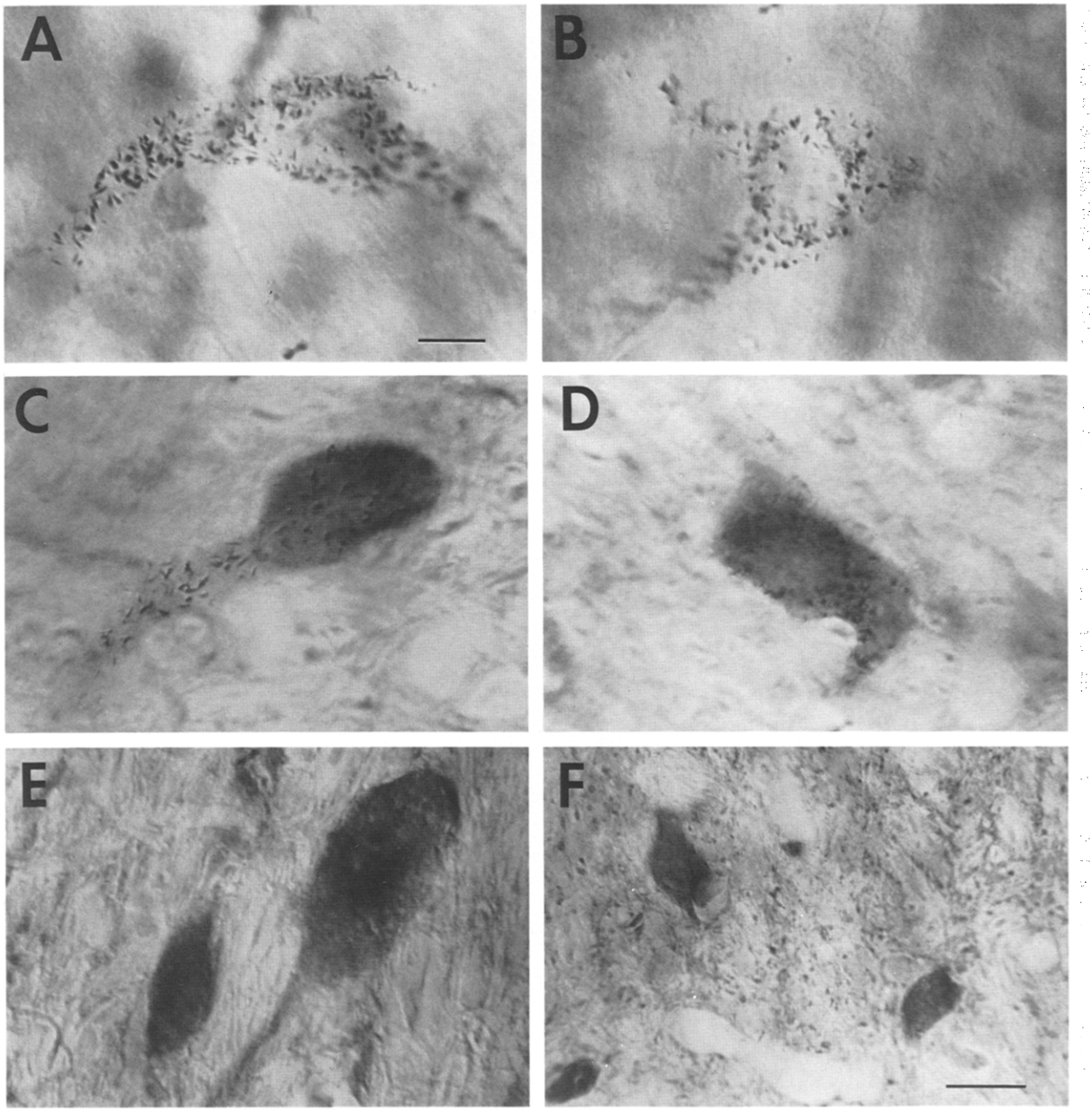

Fig. 2. Photomicrographs of pretectal cells labeled retrogradely with HRP and/or immunohistochemically with an antibody directed against GABA. (A,B) Cells labeled only by retrograde transport of HRP. (C,D) Double-labeled cells showing both retrograde HRP labeling and staining for GABA. (E,F) Cells labeled only with the anti-GABA antibody. The lower power view in F more clearly illustrates the labeled puncta in the neuropil, which we interpret as GABAergic terminals. Scale bar in A = 10 μm and also applies to B–E; in F = 20 μm.

granular black tetramethylbenzidine reaction product and the homogeneous, lavender, cytoplasmic GABA staining (see Fig. 2C,D). The cytoplasmic GABA staining was clearly identifiable between the dark tetramethylbenzidine granules. We readily discriminated these double-labeled cells from cells single labeled for HRP or GABA. In general, as is illustrated in Fig. 2A–D, we found no difference in the extent of HRP labeling between those cells labeled only with HRP and those double labeled for HRP and GABA.

We were concerned with the possibility of leakage from the retrogradely transported HRP granules into the cytoplasm during the immunohistochemical processing, because such leakage could produce the type of cytoplasmic staining that we have attributed to the GABA antibody. If so, then many or all of the cells we have interpreted as double labeled would have been incorrectly identified as such. We performed three general procedures to control against this artifact, and because of these controls we are confident that leakage of HRP from granules to the cytoplasm did not contribute to our interpretation of pretectal cells as double labeled.

Firstly, in one series of controls, we omitted the tetramethylbenzidine reaction altogether to see if diaminobenzidine, which we used to reveal the pattern of anti-GABA antibodies, could effectively reveal retrograde labeling. For cases 4–6, in which the injection sites were essentially limited to lateral geniculate nucleus, we found that exposure to diaminobenzidine alone did not reveal retrogradely labeled cells. In these cases, if the amount of HRP in the granules was insufficient to be detected by diaminobenzidine alone, then HRP leakage from the granules to the cytoplasm would also be insufficient to produce detectable labeling. When such sections were subsequently processed for anti-GABA immunohistochemistry and the diaminobenzidine reaction was used to reveal the distribution of GABA antibodies, no cells showed granular label, but many showed cytoplasmic staining. Therefore, since we always used the more sensitive tetramethylbenzidine reaction first, the less sensitive diaminobenzidine reaction used to reveal the GABA staining would not be likely to detect leakage.

Secondly, we processed some sections through the dorsal raphé nucleus in the same vials with the pretectal sections. The dorsal raphé also contained cells retrogradely labeled with HRP. After identical processing, many dorsal raphé cells showed the granular staining characteristic of the retrogradely transported HRP, and many others showed the cytoplasmic labeling characteristic of the GABA staining, but none showed both granular and cytoplasmic staining as seen in the double-labeled cells of the pretectum.

Thirdly, in some pretectal sections that were reacted for tetramethylbenzidine and that thus contained many retrogradely labeled cells, we omitted the primary antibody or bound it up using exogenous GABA bound to bovine serum albumin.[19] This provided a control, not only for leakage from the granules, but also for nonspecific staining of the primary and/or secondary antibodies. In neither case did any cells show cytoplasmic staining like that seen in GABA-positive cells.

Distribution of labeled pretectal cells

Because three of the GABA localization experiments (cases 2, 3, and 6) were performed while we were still perfecting our anti-GABA immunohistochemical procedures, many of the sections from these cases had too little or too much reaction product to be useful. We thus had only a limited series of sections available for quantitative analysis of these experiments. Also, for technical reasons, only the side of the brain ipsilateral to the HRP injection was available for analysis in cases 2–6.

Horseradish peroxidase labeling. For reasons given above, only case 1 was suitable for quantitative analysis of laterality of the projection from the pretectum to the lateral geniculate nucleus. Although we could not obtain a complete series of sections suitable for analysis from cases 2 and 3, the results from isolated sections of these experiments generally supported conclusions reached from analysis of case 1. Figure 3 illustrates the pattern of distribution of retrogradely labeled cells seen in case 1. We counted and plotted the distribution of every HRP-labeled pretectal cell on both sides in the seven sections illustrated. Ipsilateral to the HRP injection, we found labeled cells in the nucleus of the optic tract, the posterior pretectal nucleus, and the olivary pretectal nucleus. Because of the difficulty in precisely identifying pretectal nuclear borders in the coronal plane, arrows are used in Fig. 3 to indicate the general location of these cell groups.

Of the 1626 cells plotted in case 1, 1449 (89%) were ipsilateral to the injection, and 177 (11%) were contralateral (see Fig. 3). The cells labeled in the contralateral pretectum were found in the same three nuclei that contained labeled cells ipsilaterally: the nucleus of the optic tract, the posterior pretectal nucleus, and the olivary pretectal nucleus. However, as Fig. 3 indicates, the relative strength of the contralateral projection varied along the rostral–caudal axis. The highest percentages of contralaterally labeled cells were seen at rostral levels through the olivary pretectal nucleus (14–28%), and the lowest were seen at caudal levels through the nucleus of the optic tract (6–11%). We thus found that some of the projection from pretectum to the lateral geniculate nucleus derives from the contralateral side. However, since case 1 represents one of the larger HRP injections (see Fig. 1A), the possibility exists that the contralateral pathway so detected innervates only certain regions surrounding the lateral geniculate nucleus and not the lateral geniculate nucleus itself.

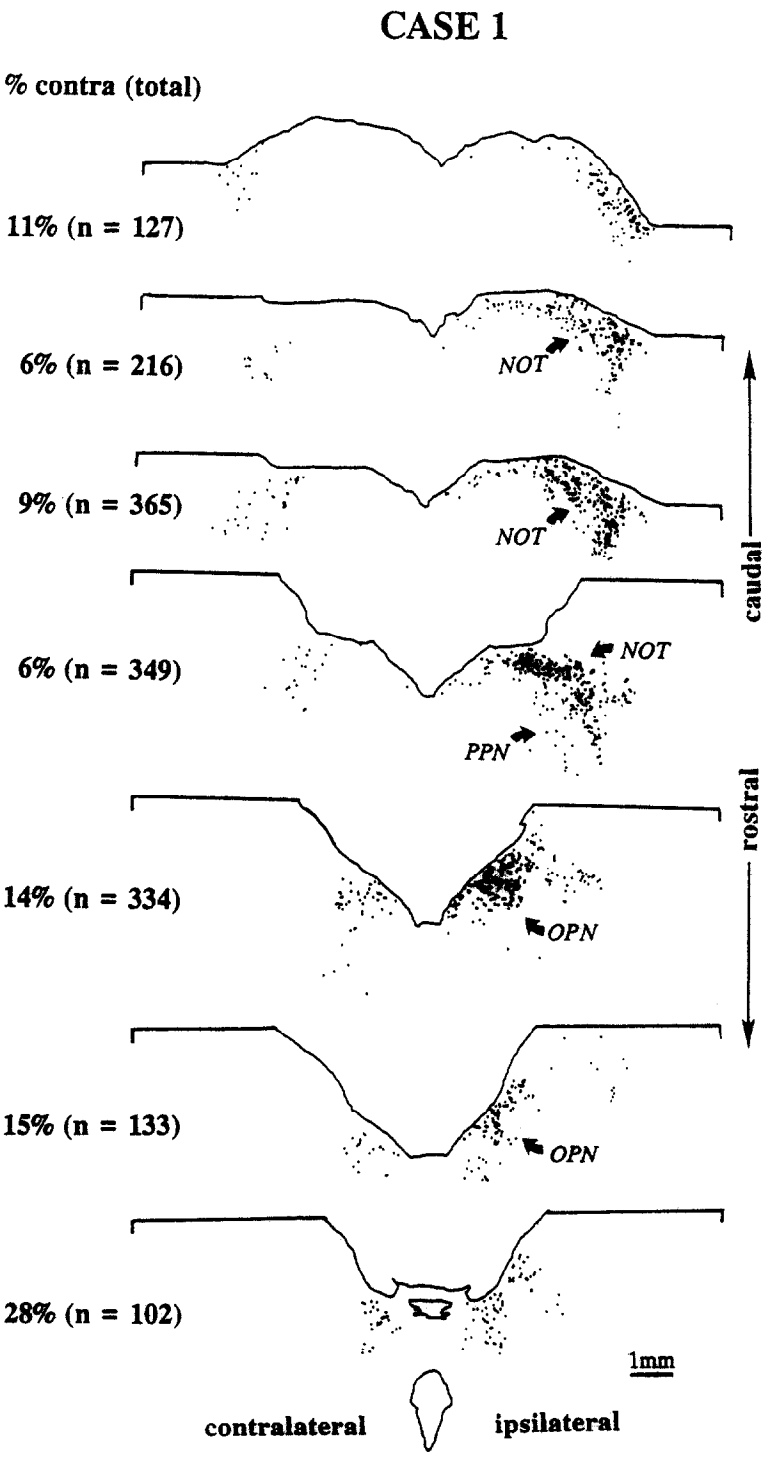

Fig. 3. Plots of retrogradely labeled cells (shown as dots) in seven sections through the pretectum of case 1 following an HRP injection into the right lateral geniculate nucleus (see Fig. 1A). Arrows indicate the approximate locations of the pretectal nuclei containing labeled cells: the nucleus of the optic tract (NOT), the posterior pretectal nucleus (PPN), and the olivary pretectal nucleus (OPN). Indicated for each section is the total number of labeled cells and the percentage of this total that is labeled on the contralateral side.

We have more completely analysed results from cases 4 and 5, in which small, iontophoretic injections of HRP were largely limited to the lateral geniculate nucleus (see Fig. 1D,E). The distributions of cells labeled with HRP are shown in Figs 4 and 5. From a series of 11 sections from each case, we counted 294 cells labeled with HRP in case 4, and 315 in case 5. Such labeled cells were mostly found in the nucleus

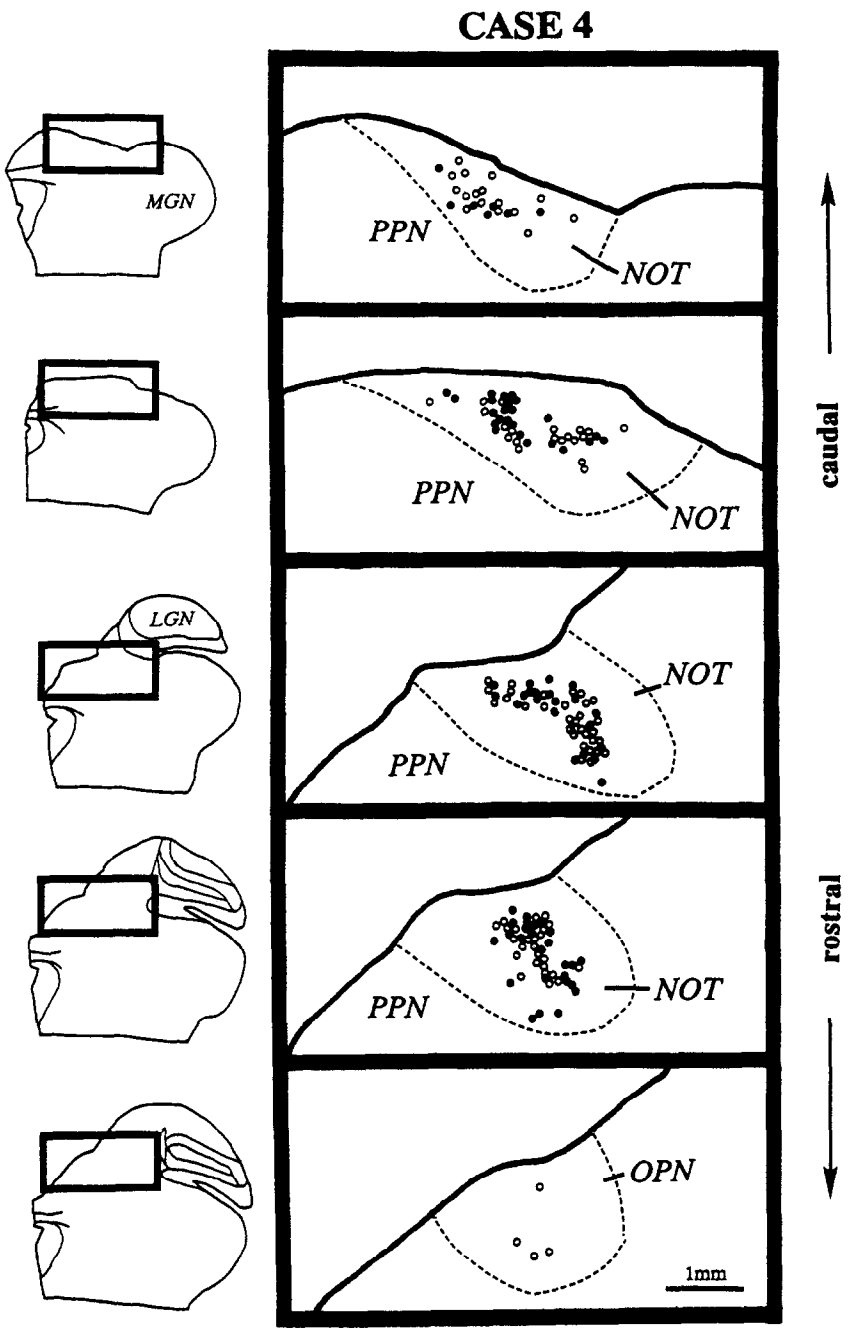

Fig. 4. Distribution of pretectal cells retrogradely labeled from the lateral geniculate nucleus in case 4. On the right is drawn a series of five sections through the pretectum showing cells that are labeled only with the retrograde tracer (open circles) or labeled also for GABA (i.e. double labeled; closed circles). To the left of each of these drawings is a lower power drawing with a rectangle enclosing the region of labeled cells shown on the right. LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus; NOT, nucleus of the optic tract; PPN, posterior pretectal nucleus; OPN, olivary pretectal nucleus.

of the optic tract, with some cells labeled in the posterior pretectal nucleus and a few labeled in the caudal pole of the olivary pretectal nucleus (see Figs 4 and 5). Although only isolated sections were available for analysis from case 6, the pattern of labeling seen there matches that seen for cases 4 and 5.

Comparison of results from the large and the small injections of HRP shows that injections confined to the lateral geniculate nucleus labeled fewer cells with a more limited distribution. As can be seen in a comparison of Fig. 3 with Figs 4 and 5, the large injection labeled six to seven times as many pretectal cells ipsilaterally as did the small injections. These

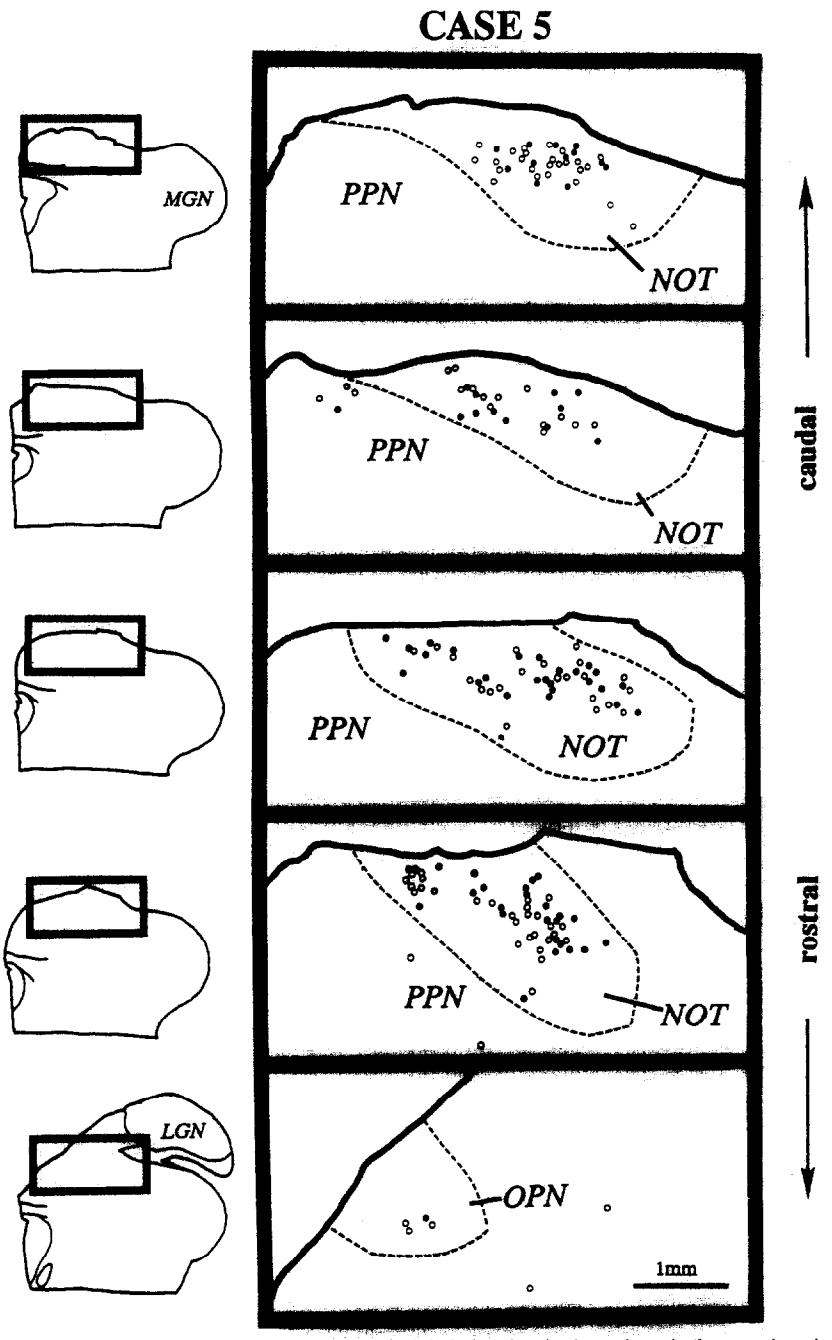

Fig. 5. Distribution of pretectal cells retrogradely labeled from the lateral geniculate nucleus in case 5; conventions and abbreviations as in Fig. 4.

small injections labeled cells mostly in the nucleus of the optic tract. After the large injections, many more cells were labeled in rostral and medial pretectal regions, especially in the olivary pretectal nucleus. This suggests that a substantial pretectal projection exists to thalamic zones beyond the lateral geniculate nucleus, and the nucleus of the optic tract provides the primary pretectal input to the lateral geniculate nucleus.

GABA labeling. Cells labeled with the anti-GABA antibody were present throughout the pretectum and the thalamus. In agreement with many earlier reports,[9,22,36,41] we found GABA immunoreactivity for small cells in the lateral geniculate nucleus and for most cells in the nearby perigeniculate nucleus and the thalamic reticular nucleus. In the pretectum, GABA-positive cells were found in all the nuclei, including the nucleus of the optic tract, the posterior pretectal nucleus, and the olivary pretectal nucleus. Because we have not counterstained out materials for Nissl substance, the percentage of neurons that stain for GABA in the various nuclei was not determined.

Table 1. Counts of pretectal cells labeled with horseradish peroxidase

Case	HRP only*	Double†	Total	Double (%)
1	1449	NA	1449	NA
2	18	9	27	33
3	96	40	136	29
4‡	171	123	294	42
5‡	179	136	315	43
6‡	16	10	26	38

*Cells labeled only with HRP.
†Cells double labeled with HRP and GABA.
‡Injection limited essentially to dorsal lateral geniculate nucleus.

Double labeling with horseradish peroxidase and GABA. In each of the five experiments for which both HRP and GABA staining was employed (i.e. cases 2–6), we found many pretectal cells labeled for both substances. The representative pattern of cells labeled only the HRP and double labeled with HRP plus GABA can be seen in Figs 4 and 5. Table 1 provides a summary of some of these data for pretectal cells ipsilateral to the injection site. The small cell numbers for cases 2, 3, and 6 are due to the limited number of sections available for analysis from these experiments (see above). Data pooled from the five experiments reveal that, of the 798 cells labeled with HRP and thus projecting to the lateral geniculate nucleus, 318 (39.9%) were also GABA-positive.

In alternate sections from cases 4 and 5, we plotted the distribution of every cell retrogradely labeled from the lateral geniculate nucleus and compared those labeled only with HRP with those double labeled for HRP and GABA. Each case was analysed by a separate observer to minimize sampling bias. Among retrogradely labeled cells in the ipsilateral pretectum, the fraction double labeled was 42% (123 of 294 cells) for case 4 and 43% (136 of 315 cells) for case 5 (see Table 1). Double-labeled cells were found throughout the pretectal regions that contained retrogradely labeled cells (see above). As shown by Figs 4 and 5, double-labeled cells and cells labeled only with HRP within the pretectal nuclei appeared to be randomly distributed with respect to each other, and there was no evidence for clustering or segregation of either cell population.

Our estimates of the number of double-labeled cells must be viewed as conservative for several reasons. In some instances it was difficult to distinguish single-from double-labeled cells. When there was a question about the staining, typically because the GABA staining was light or because the tetramethylbenzidine crystals obscured the GABA staining, the cell was always classified as labeled only by HRP. Also, as noted above, the retrograde tetramethylbenzidine reaction product penetrated the full thickness of each section, while the GABA staining did not. This limited the double labeling to cells with surface exposure. However, the lack of GABA staining in some of the retrogradely labeled cells cannot be explained solely by a failure of the antibody to penetrate the tissue: often a side by side pair of retrogradely labeled cells was located near a section surface, and one cell was immunoreactive for GABA while the other was not. Finally, there is always the possibility that cells containing GABA fail to stain for unknown reasons, so that many of the cells we deemed to be GABA-negative might in fact contain GABA.

Soma sizes of labeled pretecal cells

Horseradish peroxidase labeling. Soma sizes of the total population of pretectal cells retrogradely labeled from the lateral geniculate nucleus were measured from cases 4 and 5. This includes cells labeled only with HRP as well as those double labeled with HRP and the anti-GABA antibody. Table 2 along with Figs 6A,C and 7A,B,D,E summarizes these measurements. The pretectal cells projecting to the lateral geniculate nucleus display a unimodal size distribution (Fig. 6A,C).

GABA labeling. Soma sizes of pretectal cells that stained positively for GABA were also measured in sections from cases 4 and 5. For comparison with cells retrogradely labeled from the lateral geniculate nucleus, we measured only those cells single labeled for GABA that were found within the zone of retrogradely labeled cells. These results are summarized in Table 3 along with Figs 6B,D and 7B,C,E,F. Figure 6B,D shows that the pretectal cells with immunoreactivity for GABA, like those retrogradely labeled from the lateral geniculate nucleus, have a unimodal soma size distribution.

Double labeling with horseradish peroxidase and GABA. The soma sizes of double-labeled pretectal cells are shown in Tables 2 and 3 and some size histograms are shown in Fig. 7B,E. As is the case for the other cell populations, soma size is distributed

Table 2. Soma sizes of pretectal cells labeled with horseradish peroxidase*

	Case 4	Case 5	Total
HRP only†	$n = 172$	$n = 136$	$n = 308$
	$340.5 \pm 120.3\ \mu m^2$	$318.7 \pm 90.9\ \mu m^2$	$330.9 \pm 108.8\ \mu m^2$
Double‡	$n = 122$	$n = 130$	$n = 252$
	$367.7 \pm 131.7\ \mu m^2$	$333.4 \pm 121.7\ \mu m^2$	$350.0 \pm 127.8\ \mu m^2$
Total	$n = 294$	$n = 266$	$n = 560$
	$351.8 \pm 125.8\ \mu m^2$	$325.9 \pm 107.3\ \mu m^2$	$339.5 \pm 118.1\ \mu m^2$

*Mean ± S.D.
†Cells labeled only with HRP.
‡Cells double labeled with HRP and GABA.

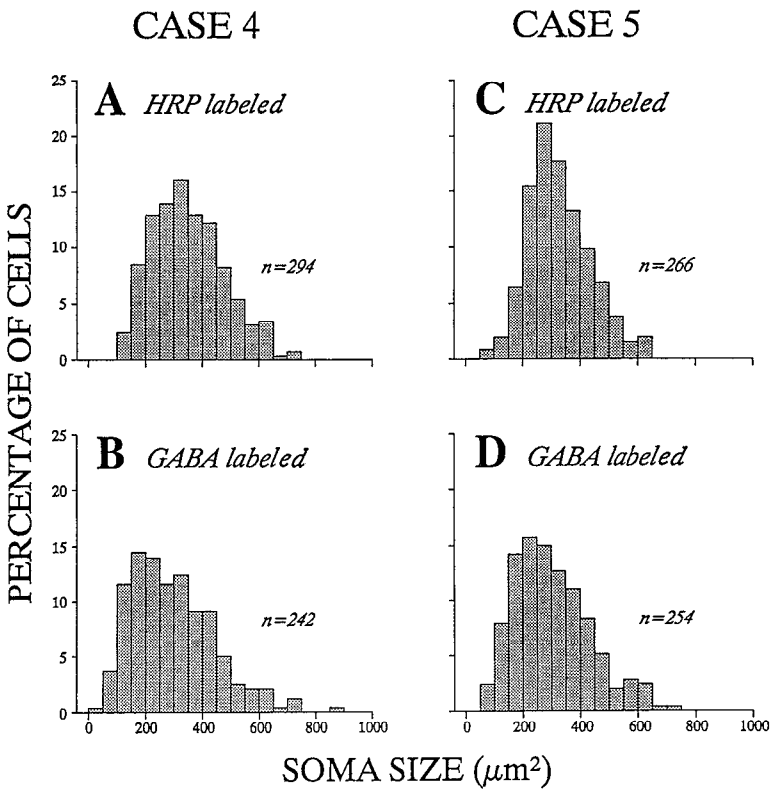

Fig. 6. Histograms showing cross-sectional soma areas of pretectal cells labeled retrogradely from the lateral geniculate nucleus with HRP (HRP labeled) and/or labeled with an antibody directed against GABA (GABA labeled). The total number of cells for each histogram is indicated. Cells double labeled for both markers are included both as "HRP labeled" and "GABA labeled". (A) Cells from case 4 labeled with HRP. (B) Cells from case 4 labeled positively for GABA. (C) Cells from case 5 labeled with HRP. (D) Cells from case 5 labeled positively for GABA.

unimodally for the double-labeled cells. Statistical analyses comparing soma size distributions of various cell populations in cases 4 and 5 lead to several interesting conclusions. All analyses are based on the Mann–Whitney U-test. Firstly, cells immunoreactive only for GABA are smaller than retrogradely labeled cells, whether the latter are double labeled with GABA and HRP ($P < 0.001$ for cases 4 and 5) or labeled only with HRP ($P < 0.001$ for both cases 4 and 5). Secondly, double-labeled cells are slightly larger in both cases than are cells labeled only with HRP, but the differences are not compelling ($P < 0.05$ for case 4 and $P > 0.1$ for case 5). Thirdly, although the GABAergic projection neurons may be relatively large, as a population, GABAergic cells are smaller than those labeled only with HRP ($P < 0.001$ for case 4 and $P < 0.01$ for case 5).

DISCUSSION

We studied the projection from the pretectum to the lateral geniculate nucleus in cats, and formed three main conclusions. Firstly, we confirm that the projection arises from several pretectal nuclei: the nucleus of the optic tract, the posterior pretectal nucleus, and the olivary pretectal nucleus, with most of the projection arising from the nucleus of the optic tract. Secondly, we found that 40% of the projection cells, and perhaps more, are GABAergic. Thirdly, while the majority of the pathway is ipsilateral, some may be contralateral.

GABA-positive cells that project to the lateral geniculate nucleus

Our finding that the pathway from the pretectum to the lateral geniculate nucleus is at least partly GABAergic may seem surprising, because GABAergic innervation to thalamic relay nuclei was previously thought to be exclusively thalamic in origin.[9,22,38,41] However, as noted in the Introduction, such a GABAergic pathway was predicted by the large number and size of pretectal cells staining for antibodies to glutamate decarboxylase[11,50] and by the morphology of synaptic terminals in the lateral geniculate nucleus that were labeled from the pretectum.[4] There are two provisos to this conclusion that are considered in the following paragraphs.

Firstly, there is the possibility that the double labeling reflects transneuronal retrograde transport of the HRP into GABAergic cells that are not projection cells. We think this unlikely, because, with the short survival times we used, we would expect any such transneuronal transport to label few cells,[13] and the density of label in these cells would be weak. As

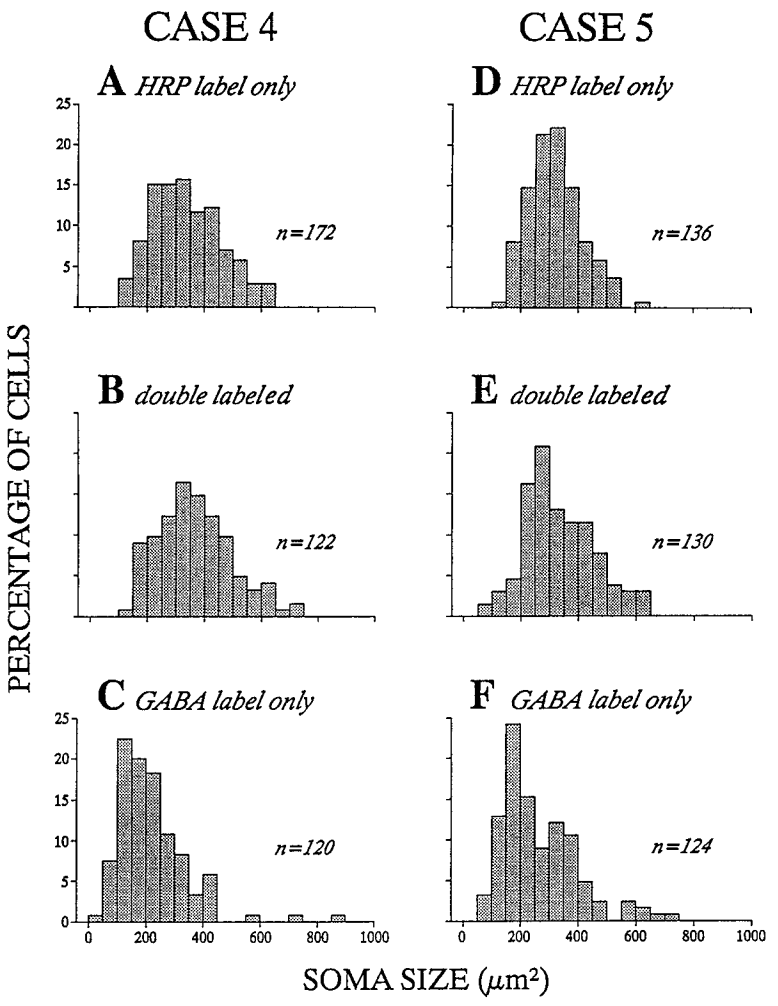

Fig. 7. Histograms showing cross-sectional soma areas of pretectal cells labeled in cases 4 and 5. The total number of cells for each histogram is indicated. Unlike Fig. 6, the histograms here separately illustrate sizes for each of the three types of labeled cells: labeled only with HRP retrogradely transported from the lateral geniculate nucleus (HRP label only), labeled only by an antibody directed against GABA (GABA label only), and double labeled for both markers (double labeled). (A) Cells from case 4 labeled only with HRP. (B) Double-labeled cells in case 4. (C) Cells from case 4 labeled only for GABA. (D) Cells from case 5 labeled only with HRP. (E) Double-labeled cells in case 5. (F) Cells from case 5 labeled only for GABA.

we have noted in Results, roughly half of the GABA-positive cells in pretectum are double labeled, and these are as densely labeled with HRP as are those labeled only with HRP. Although orthograde transneuronal transport of HRP conjugated to wheat-germ agglutinin is well known, most studies failed to find evidence for retrograde transneuronal transport of this label in adult animals (e.g. Ref. 10). One study that has described retrograde transneuronal transport of HRP conjugated to wheat-germ agglutinin in spinal cord,[13] made use of a more efficient labeling method (application of label directly to the stump of cut spinal nerves) and longer survival times than we used to observe significant transneuronal transport. Furthermore, by using *Phaseolus vulgaris* leucoagglutinin as an orthograde label, we have shown that pretectal axon terminals innervating the cat's lateral geniculate nucleus have features of F-terminals,[4] which previous work has shown to generally be GABA-positive.[9,32,33,35]

Secondly, even though our smallest HRP injections were mostly limited to the lateral geniculate nucleus, there was some spillover to surrounding tissue, and there is always the possibility that fibers of passage from pretectum to other targets were labeled. This raises the possibility that the retrogradely labeled pretectal cells do not actually terminate in the lateral geniculate nucleus. While it seems unlikely that all of the retrograde labeling could be due to axons terminating outside of the lateral geniculate nucleus, particularly since we and others (Refs 4,12; but see Ref 2). have seen clear evidence from orthograde labeling of a pretectal projection to this nucleus, some of the labeling might well represent cells not projecting to the lateral geniculate nucleus. Even so, this would still represent an ascending projection from the pretectum

Table 3. Soma sizes of pretectal cells labeled with GABA*

	Case 4	Case 5	Total
GABA only†	$n = 120$	$n = 124$	$n = 244$
	$224.0 \pm 124.2\ \mu m^2$	$266.5 \pm 130.8\ \mu m^2$	$245.6 \pm 129.4\ \mu m$
Double‡	$n = 122$	$n = 130$	$n = 252$
	$367.7 \pm 131.7\ \mu m^2$	$333.4 \pm 121.7\ \mu m^2$	$350.0 \pm 127.8\ \mu m^2$
Total	$n = 242$	$n = 254$	$n = 496$
	$296.4 \pm 146.8\ \mu m^2$	$300.8 \pm 130.6\ \mu m^2$	$298.6 \pm 138.7\ \mu m^2$

*Mean ± S.D.
†Cells labeled only with GABA.
‡Cells double labeled with HRP and GABA.

to the thalamus, much of which involves cells that label positively for GABA.

We thus conclude that a large fraction of pretectal cells projecting to the thalamus contain GABA, and many of these provide innervation to the lateral geniculate nucleus. Double-labeling studies of the descending pretectal projection to the inferior olive indicate that none of these projection cells stains positively for GABA.[20,37] The population of pretectal cells projecting to the inferior olive is separate from that projecting to thalamus.[42] Thus pretectal projection cells that are GABAergic may be limited to the ascending thalamic pathway.

GABA-negative cells that project to the lateral geniculate nucleus

About 60% of the pretectal cells that we retrogradely labeled from the lateral geniculate nucleus did not stain positively for GABA. This is a negative result, and we thus cannot draw firm conclusions about this GABA-negative population. Our results may reflect a failure of our method to stain every cell that contains GABA (false negatives), and indeed we cannot rule out the possibility that every such projection cell is GABAergic. However, in case 5, we included sections through the thalamic reticular nucleus, and we processed them immunohistochemically along with the pretectal sections. Many of the thalamic reticular cells were retrogradely labeled from the lateral geniculate nucleus from the same injection of HRP that labeled the pretectal cells; 75% of these retrogradely labeled thalamic reticular cells were also positively labeled for GABA,[5] a much higher percentage than the roughly 40% of pretectal projection cells we similarly labeled. We thus conclude that there may be both GABAergic and non-GABAergic populations of pretectal cells that project to the lateral geniculate nucleus.

GABA-positive cells that do not project to lateral geniculate nucleus

Previous studies of GABA immunohistochemistry in the cat's pretectum showed that the population of GABA-positive cells is smaller in soma size than is the unlabeled population, suggesting that at least some small GABAergic cells may serve as local circuit cells or interneurons.[11,20,50] Our results are consistent with this view: we describe a population of pretectal cells that stain positively for GABA but that are not retrogradely labeled from the lateral geniculate nucleus; these cells, as a population, are much smaller in size than are those projecting to the lateral geniculate nucleus, although a few individual cells are very large (see Fig. 7C,F). These results indicate that there may be at least two different types of GABA-positive cells in the cat's pretectum: projection cells and interneurons. However, it is possible that the smaller GABAergic cells are projection cells with targets other than the lateral geniculate nucleus or inferior olive.[26,42]

Role of pretectal innervation in the lateral geniculate nucleus

Brainstem stimulation generally facilitates retinogeniculate transmission.[45,46,48,49] This has been documented best for the cholinergic inputs from the parabrachial region of the midbrain, and an important feature of its control of retinogeniculate transmission seems to be the ability of acetylcholine to inhibit local GABAergic inhibitory neurons,[8,30] thereby disinhibiting the relay cells. A GABAergic input from the pretectum could facilitate transmission in a similar way, and our preliminary morphological studies of the organization of the input from pretectum to the lateral geniculate nucleus support this view.

As noted above, we have used *Phaseolus vulgaris* leucoagglutinin for orthograde labeling of pretectal terminals in the lateral geniculate nucleus. We find that pretectal axons also innervate the perigeniculate nucleus and that the primary postsynaptic targets of pretectal axons within the lateral geniculate nucleus have features of GABAergic interneurons.[4] Therefore, any GABAergic innervation from the pretectum may result in a disinhibition of geniculate projection cells and thereby facilitate retinogeniculate transmission.

What, then, is the information used by this pretectal input to control retinogeniculate transmission? The nucleus of the optic tract, the olivary pretectal nucleus, and the posterior pretectal nucleus all receive retinal innervation from ganglion cells with morphological and functional characteristics of X, Y, and W cells.[1,11,18,23,24,26,43] The nucleus of the optic tract, which is the main source of pretectal input to the lateral geniculate nucleus (the present study

and Refs 12, 21, 24, 26, 51; however, see Ref. 2), has received particular attention because of its involvement with retinal slip signals and the slow phase of optokinetic nystagmus.[14] Cells in the nucleus of the optic tract respond optimally to moving objects.[1,15–18,44] Perhaps such visual stimuli not only serve to elicit eye movements appropriate to tracking a target, but may also serve to facilitate retinogeniculate transmission of information about the target. Thus, the pretectum, through its descending projection to the midbrain and pons and through its ascending projection to thalamus, may be acting to co-ordinate eye movements with visual perception.

Acknowledgements—We thank D. J. Uhlrich and N. Tamamaki for their help with the HRP injections and M. Ramsay for assistance with the immunohistochemistry. This work was supported by USPHS grants EYO3604 and EYO3038; M.E.B. was supported by USPH postdoctoral fellowship EYO6101.

REFERENCES

1. Ballas I. and Hoffman K.-P. (1985) A correlation between receptive field properties and morphological structures in the pretectum of the cat. *J. comp. Neurol.* **238,** 417–428.
2. Berman N. (1977) Connections of the pretectum in the cat. *J. comp. Neurol.* **174,** 227–254.
3. Bloomfield S. A. and Sherman S. M. (1988) Postsynaptic potentials recorded in neurons of the cat's lateral geniculate nucleus following electrical stimulation of the optic chiasm. *J. Neurophysiol.* **60,** 1924–1945.
4. Cucchiaro J. B., Uhlrich D. J. and Sherman S. M. (1989) Synapses from the pretectum in the geniculate A-laminae of the cat. *Soc. Neurosci. Abstr.* **15,** 1392.
5. Cucchiaro J. B., Uhlrich D. J. and Sherman S. M. (1990) A projection from the thalamic reticular nucleus to the dorsal lateral geniculate nucleus in the cat: a comparison with the perigeniculate projection. *Soc. Neurosci. Abstr.* **16,** 159.
6. Distler C. and Hoffmann K.-P. (1989) The pupillary light reflex in normal and innate microstrabismic cats I: Behavior and receptive-field analysis in the nucleus praetectalis olivaris. *Visual Neurosci.* **3,** 127–138.
7. Distler C. and Hoffmann K.-P. (1989) The pupillary light reflex in normal and innate microstrabismic cats. II: Retinal and cortical input to the nucleus praetectalis olivaris. *Visual Neurosci.* **3,** 139–153.
8. Eysel U.-T., Pape H.-C. and Van Schayack R. (1986) Excitatory and differential disinhibitory actions of acetylcholine in the lateral geniculate nucleus of the cat. *J. Physiol., Lond.* **370,** 233–254.
9. Fitzpatrick D., Penny G. R. and Schmechel D. E. (1984) Glutamic acid decarboxylase-immunoreactive neurons and terminals in the lateral geniculate nucleus of the cat. *J. Neurosci.* **4,** 1809–1829.
10. Gerfen C. R., O'Leary D. D. M. and Cowan W. M. (1982) A note on the transneuronal transport of wheat germ agglutin-conjugated horseradish peroxidase in the avian and rodent visual systems. *Expl Brain Res.* **48,** 443–448.
11. Giolli R. A., Peterson G. M., Ribak C. E., McDonald H. M., Blanks R. H. I. and Fallon J. H. (1985) GABAergic neurons comprise a major cell type in rodent visual relay nuclei: an immunocytochemical study of pretectal and accessory optic nuclei. *Expl Brain Res.* **61,** 194–203.
12. Graybiel A. M. and Berson D. M. (1980) Autoradiographic evidence for a projection from the pretectal nucleus of the optic tract to the dorsal lateral geniculate complex in the cat. *Brain Res.* **195,** 1–12.
13. Harrison P. J., Hultborn H., Jankowska E., Katz R., Storai B. and Zytnicki D. (1984) Labelling of interneurons by retrograde transsynaptic transport of horseradish peroxidase from motoneurones in rats and cats. *Neurosci. Lett.* **45,** 15–19.
14. Hoffmann K.-P. (1981) Neuronal responses related to optokinetic nystagmus in the cat's nucleus of the optic tract. In *Progress in Oculomotor Research* (eds Fuchs and Becker), pp. 443–454.
15. Hoffmann K.-P., Behrend K. and Schopmann A. (1976) A direct afferent visual pathway from the nucleus of the optic tract to the inferior olive in the cat. *Brain Res.* **115,** 150–153.
16. Hoffmann K.-P. and Schopmann A. (1975) Retinal input to direction selective cells in the nucleus tractus opticus of the cat. *Brain Res.* **99,** 359–366.
17. Hoffmann K.-P. and Schopmann A. (1981) A quantitative analysis of the direction-specific response of neurons in the cat's nucleus of the optic tract. *Expl Brain Res.* **42,** 146–157.
18. Hoffmann K.-P. and Stone J. (1985) Retinal input to the nucleus of the optic tract of the cat assessed by antidromic activation of ganglion cells. *Expl Brain Res.* **59,** 395–403.
19. Holdefer R. N., Norton T. T. and Mize R. R. (1988) Laminar organization and ultrastructure of GABA-immunoreactive neurons and processes in the dorsal lateral geniculate nucleus of the tree shrew (*Tupaia belangeri*). *Visual Neurosci.* **1,** 189–204.
20. Horn A. K. E. and Hoffmann K.-P. (1987) Combined GABA-immunocytochemistry and TMB– HRP histochemistry of pretectal nuclei projecting to the inferior olive in rats, cats and monkeys. *Brain Res.* **409,** 133–138.
21. Itoh K. (1977) Efferent projections of the pretectum in the cat. *Expl Brain Res.* **30,** 89–105.
22. Jones E. G. (1985) *The Thalamus.* Plenum Press, New York.
23. Kanaseki T. and Sprague J. M. (1974) Anatomical organization of pretectal nuclei and tectal laminae in the cat. *J. comp. Neurol.* **158,** 319–338.
24. Koontz M. A., Rodieck R. W. and Farmer S. G. (1985) The retinal projection to the cat pretectum. *J. comp. Neurol.* **236,** 42–59.
25. Kubota T., Morimoto M., Kanaseki T. and Inomata H. (1987) Projection from the pretectal nuclei to the dorsal lateral geniculate nucleus in the cat: a wheatgerm agglutinin–horseradish peroxidase study. *Brain Res.* **421,** 30–40.
26. Kubota T., Morimoto M., Kanaseki T. and Inomata H. (1988) Visual pretectal neurons projecting to the dorsal lateral geniculate nucleus and pulvinar nucleus in the cat. *Brain Res. Bull.* **20,** 573–579.
27. Livingstone M. S. and Hubel D. H. (1981) Effects of sleep and arousal on the processing of visual information in the cat. *Nature* **291,** 554–561.
28. McCormick D. A. and Prince D. A. (1986) Acetylcholine induces burst firing in thalamic neurones by activating a potassium conductance. *Nature* **319,** 402–405.
29. McCormick D. A. and Prince D. A. (1987) Actions of acetylcholine in the guinea pig and cat medial and lateral geniculate nuclei, *in vitro*. *J. Physiol., Lond.* **392,** 147–165.

30. McCormick D. A. and Pape H.-C. (1988) Acetylcholine inhibits identified interneurons in the cat lateral geniculate nucleus. *Nature* **334,** 246–248.
31. Mesulam M.-M. (1978) Tetramethylbenzedine for horseradish peroxidase neurohistochemistry. A noncarcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J. Histochem. Cytochem.* **26,** 106–117.
32. Montero V. M. (1986) Localization of gamma-aminobutyric acid (GABA) in type 3 cells and demonstration of their source to F2 terminals in the cat lateral geniculate nucleus: a Golgi-electron microscopic GABA-immunocytochemical study. *J. comp. Neurol.* **254,** 228–245.
33. Montero V. M. (1987) Ultrastructural identification of synaptic terminals from the axon of type 3 interneurons in the cat lateral geniculate nucleus. *J. comp. Neurol.* **264,** 268–283.
34. Montero V. M. and Singer W. (1984) Ultrastructure and synaptic relations of neural elements containing glutamic acid decarboxylase (GAD) in the perigeniculate nucleus of the cat. *Expl Brain Res.* **56,** 115–125.
35. Montero V. M. and Singer W. (1985) Ultrastructural identification of somata and neural processes immunoreactive to antibodies against glutamic acid decarboxylase (GAD) in the dorsal lateral geniculates nucleus of the cat. *Expl Brain Res.* **59,** 151–165.
36. Montero V. M. and Zempel J. (1985) Evidence for two types of GABA-containing interneurons in the A-laminae of the cat lateral geniculate nucleus: a double-label HRP and GABA-immunocytochemical study. *Expl Brain Res.* **60,** 603–609.
37. Nunes Cardozo J. J. and Van der Want J. J. L. (1990) Ultrastructural organization of the retino-pretecto-olivary pathway in the rabbit: a combined WGA–HRP tracing and GABA immunocytochemical study. *J. comp. Neurol.* **291,** 313–327.
38. Oertel W. H., Graybiel A. M., Mugnaini E., Elde R. P., Schmechel D. E. and Kopin I. J. (1983) Coexistence of glutamic acid decarboxylase-like and somatostatin-like immunoreactivity in neurons of the feline nucleus reticularis thalami. *J. Neurosci.* **3,** 1322–1332.
39. Ottersen O. P. and Storm-Mathieson J. (1984) GABA-containing neurons in the thalamus and pretectum of the rodent. An immunocytochemical study. *Anat. Embryol.* **170,** 197–207.
40. Penny G. R., Conley M., Schmechel D. E. and Diamond I. T. (1984) The distribution of glutamic acid decarboxylase immunoreactivity in the diencephalon of the opossum and rabbit. *J. comp. Neurol.* **228,** 38–56.
41. Rinvik E., Ottersen O. P. and Storm-Mathisen J. (1987) Gamma-aminobutyrate-like immunoreactivity in the thalamus of the cat. *Neuroscience* **21,** 781–805.
42. Robertson R. T. (1983) Efferents of the pretectal complex: separate populations of neurons project to lateral thalamus and to inferior olive. *Brain Res.* **258,** 91–95.
43. Scalia F. (1972) The termination of retinal axons in the pretectal region of mammals. *J. comp. Neurol.* **145,** 223–258.
44. Schoppmann A. (1985) A developmental study of retinal afferents and visual responses in the cat pretectum. *Expl Brain Res.* **60,** 350–362.
45. Sherman S. M. and Koch C. (1986) The control of retinogeniculate transmission in the mammalian lateral geniculate nucleus. *Expl Brain Res.* **63,** 1–20.
46. Sherman S. M. and Koch C. (1990) Thalamus. In *Synaptic Organization of the Brain*, 3rd edn (ed. Shepherd G. M.), pp. 246–278. Oxford University Press, New York.
47. Sillito A. M., Kemp J. A. and Berardi N. (1983) The cholinergic influence on the function of the cat dorsal lateral geniculates nucleus (dLGN). *Brain Res.* **280,** 299–307.
48. Singer W. (1973) Brainstem stimulation and the hypothesis of presynaptic inhibition in cat lateral geniculate nucleus. *Brain Res.* **61,** 55–68.
49. Singer W. (1977) Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiol. Rev.* **57,** 386–420.
50. Weber J. T. and Chen I.-L. (1984) GAD immunoreactivity in the pretectal complex of the cat. *Soc. Neurosci. Abstr.* **10,** 576.
51. Weber J. T. and Harting J. K. (1980) The efferent projections of the pretectal complex: an autoradiographic and horseradish peroxidase analysis. *Brain Res.* **194,** 1–28.

(*Accepted* 3 *October* 1990)