

# Neurotransmitters Contained in the Subcortical Extraretinal Inputs to the Monkey Lateral Geniculate Nucleus

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## ABSTRACT

The lateral geniculate nucleus (LGN) is the thalamic relay of retinal information to cortex. An extensive complement of nonretinal inputs to the LGN combine to modulate the responsiveness of relay cells to their retinal inputs, and thus control the transfer of visual information to cortex. These inputs have been studied in the most detail in the cat. The goal of the present study was to determine whether the neurotransmitters used by nonretinal afferents to the monkey LGN are similar to those identified in the cat. By combining the retrograde transport of tracers injected into the monkey LGN with immunocytochemical labeling for choline acetyl transferase, brain nitric oxide synthase, glutamic acid decarboxylase, tyrosine hydroxylase, or the histochemical nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase reaction, we determined that the organization of neurotransmitter inputs to the monkey LGN is strikingly similar to the patterns occurring in the cat. In particular, we found that the monkey LGN receives a significant cholinergic/nitroergic projection from the pedunculopontine tegmentum,  $\gamma$ -aminobutyric acid (GABA)ergic projections from the thalamic reticular nucleus and pretectum, and a cholinergic projection from the parabrachial nucleus. The major difference between the innervation of the LGN in the cat and the monkey is the absence of a noradrenergic projection to the monkey LGN. The segregation of the noradrenergic cells and cholinergic cells in the monkey brainstem also differs from the intermingled arrangement found in the cat brainstem. Our findings suggest that studies of basic mechanisms underlying the control of visual information flow through the LGN of the cat may relate directly to similar issues in primates, and ultimately, humans. *J. Comp. Neurol.* 424:701–717, 2000. © 2000 Wiley-Liss, Inc.

**Indexing terms:** acetylcholine; nitric oxide;  $\gamma$ -aminobutyric acid; noradrenaline; pedunculopontine tegmentum

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The lateral geniculate nucleus (LGN) is the thalamic relay of retinal information to cortex. The LGN performs this function dynamically, as a consequence of complex interactions of the intrinsic membrane properties of the LGN cells with an extensive complement of nonretinal inputs to the LGN that arise from cortical and subcortical loci (for reviews see Sherman and Koch, 1986; Sherman and Guillery, 1996).

Nonretinal inputs have been examined in the most detail in the cat. In this species, the retinal input to thalamocortical relay cells makes up only 5–10% of the total syn-

apses, and the remaining inputs are made up of  $\gamma$ -aminobutyric acid (GABA)ergic inputs from interneu-

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rons, the thalamic reticular nucleus and the pretectum, glutamatergic inputs from cortex, histaminergic inputs from the hypothalamus, and cholinergic/nitroergic, noradrenergic, and serotonergic inputs from the midbrain and pons (De Lima and Singer, 1987; Smith et al., 1988; Fitzpatrick et al., 1989; Montero, 1990; Uhlich and Cucchiaro, 1992; Uhlich et al., 1993; Bickford et al., 1993, 1994, 1999; Erişir et al., 1997, 1998). These inputs combine to modulate the responsiveness of relay cells to their retinal inputs, and thus control the transfer of visual information to cortex.

The monkey remains an important species for the study of the visual system, particularly at the cortical level, but we do not understand the microcircuitry of the monkey LGN to the same level of detail as we do for the cat (Wilson, 1993). Recently, the sources of subcortical input to the monkey LGN were identified and found to be nearly identical to those previously described in other species (Wilson et al., 1995). The goal of the present study was to determine whether the neurotransmitters used by these afferents are also similar to those identified in the cat. This information is important for determining how the organization of the LGN may vary across species, and thus to what extent the results of studies in the cat can be applied to the monkey and human.

## MATERIALS AND METHODS

Portions of the brains of five macaque monkeys were used for these experiments. Two of these monkeys received unilateral tracer injections in the LGN. In one *Macaca mulatta* monkey, a pressure injection of fast blue was placed primarily in the parvocellular geniculate layers. In one *Macaca fascicularis* monkey, dual pressure injections were placed in the parvocellular (diamidino yellow) and magnocellular (fast blue) layers. Tissue from these monkeys was subsequently reacted for glutamic acid decarboxylase (GAD), choline acetyl transferase (ChAT), brain nitric oxide synthase (BNOS), and tyrosine hydroxylase (TH). Tissue from three additional monkeys (two *Macaca mulatta* and one *Macaca fascicularis* monkey) was used for examining the distribution of the above transmitters and enzymes and for double-label immunocytochemistry and histochemistry (ChAT and BNOS, ChAT and nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase, BNOS and NADPH-diaphorase).

### Tracer injections

The monkeys used for the injections of the fluorescent dyes fast blue and diamidino yellow had been previously used for extracellular recordings in the lateral geniculate nucleus as part of another project, and as such the nucleus was quite thoroughly mapped. Data were collected at the State University of New York at Stony Brook by using single-unit recording techniques in chronically prepared monkeys, treated in accordance with the institutional guidelines for animal care. The techniques were adapted from those of Gnadt and Mays (1995). Neurons in the lateral geniculate nucleus were classified according to their small visual receptive fields, predictable ocular dominance switching as we progressed through the laminae, and a change to larger receptive fields with better contrast sensitivity as we passed from parvocellular to magnocellular laminae. First, recordings were made in the parvocellular or magnocellular laminae at predetermined coordinates.

The electrode was then withdrawn and a 1- $\mu$ l Hamilton syringe was lowered at the same coordinates and to the same depth as the electrode. Approximately 200 nl of a 3% aqueous diamidino yellow (Sigma, St. Louis, MO) solution or a 2% aqueous fast blue (EMS-Polyloy, GmbH, Germany) solution was injected over a 30-minute period. After a survival period of 2 weeks, the animal was given an overdose of sodium pentobarbital (30 mg/kg) and then perfused with heparinized saline followed by a 3% or 4% buffered (pH 7.4) paraformaldehyde solution. The brain was removed, and after cryoprotection in glycerol (10%), 30- $\mu$ m sections were cut on a freezing microtome and either mounted onto gelatinized slides or placed in a 10% glycerol in 0.1 M phosphate buffer solution (pH 7.4) for storage prior to use in immunocytochemical experiments.

### Immunocytochemistry and histochemistry

Tissue in this study was incubated with a number of different antibodies, and the methods used to visualize their distribution varied. For single-label immunocytochemistry in brains that were not injected, antibodies were tagged with horseradish peroxidase (HRP), which was then reacted with diaminobenzidine (DAB) for examination using transmitted white light. For double-labeling with ChAT or BNOS, and NADPH-diaphorase, antibodies were tagged with fluorescein or Alexa 488 (Molecular Probes, Eugene, OR), and subsequently reacted for NADPH-diaphorase activity. The tissue was then examined alternately with epifluorescent blue light (to examine the distribution of ChAT or BNOS) and transmitted white light (to examine the distribution of NADPH-diaphorase reaction product). For immunocytochemical labeling of cells that contained fast blue or diamidino yellow, antibodies were tagged with rhodamine or Alexa 546 (Molecular Probes). The tissue was then alternately viewed with epifluorescent ultraviolet light (to detect the distribution of diamidino yellow and fast blue) and epifluorescent green light (to detect the distribution of antibody labeling). For double immunocytochemical labeling of ChAT and BNOS, the ChAT antibody was tagged with Alexa 546 and the BNOS antibody was tagged with Alexa 488. The tissue was then alternately examined with epifluorescent green light (to view ChAT labeling) or blue light (to view BNOS labeling). Examples of the various labeling methods are shown in Figure 1.

**Immunocytochemistry.** Sections were incubated for 30 minutes in 10% normal goat serum (NGS) or normal rabbit serum (NRS) in phosphate-buffered saline (PBS; 0.01 M  $\text{PO}_4$  buffer, pH 7.4, 0.9% NaCl). Sections were then transferred to their primary antibody solutions (diluted in 1% NGS or NRS in PBS) and incubated overnight at 4°C. The following antibody dilutions were used: a polyclonal rabbit anti-GAD (67) 1:2,000 (Chemicon International Incorporated, Temecula, CA), a monoclonal mouse anti-BNOS 1:500–1:1,000 (Sigma), or a polyclonal rabbit anti-BNOS 1:1,000 (Transduction Laboratories, Lexington, KY), a monoclonal rabbit anti-TH 1:1,000 (Boehringer Mannheim, Indianapolis, IN), and a polyclonal goat anti-ChAT 1:125–1:300 (Chemicon). The next day, the tissue was rinsed 3 times (10 minutes each) in phosphate buffer (PB; 0.1 M, pH 7.4), and incubated 1 hour in biotinylated or fluorescently tagged secondary antibodies (Vector Laboratories; Burlingame, CA; biotinylated antibodies: goat anti-rabbit, goat anti-rat, goat anti-mouse, or rabbit-anti-

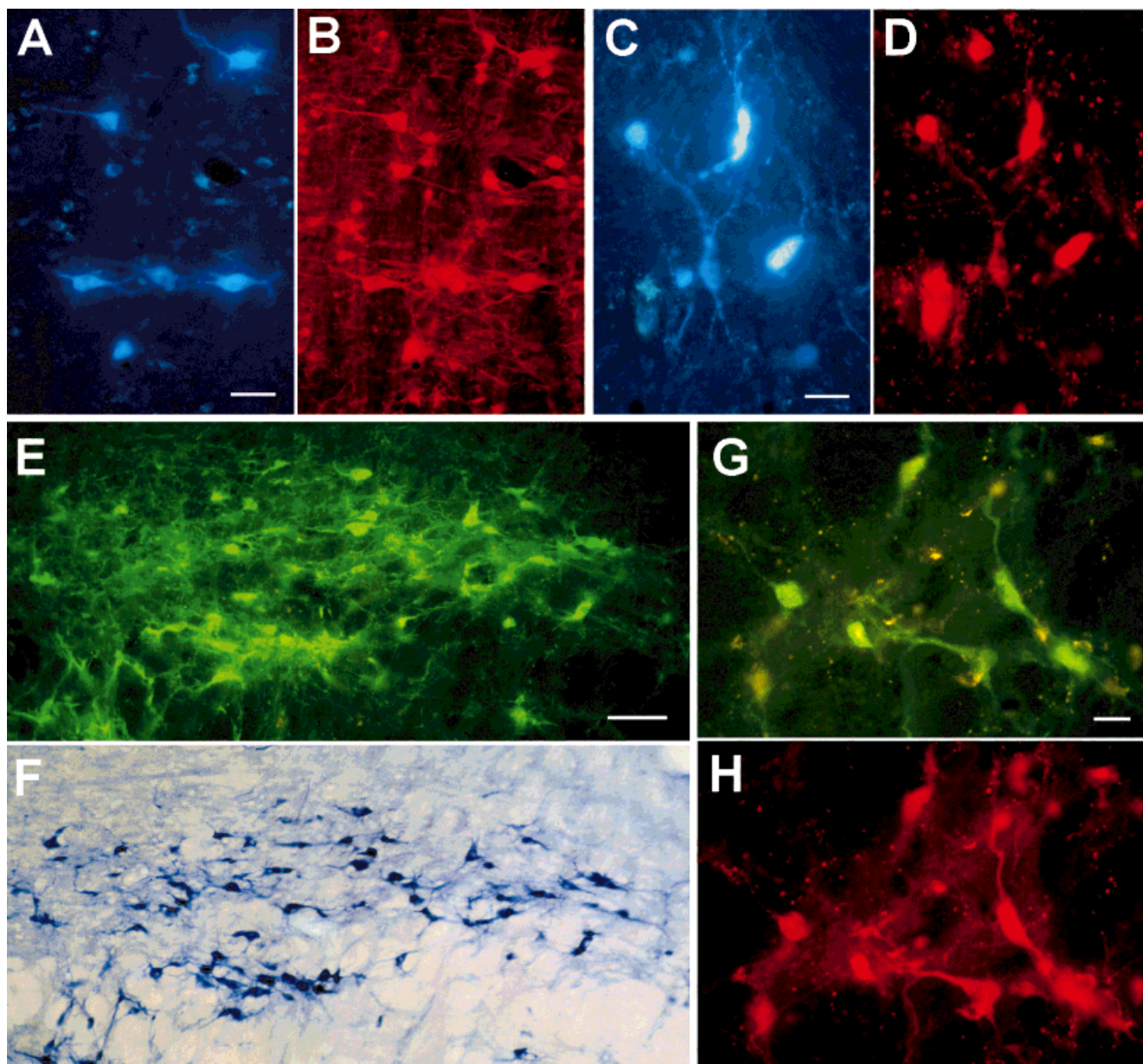


Fig. 1. Double-labeling methods used in the study are illustrated in pairs of photomicrographs of fluorescently labeled tissue. **A:** Cells in the pendunculopontine tegmentum (PPT) labeled with fast blue from an injection in the lateral geniculate nucleus (LGN) are revealed by using ultraviolet light. **B:** The same PPT cells illustrated in A are labeled with an antibody against choline acetyl transferase (ChAT) that has been tagged with Alexa 546 and revealed by illuminating the section with green light. **C:** Cells in the thalamic reticular nucleus (TRN) labeled with fast blue from an injection in the LGN are revealed by using ultraviolet light. **D:** The same TRN cells illustrated in C are labeled with an antibody against glutamic acid decarboxylase (GAD) that has been tagged with Alexa 546 and revealed by illuminating the section with green light. **E:** Cells in the PPT labeled with an antibody against brain nitric oxide synthase (BNOS) tagged with Alexa 488 revealed with blue light. **F:** The same PPT cells illustrated in E are also labeled with the nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase reaction revealed with white light. **G:** Cells in the PPT labeled with an antibody against BNOS tagged with Alexa 488 revealed with blue light. **H:** The same PPT cells illustrated in G are labeled with an antibody against ChAT that has been tagged with Alexa 546 and revealed by illuminating the section with green light. Scale bar = 30  $\mu$ m in A (also applies to B); 20  $\mu$ m in C (also applies to D); 100  $\mu$ m in E (also applies to F); 20  $\mu$ m in G (also applies to H).

goat) or Molecular Probes rabbit anti-mouse-Alexa 488 diluted 1:100 in 1% NGS or NRS in PBS. The sections were then rinsed 3 times (10 minutes each) in PB and incubated for 1 hour in a 1:100 dilution of avidin and biotinylated-horseradish peroxidase complex (ABC; Vector), or avidin-rhodamine, avidin-fluorescein (Vector), or

avidin-Alexa 546 (Molecular Probes) in 1% NGS or NRS in PBS. For sections labeled with HRP, the tissue was rinsed and reacted with a nickel-intensified diaminobenzidine solution. Following further rinses, these sections were mounted onto slides, dehydrated, and coverslipped for subsequent examination and photography by using trans-

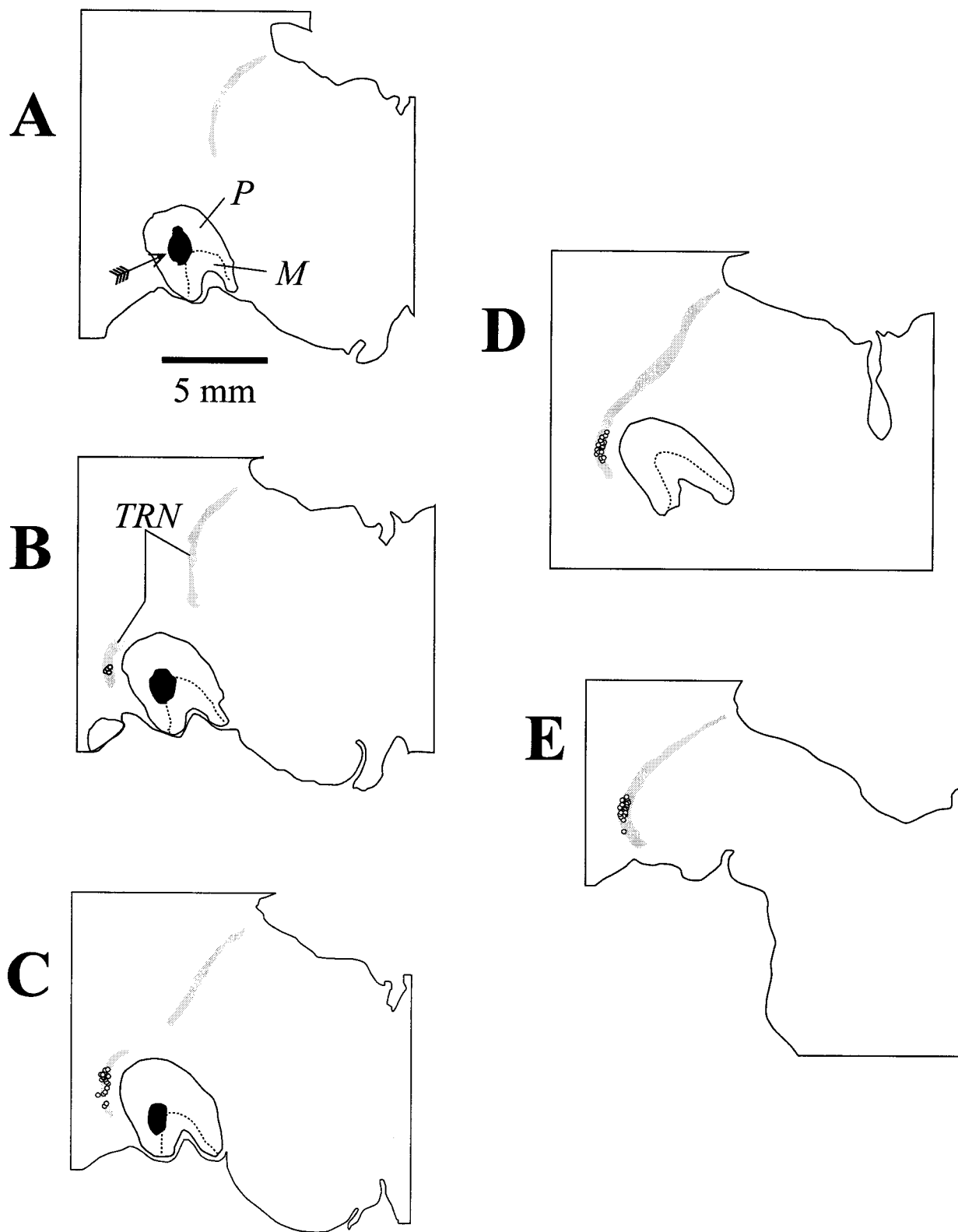


Fig. 2. **A-K:** The distribution of cells (open circles) labeled by retrograde transport from an injection of fast blue (arrow) in the lateral geniculate nucleus are plotted in sections arranged from rostral (A) to

caudal (K). The dashed line indicates the border between the parvocellular laminae (P) and the magnocellular laminae (M). TRN, thalamic reticular nucleus.



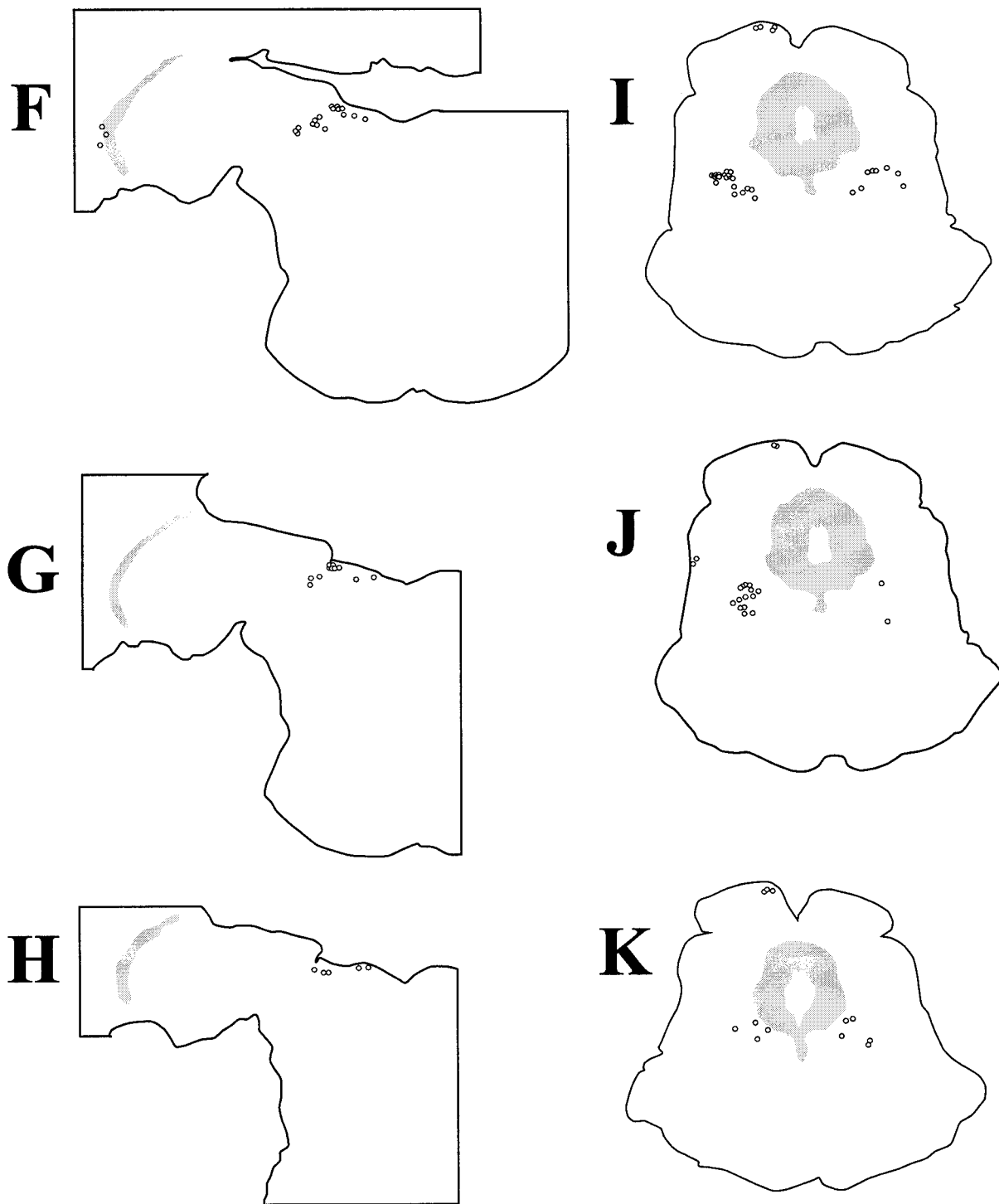


Figure 2 (Continued)

mitted light. For sections tagged with fluorescent labels, the sections were rinsed, mounted onto slides, briefly dehydrated and coverslipped with Vectashield mounting medium (Vector) for examination and photography by using epifluorescent illumination.

**NADPH-diaphorase reaction.** Sections were rinsed with Tris buffer (0.1 M, pH 6.0) and incubated in a solution of 0.01% NADPH (Sigma), 0.025% nitroblue tetrazolium (Sigma), and 0.1% Triton-X (Sigma) in Tris buffer for 2–4 hours at 37°C. The sections were rinsed, mounted,

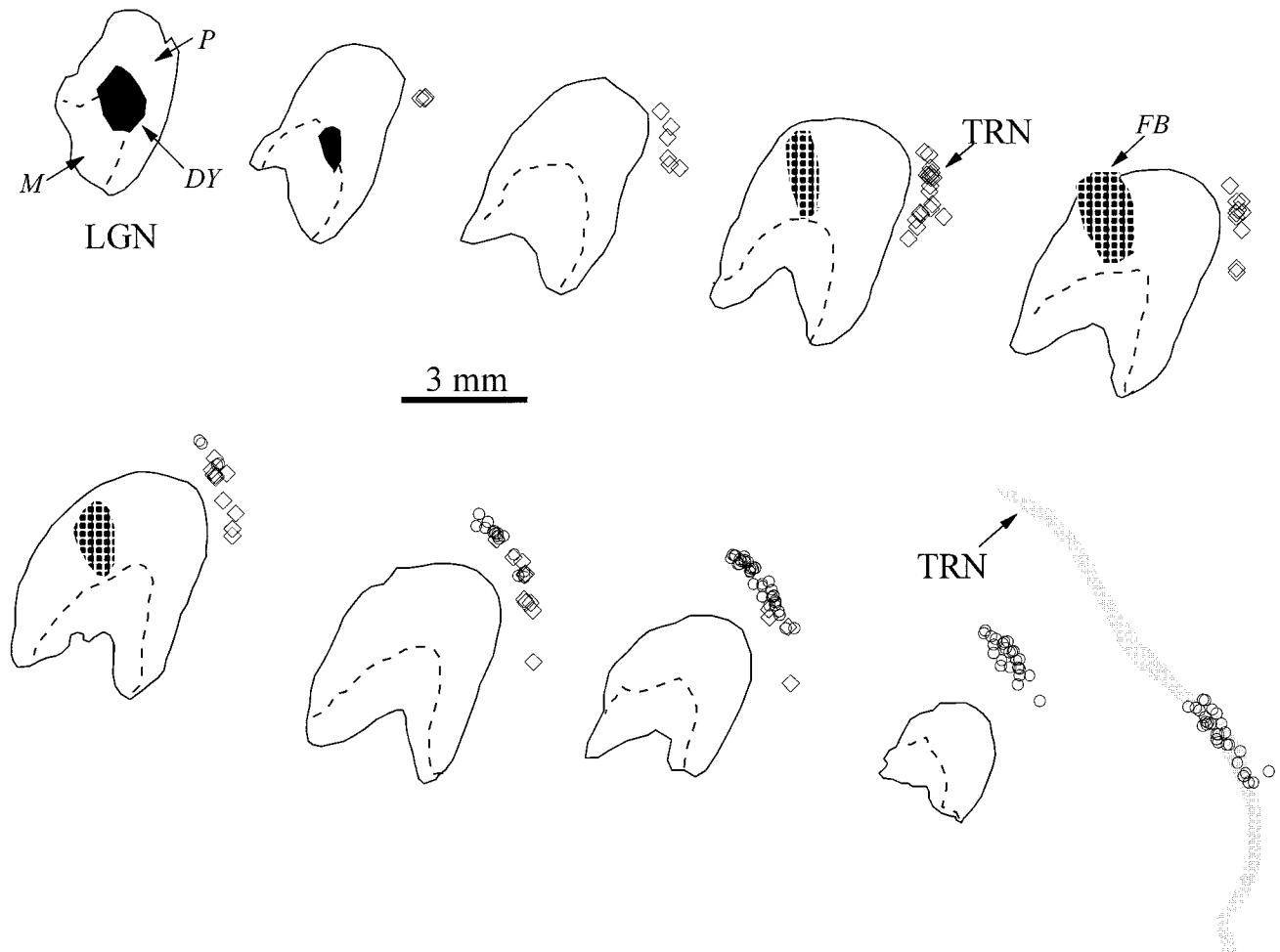


Fig. 3. The distribution of cells labeled by retrograde transport from injections of both diamidino yellow (DY, black) and fast blue (FB, hatching) in the lateral geniculate nucleus (LGN) are plotted in sections arranged from rostral to caudal. The dashed line indicates the border between the parvocellular laminae (P) and the magnocellular

laminae (M) of the LGN. Cells labeled with FB (open circles) DY (open diamonds) or both (filled squares) are located in the thalamic reticular nucleus (TRN), nucleus of the optic tract (NOT), superior colliculus (SC), parabigeminal nucleus (PBG), and the pedunculo pontine tegmentum (PPT).

air-dried, briefly dehydrated, and coverslipped with Vectashield (Vector).

**Computer-generated figures.** Light level photographs were taken using color slide film. Photographic slides to be used for figures were digitized by using a SprintScan slide scanner (Polaroid Corporation, Cambridge, MA). By using Photoshop software (Adobe Systems Incorporated, San Jose, CA), the brightness and contrast were adjusted to optimize the images. For black and white images, the color was removed by using Photoshop software.

## RESULTS

### Subcortical projections to the monkey LGN

Figure 2 illustrates the distribution of cells labeled by retrograde transport from an injection of fast blue in the monkey LGN. As previously reported by Wilson et al. (1995), excluding the retina, seven subcortical areas

project to the LGN of the monkey: the tuberomammillary nucleus of the hypothalamus, the thalamic reticular nucleus, the nucleus of the optic tract of the pretectum, the superior colliculus, the parabigeminal nucleus, the pedunculo pontine tegmentum (PPT), and the dorsal raphe nucleus. The only region identified as projecting to the LGN in other species, which is not labeled following tracer injections into the LGN of the monkey, is the locus coeruleus.

Cells labeled in the thalamic reticular nucleus and superior colliculus were ipsilateral to the injection site and no contralateral labeled cells were seen. The majority of cells labeled in the pretectum and PPT were ipsilateral, but significant numbers of contralateral cells were labeled. Cells in the parabigeminal nucleus were primarily ipsilateral, but a small number of contralateral cells were also labeled. The few cells labeled in the hypothalamus and dorsal raphe nucleus were approximately evenly distributed on either side of the midline.

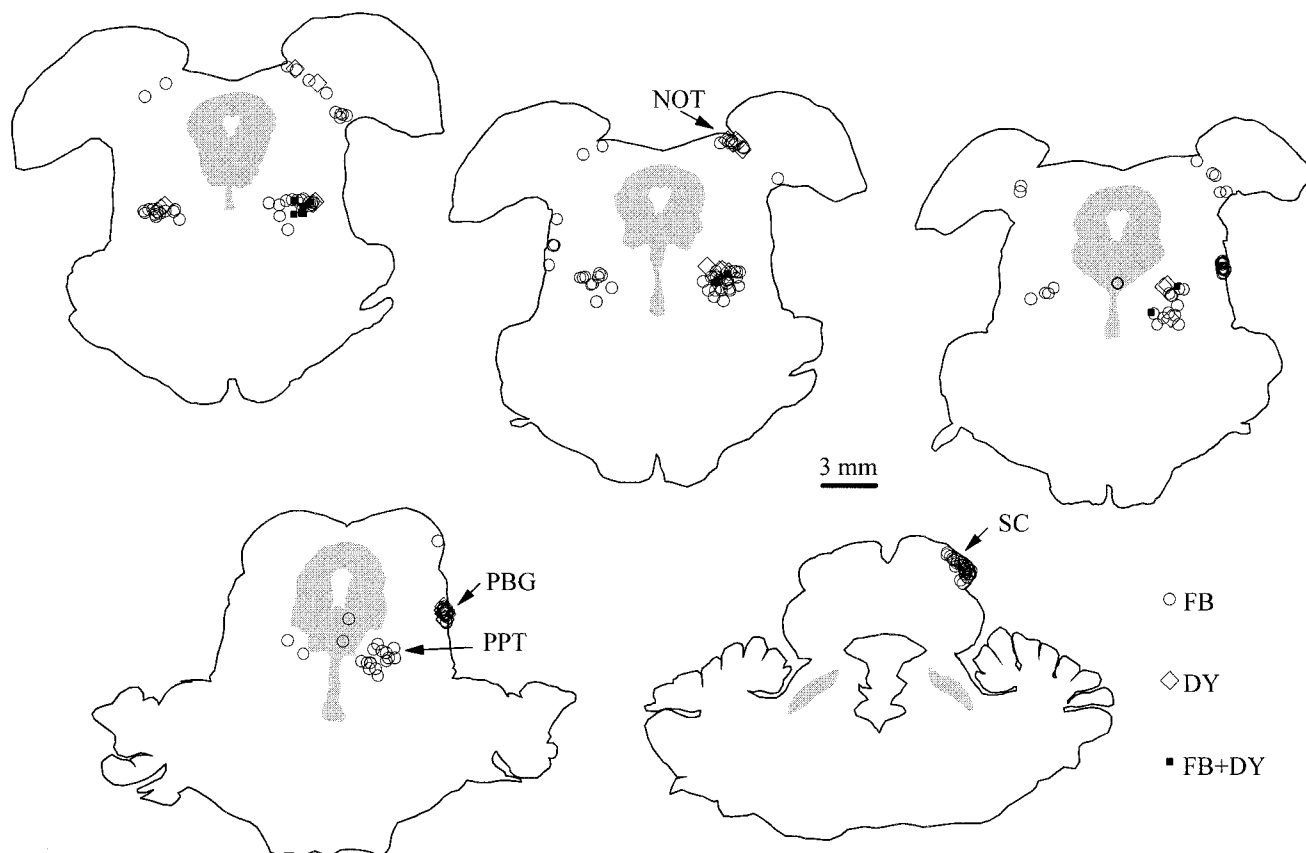


Figure 3 (Continued)

### Topography of projections to the LGN

Following dual injections of fast blue and diamidino yellow unilaterally but in widely spaced foci into the LGN of one animal (Fig. 3), the only area that contained double-labeled cells was the PPT (Fig. 3). This suggests that, as in the cat (Uhlrich et al., 1988), single PPT axons have widespread projections within the LGN. However, only cells ipsilateral to the dual injections were double-labeled. This, and the fact that more ipsilateral than contralateral pedunculopontine tegmentum cells were labeled in both cases, suggests that contralateral PPT projections are more restricted than are ipsilateral ones.

In contrast to the labeling in the PPT, the cell labeling within the thalamic reticular nucleus and superior colliculus indicates that these areas project to restricted, topographic regions within the LGN. As illustrated in Figure 3, an injection in the rostral LGN resulted in cell labeling in more rostral areas of the thalamic reticular nucleus, and an injection in the caudal LGN resulted in cell labeling in more caudal regions of the thalamic reticular nucleus. In the superior colliculus, small restricted clusters of cells were labeled in the superficial layers following injections in the LGN (Fig. 3).

Cells labeled with diamidino yellow and fast blue were intermingled in the nucleus of the optic tract, but were never observed in the same cell. This pattern of labeling suggests that pretectogeniculat axons are nontopographic, but sparser than PPT projections. In fact, antero-

grade labeling studies have shown that the projections from the nucleus of the optic tract to the LGN are fairly dense in the magnocellular laminae, but sparse in the parvocellular laminae where the majority of our injections were placed (Büttner-Ennever et al., 1996). The small numbers of cells labeled in the hypothalamus and the dorsal raphe nucleus preclude any analysis of topography, but suggest that these projections to the LGN are sparse.

### GABAergic projections to the monkey LGN

As illustrated in Figure 4A–C, the monkey LGN is densely stained with an antibody directed against GAD. In addition to small GABAergic interneurons, the neuropil in all laminae of the LGN contains dense punctate staining, which presumably represents GABAergic axonal and dendritic terminals (Wilson, 1993). GAD staining in the subcortical areas projecting to the LGN reveals two potential sources of GABAergic projections: the thalamic reticular nucleus (Fig. 4A) and the nucleus of the optic tract (Fig. 4D,E). Both of these areas contain cells stained for GAD, and have previously been identified as sources of GABAergic projections to the LGN in the cat (Cucchiari et al., 1991a,b, 1993; Uhlrich et al., 1991; Wahle et al., 1994; Bickford et al., 1994; Uhlrich and Manning, 1995).

Figure 5 illustrates that cells in the thalamic reticular nucleus and nucleus of the optic tract that were labeled by retrograde transport from fast blue and/or diamidino yellow injections in the LGN also contain GAD. In these

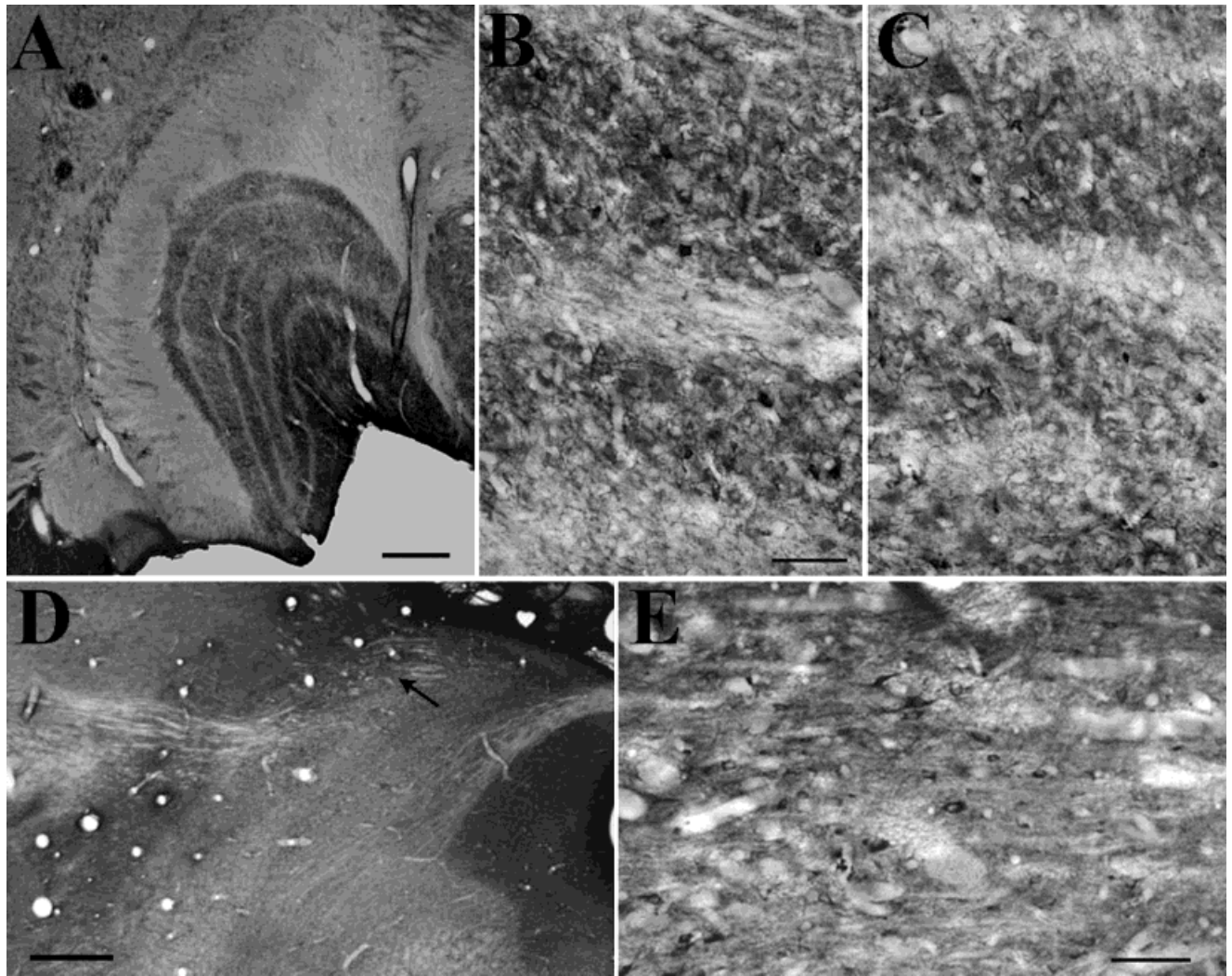


Fig. 4. The potential sources of gamma aminobutyric acid (GABA)ergic innervation of the lateral geniculate nucleus (LGN) are illustrated in sections stained for glutamic acid decarboxylase (GAD) by using immunohistochemical methods. **A:** The LGN and surrounding thalamic reticular nucleus stain densely for GAD. **B,C:** GAD staining in the magnocellular (B) and parvocellular layers (C) of the

LGN reveals numerous puncta and small interneurons. **D,E:** GAD stains a population of cells in the nucleus of the optic tract. The arrow indicates an area in D that is shown at higher magnification in E (scale bar). Scale bars = 1 mm in A; 100  $\mu$ m in B (also applies to C); 1 mm in D; 100  $\mu$ m in E.

sections, the GAD antibody was tagged with rhodamine. To determine whether cells were labeled with fast blue, diamindino yellow, and/or rhodamine, sections were alternately viewed under ultraviolet or green epifluorescent illumination (see Fig. 1C, D). Of a sample of 166 thalamic reticular nucleus (TRN) cells labeled by retrograde transport from the LGN injections, 144 (87%) were also labeled for GAD, and of a sample of 75 nucleus of the optic tract (NOT) cells labeled by retrograde transport from the LGN injections, 38 (51%) were also labeled for GAD.

Evidence from other species (Houser et al., 1980) suggests that the failure to observe all retrogradely labeled cells in the thalamic reticular nucleus as also containing GAD reflects a failure of the GAD antibody to penetrate the entire thickness of the sections. However, the higher percentage of double-labeled cells in the thalamic reticular nucleus suggests that lack of antibody penetration

cannot account for the lack of GAD labeling in the population of pretectogeniculate cells. Perhaps part of the projection from the pretectum to the LGN is not GABAergic, or arises from cells that contain lower levels of GAD which can only be detected with more sensitive techniques such as in situ hybridization (Wahle et al., 1994).

#### Cholinergic/Nitroergic projections to the monkey LGN

Figure 6A illustrates the dense innervation of the monkey LGN by cholinergic axons. These axons were distributed throughout the magnocellular and parvocellular layers, as well as in the interlaminar zones (see also Wilson et al., 1999). Figure 6B illustrates that the monkey LGN is also innervated by axons that contain the enzyme BNOS, the synthesizing enzyme for nitric oxide. However, unlike in the cat (Bickford et al., 1993, 1999), we found no cells



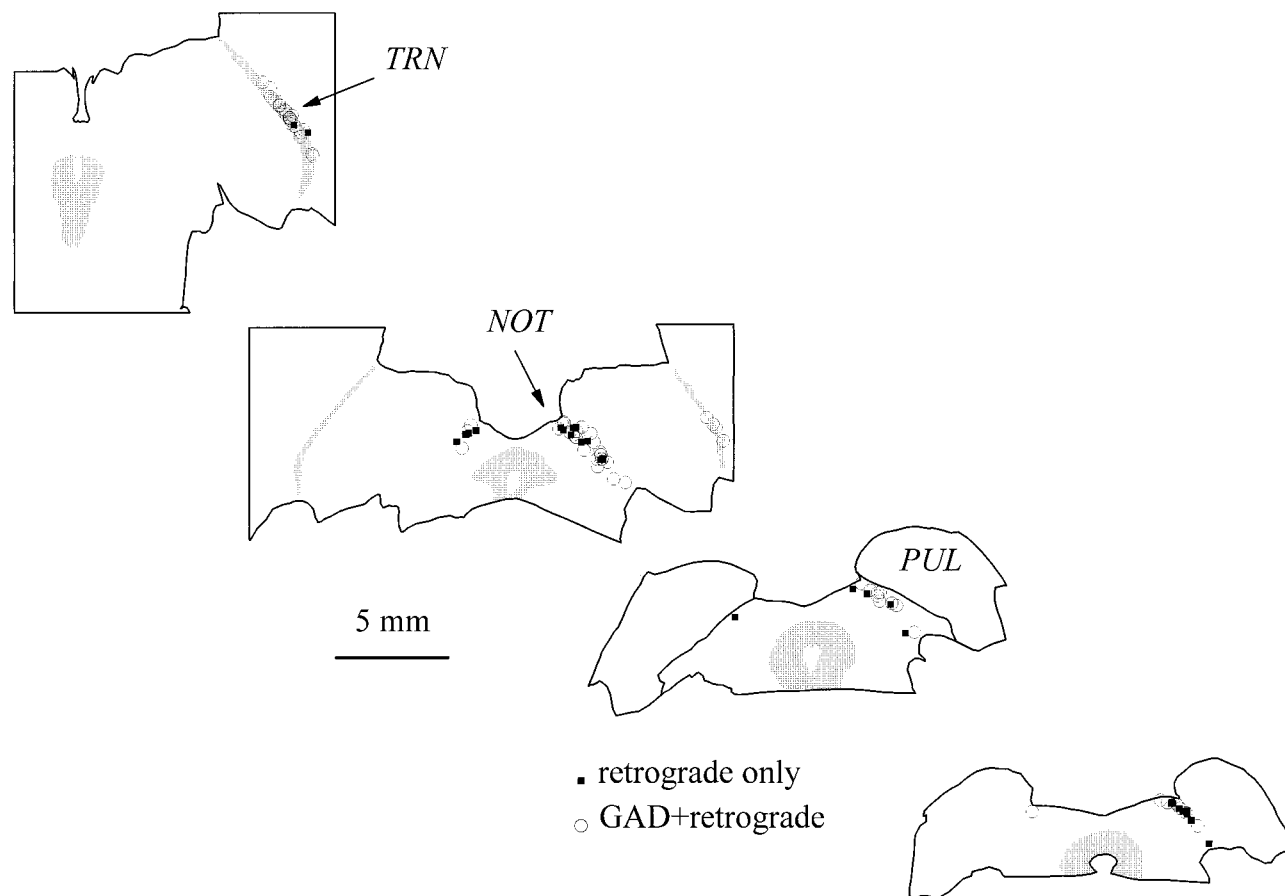


Fig. 5. Most cells in the nucleus of the optic tract (NOT) and the thalamic reticular nucleus (TRN) that project to the lateral geniculate nucleus are gamma aminobutyric acid (GABA)ergic. The distribution of cells labeled by retrograde transport from the injections illustrated

in Figure 3 are plotted in sections that were also stained for glutamic acid decarboxylase (GAD) by using an antibody tagged with rhodamine. Cells that contained fast blue or diamidino yellow and GAD are indicated by the open circles. PUL, pulvinar nucleus.

that stained for BNOS. The distribution and morphology of axons stained with the ChAT antibody or the BNOS antibody were very similar, although the BNOS fibers tended to be less densely distributed within the interlaminar zones. This is consistent with the idea that many axons in the LGN contain both ChAT and BNOS, as has previously been reported for the cat (Erişir et al., 1997).

In the cat LGN, the source of cholinergic/nitroergic axons has been identified as a group of cells that are located in the region of the brachium conjunctivum, or parabrachial region (Bickford et al., 1993; Erişir et al., 1997). In the primate, this region is most commonly referred to as the PPT (Fitzpatrick et al., 1988; Wilson et al., 1995). As in the cat parabrachial region, we found that cells in the PPT stain with antibodies against ChAT or BNOS or with the NADPH-diaphorase reaction. We found that the NADPH-diaphorase reaction indicates the presence of BNOS; all cells labeled with the NADPH-diaphorase reaction could also be labeled with an antibody against BNOS (Fig. 1G, H; 200 of 200 sampled). In addition, most PPT cells immunocytochemically labeled for BNOS also stained immunocytochemically for ChAT (Fig. 1E, F; 202 of 219 or 92%) and most PPT cells stained for CHAT also labeled for BNOS (202 of 206 or 98%). Similarly, in sections stained

immunocytochemically for ChAT, and then histochemically for NADPH-diaphorase, the majority of ChAT-stained cells contained the NADPH-diaphorase reaction product (320 of 485 or 68%; Fig. 7). The lower percentage of double-labeled cells in material stained with the NADPH-diaphorase reaction probably reflects the fact that the immunocytochemical method is a more sensitive method of detecting BNOS (Bickford et al., 1999).

As illustrated in Figures 2 and 3, the vast majority of cells in the PPT that project to the LGN are located specifically in the region of cholinergic/nitroergic cells. In fact, within the PPT, nearly 100% (140 of 145 sampled) of cells labeled by retrograde transport from injections in the LGN were also labeled with a ChAT antibody tagged with rhodamine (Figs. 1A, B; 8). Thus, as in the cat, the cells in and around the brachium conjunctivum are a source of acetylcholine and nitric oxide in the LGN of the monkey.

### Cholinergic projections to the LGN

The parabrachial nucleus (PBG) also contains a dense population of cholinergic cells and nearly 100% (80 of 86 sampled) of the cells that were labeled by retrograde transport from injections in the LGN were also labeled with a ChAT antibody tagged with rhodamine (Fig. 8). In

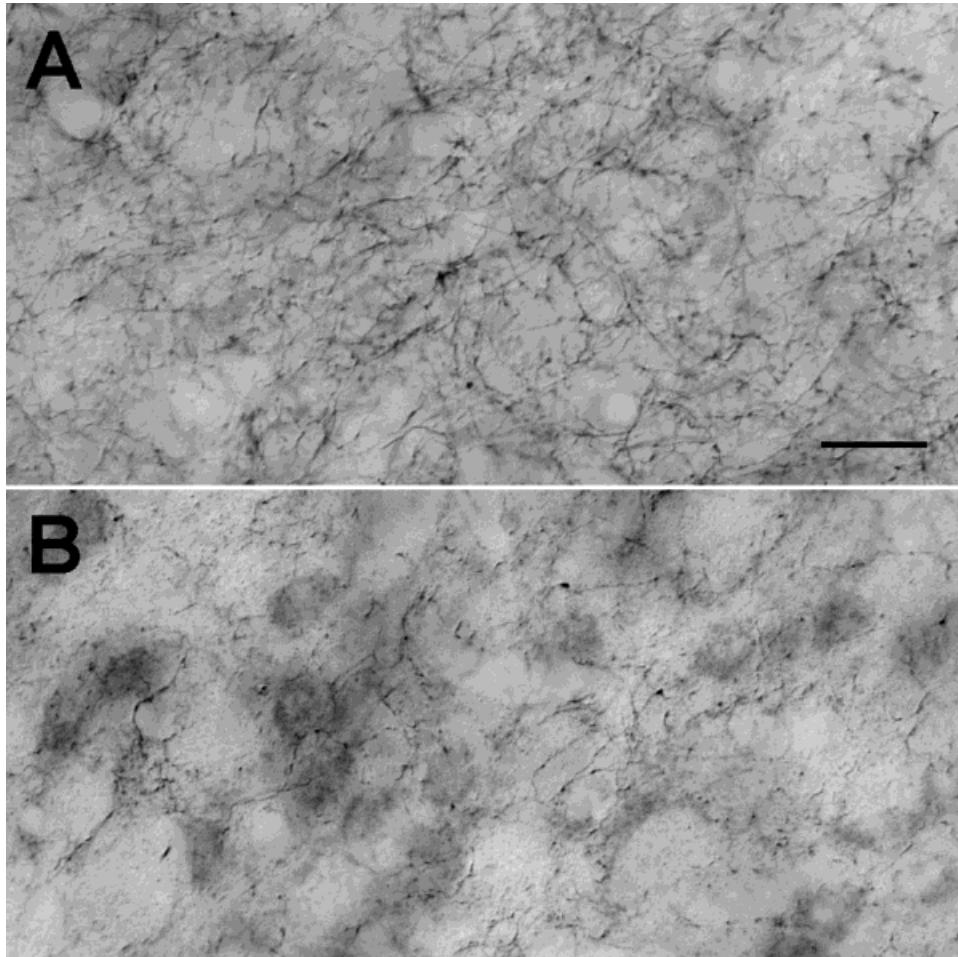


Fig. 6. The lateral geniculate nucleus contains fibers that stain for choline acetyl transferase (A) and brain nitric oxide synthase (B). Photomicrographs illustrate immunohistochemical staining in the parvocellular laminae. Scale bar = 30  $\mu$ m (applies to A,B).

contrast to the PPT, we found no BNOS staining in the parabigeminal nucleus. Therefore, the cholinergic axons in the LGN that arise from the PBG most likely do not contain BNOS. Results from previous anterograde transport studies have shown that the PBG primarily innervates the interlaminar zones (Fitzpatrick et al., 1988; Harting et al., 1991b). This likely accounts for some of the cholinergic fibers that we observed in the interlaminar zones.

#### **Lack of noradrenergic projections to the LGN**

In contrast to the dense cholinergic/nitroergic innervation of the monkey LGN, we detected no LGN labeling in sections incubated with an antibody against tyrosine hydroxylase, an enzyme used in the production of noradrenaline (data not shown, see also Morrison and Foote, 1986; Rico and Cavada, 1998). As shown in Figures 9–11, brainstem cells that contain tyrosine hydroxylase surround, but are separate from, the cholinergic/nitroergic cells of the pedunculopontine tegmentum. This suggests that the majority of brainstem projections to the LGN are cholinergic,

and confirms the lack of noradrenergic innervation of the monkey LGN. This constitutes the primary difference between neurotransmitters found in the LGN of the monkey versus those reported in other species (Morrison, and Foote, 1986; De Lima and Singer, 1987; Fitzpatrick et al., 1989; Papadopoulos and Parnavelas, 1990; Rico and Cavada, 1998).

#### **DISCUSSION**

Because the monkey is a significant species for the study of the visual system, it is important to establish how the circuitry of the LGN can modulate the transfer of visual information from the retina, through the LGN, to the cortex. As a starting point for understanding the effects of extraretinal inputs to the monkey LGN, we sought to identify the neurotransmitters used in these projections. We used methods similar to those we have used previously to examine extraretinal inputs to the cat LGN to determine the similarities and/or differences between the organization of the LGN in these two species. The present results indicate that the subcortical innervation of

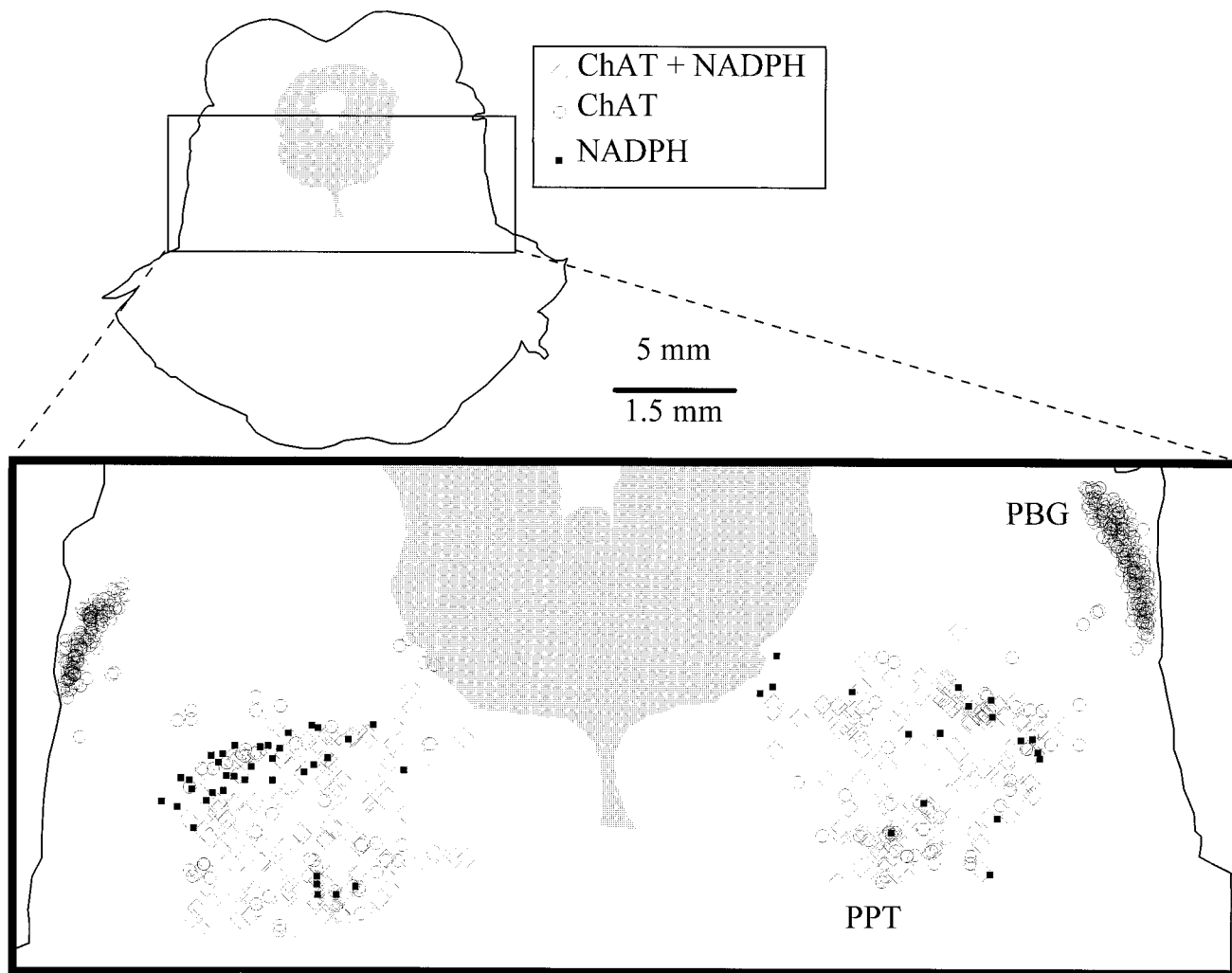


Fig. 7. Most cholinergic cells in the pedunclopontine tegmentum (PPT) are nitroergic. The plot shows the distribution of cells that stain for choline acetyltransferase (ChAT), nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase, or both in a section stained immunocytochemically for ChAT, and then histochemically for

NADPH-diaphorase. The majority of ChAT-stained cells in the PPT contained the NADPH-diaphorase reaction product (320 of 485 or 68%). In contrast, none of the ChAT-stained cells in the parabigeminal nucleus (PBG) contained the NADPH-diaphorase reaction product.

the monkey LGN is very similar to that found in the cat. In particular, we found that the monkey LGN receives a significant cholinergic/nitroergic projection from the PPT, GABAergic projections from the TRN and pretectum, and a cholinergic projection from the parabigeminal nucleus. The major difference between the innervation of the LGN in the cat and the monkey is the absence of a noradrenergic projection to the monkey LGN. The segregation of the noradrenergic cells and cholinergic cells in the monkey brainstem also differs from the intermingled arrangement found in the cat brainstem.

### Cholinergic/nitroergic projections

We found that a significant population of fibers in the monkey LGN can be stained with an antibody against ChAT, and that the majority of cells in the PPT and parabigeminal nucleus labeled by retrograde transport from injections in the LGN also stain for ChAT. This

confirms and extends the results of a recent study that showed that dense cholinergic fibers in the macaque LGN make multiple synaptic contacts with both relay cells and interneurons (Wilson et al., 1999).

We also found that similar fibers in the LGN could be stained with an antibody against BNOS. These fibers were more difficult to detect, probably due to the sensitivity of this antibody to fixative conditions (Bickford et al., 1999). However, the resulting stained fibers were very similar in morphology and distribution to the ChAT fibers, suggesting that individual fibers colocalize ChAT and BNOS. In fact, when we examined the cholinergic PPT cells, we found that nearly 100% contained BNOS. This strongly suggests that the majority of PPT fibers in the monkey LGN contain both ChAT and BNOS. In contrast, none of the cholinergic cells in the parabigeminal nucleus stained for BNOS. Because the parabigeminal nucleus primarily projects to the intralaminar zones of the monkey LGN

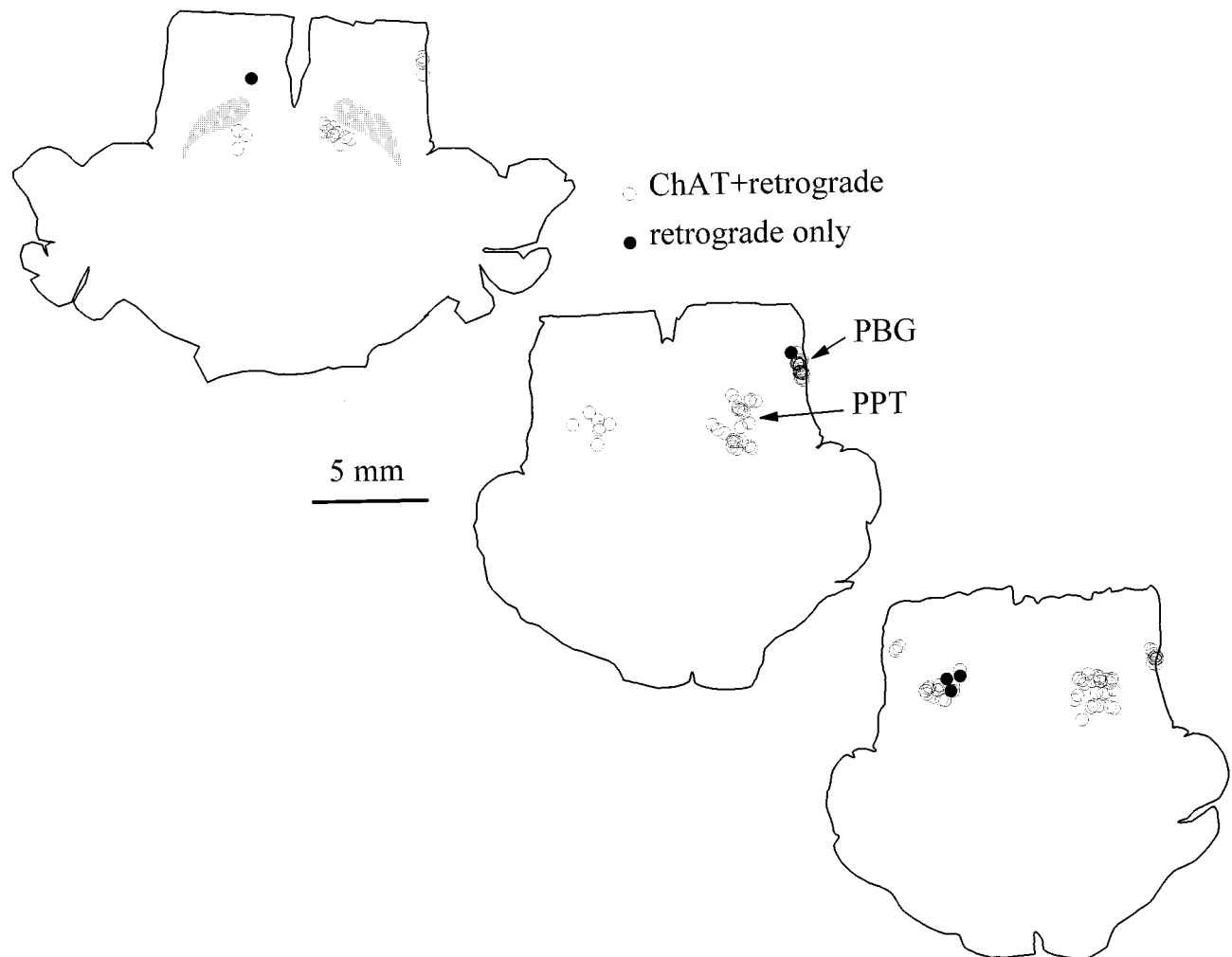


Fig. 8. Most cells in the parabrachial nucleus (PBG) and the pedunculopontine tegmentum (PPT) that project to the lateral geniculate nucleus are cholinergic. The distribution of cells labeled by retrograde transport from the injections illustrated in Figure 3 are

plotted in sections that were also stained for choline acetyl transferase (ChAT) by using an antibody tagged with rhodamine. Cells that contained fast blue or diamidino yellow and ChAT are indicated by the open circles.

(Harting et al., 1991b), this projection likely accounts for some of the cholinergic fibers that we observed in the interlaminar zones. However, it appears that the PPT also innervates the interlaminar zones to some extent because we observed some BNOS-labeled fibers in these regions.

The presence of BNOS in the cholinergic cells of the PPT appears to be a ubiquitous feature of vertebrates. Previous studies showed that the cholinergic cells of the parabrachial region (PBR) or PPT of the turtle, rat, cat, dog, monkey, baboon, and human stain with the NADPH-diaphorase reaction (Vincent et al., 1983; Reiner and Vincent, 1987; Bickford et al., 1993; Guela et al., 1993; Powers and Kaeppler, 1995; Tafti et al., 1997). The present study, and our previous results in the cat (Erişir et al., 1997), suggest that, at least within the PPT, that the NADPH-diaphorase reaction is an indicator for the presence of BNOS.

In the rat, there is substantial evidence that acetylcholine (ACh) and nitric oxide (NO) are coreleased from terminals originating in the brainstem (Saito et al., 1977;

McCarley et al., 1978; Sakai and Jouvet, 1980; El Mansari et al., 1989; Steriade et al., 1990; Williams et al., 1994, 1997; Miyazaki et al., 1996). The effects of this release in the LGN have been studied in most detail in the cat, where the primary effect of PBR stimulation is to change the firing mode of the relay cells from the relatively hyperpolarized "burst mode" of slow wave sleep or periods of relative inattentiveness, to a more depolarized "tonic mode" characteristic of periods of attentive wakefulness or REM sleep (Lu et al., 1993). This transition is presumably triggered by the combined effects of ACh binding to nicotinic and muscarinic receptors on relay cells, interneurons, and TRN cells (Eysel et al., 1986; McCormick and Prince, 1986, 1987; Francesconi et al., 1988; McCormick and Pape, 1988; McCormick, 1989), as well as influences of NO on the membrane properties of thalamocortical cells (Pape and Mager, 1992; Cudeiro et al., 1994a,b, 1996). Thus, a variety of studies have suggested that the cholinergic/nitrergic projections to the LGN mediate a complex enhancement of thalamocortical cell responses.



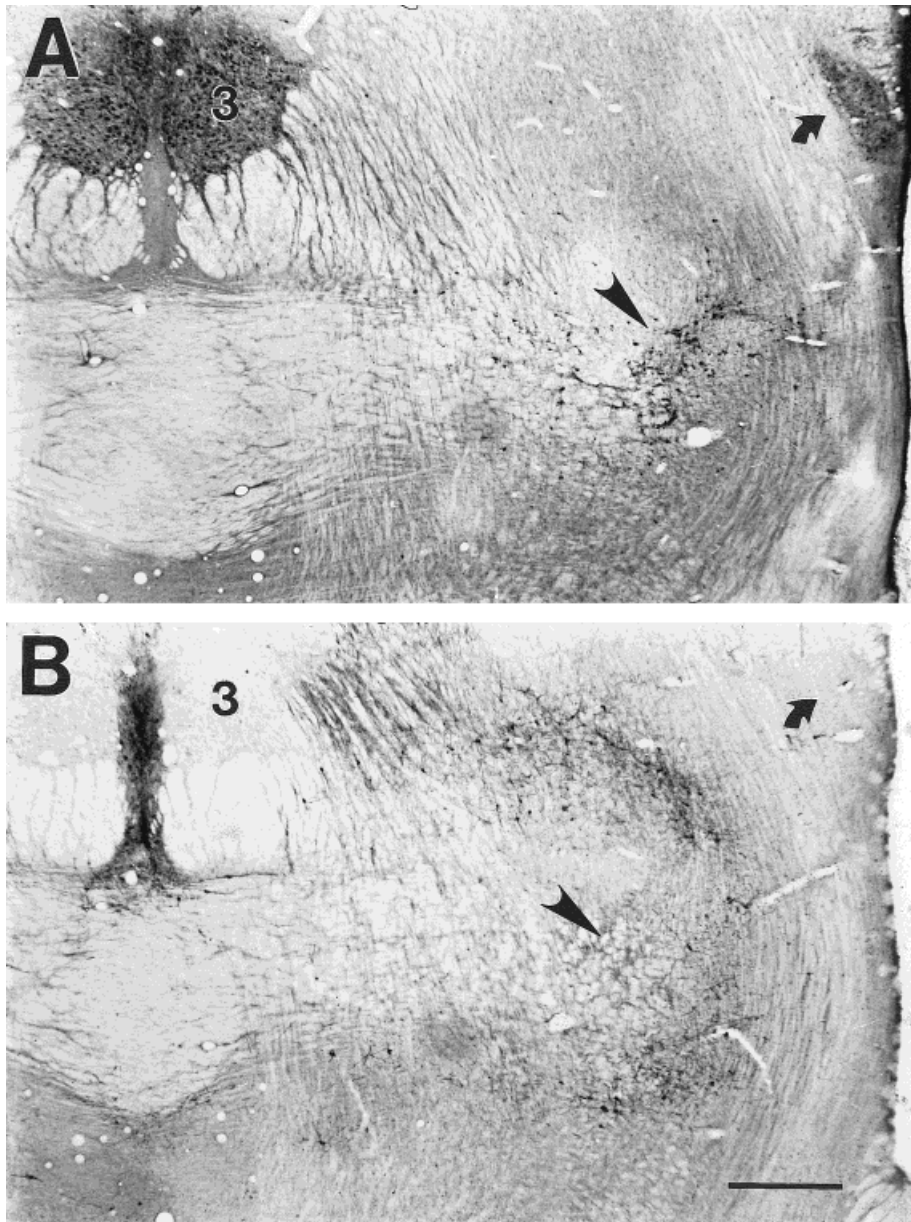


Fig. 9. In the pedunculopontine tegmentum (PPT), cholinergic cells are segregated from noradrenergic cells. **A:** A section stained for choline acetyl transferase illustrates the distribution of cholinergic cells in the PPT (arrowhead), the oculomotor nucleus (3), and the

parabrachial nucleus (curved arrow). **B:** An adjacent section stained for tyrosine hydroxylase illustrates that nearby noradrenergic cells are segregated from the PPT (arrowhead). Scale bar = 1 mm (applies to A,B).

In vivo, stimulation of the parabrachial region increases both the strength of response to receptive field centers as well as the strength of inhibition evoked by stimulation of surrounding regions. These effects would be expected to result in the transmission of a sharper visual image (Uhlrich et al., 1995). However, to ultimately understand how the cholinergic/nitrgergic projections affect visual perception, it will be necessary to carry out experiments in awake, behaving monkeys. The finding that projections similar to those studied in detail in the cat also exist in the monkey sets the stage for future behavioral tests of the cholinergic and/or nitrgergic effects on visual processing.

### GABAergic projections

We identified two extrinsic GABAergic inputs to the monkey LGN, from the TRN and from the nucleus of the optic tract. The fact that cells in the TRN retrogradely labeled from the LGN were stained with the GAD antibody was not surprising. In fact, it has been shown that terminals in the monkey LGN labeled from injections of biocytin in the TRN can be labeled with antibodies to GABA (Feig et al., 1998). Our results also confirm previous anterograde tracing studies in the primate that have shown a high degree of topography in the

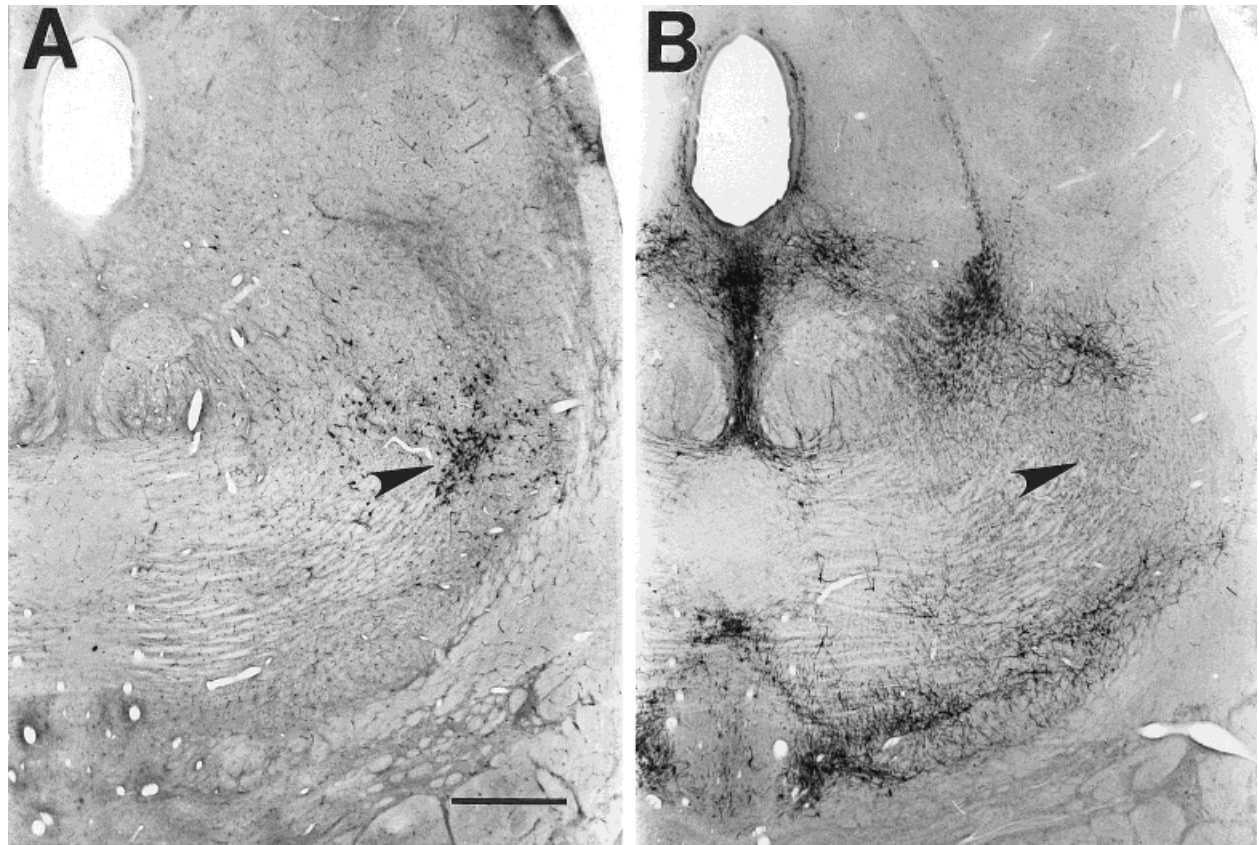


Fig. 10. In the pedunculopontine tegmentum (PPT), nitroergic cells are segregated from noradrenergic cells. **A:** A section stained for brain nitric oxide synthase illustrates the distribution of nitroergic cells in

the PPT (arrowhead). **B:** An adjacent section stained for tyrosine hydroxylase illustrates that nearby noradrenergic cells are segregated from the PPT (arrowhead). Scale bar = 1 mm (applies to A,B).

projections from the TRN to the LGN (Harting et al., 1991c).

We also found that most of the pretectogeniculate cells are GABAergic, as was previously described for the cat (Cucchiari et al., 1991a; Wahle et al., 1994). In primates, anterograde studies have described this projection as primarily innervating the parvocellular lamina (Harting et al., 1986) or the magnocellular lamina (Büttner-Ennever et al., 1996). Our results using retrograde tracers cannot completely clarify these differences. In one case, there were more pretectal cells labeled from a fast blue injection centered in the parvocellular layers than were labeled from a diamidino yellow injection centered more in the magnocellular layers. However, the diamidino yellow injection site was slightly smaller than the fast blue injection site, and differences in the uptake and transport of these two tracers make it difficult to directly compare the resulting number of labeled pretectogeniculate cells. Differences in the distribution of pretectogeniculate axons in the rostral and caudal areas of the LGN have also been documented (Büttner-Ennever et al., 1996) and may account for differences in the number of labeled cells in the pretectum. On the other hand, because pretectal cells labeled by both of these tracer injections were intermingled we can conclude that the pretectogeniculate projection is not as highly topographic as the projection from the TRN, and that it projects to some extent to both the magnocellular and parvocellular laminae.

The pretectal cells that project to the cat LGN respond to rapid displacements of large textured stimuli, and during saccadic eye movements in both the light and dark (Schmidt and Hoffman, 1992). Similar responses have been recorded in the monkey nucleus of the optic tract (Mustari and Fuchs, 1990). In the cat LGN, pretectal terminals primarily contact interneurons (Cucchiari et al., 1993). Thus, the pretectogeniculate projection may serve to disinhibit thalamocortical cells following saccadic eye movements. In the galago LGN, however, pretectal terminals have been shown to directly contact thalamocortical cell dendrites (Feig and Harting, 1994). If the pretectogeniculate projection in this species is GABAergic, it may suppress LGN responses during saccadic movements. The function of the pretectogeniculate projection in the macaque remains unclear. Because we found that the majority of macaque pretectogeniculate cells are GABAergic, it will be of interest to determine the postsynaptic targets of these terminals.

### Other neurotransmitter systems

The small number of cells labeled by retrograde transport in the dorsal raphe and the hypothalamus precluded an analysis of the neurotransmitters used in these projections. However, previous studies strongly suggest that serotonergic and histaminergic fibers emanate from these respective cell groups, and each group provides a moderately dense innervation of the monkey LGN (Pasik



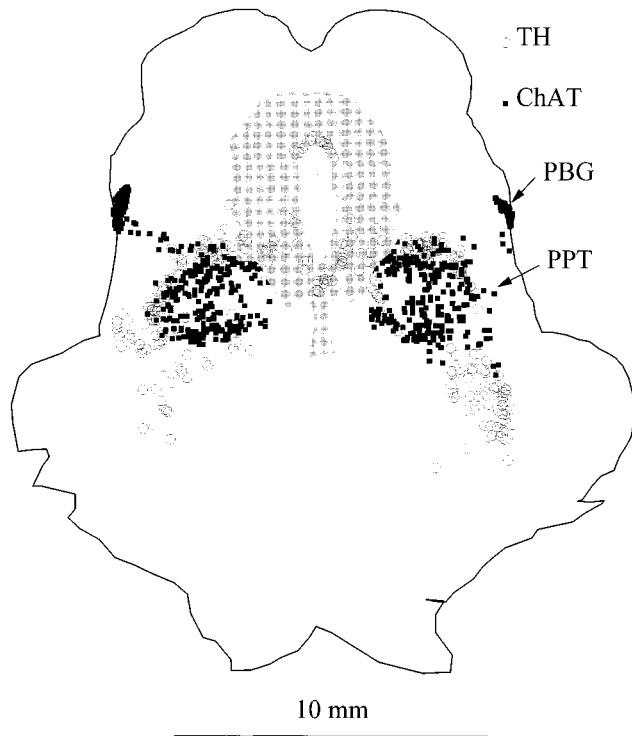


Fig. 11. Noradrenergic cells are adjacent but segregated from cholinergic cells of the pendunculo-pontine tegmentum (PPT) as illustrated in this superimposed plot of the distribution of cells stained for choline acetyl transferase (ChAT) and tyrosine hydroxylase (TH) in adjacent sections. PBG, parabigeminal nucleus.

et al., 1988; Wilson and Hendrickson, 1988; Manning et al., 1996; Wilson et al., 1999). Because our retrograde tracing indicates that few cells in the dorsal raphe and hypothalamus project to the monkey LGN, significant axonal projections may radiate from individual cells. The serotonergic and histaminergic fibers in the LGN rarely participate in conventional synaptic contacts (Wilson and Hendrickson, 1988; Wilson et al., 1999). Instead, these fibers appear to release their neurotransmitters through en passant varicosities to provide a more general modulation of LGN activity. Thus, the activity of single cells in the dorsal raphe and hypothalamus may have significant contributions to the modulation of LGN activity.

We also confirmed that the superior colliculus provides a topographic projection to the monkey LGN (Wilson et al., 1995), but the small number of cells that were labeled did not allow us to identify the neurotransmitter(s) used in this projection. To date, the neurotransmitter used in the tectogeniculat pathway has not been defined in any species. Anterograde tracing studies have shown that the tectogeniculat projection is precisely limited to the small cells of the S laminae, interlaminar zones, or koniocellular laminae (Fitzpatrick et al. 1980; Harting et al., 1991a).

### Absence of noradrenaline in the monkey LGN

In the cat, there is a small but detectable projection from the noradrenergic cells of the brainstem to the LGN (Smith et al., 1988; Fitzpatrick et al., 1989), and in vitro

studies in the cat, ferret, and guinea pig have shown that the application of noradrenaline to LGN cells contributes to the switch from the "burst mode" to the "tonic mode" of firing (McCormick and Prince, 1988; McCormick and Pape, 1990; McCormick, 1992; Lee and McCormick, 1996). Thus, in some species, a noradrenergic projection may participate in the modulation of LGN activity. However, in the monkey, it appears that there are no noradrenergic projections to the LGN. It was previously reported that the LGN was unstained with antibodies against tyrosine hydroxylase (Morrison and Foote, 1986; Rico and Cavad, 1998) and the present study confirms this lack of labeling. In fact, we have shown that the vast majority of projections from the brainstem to the monkey LGN arise from the cholinergic/nitric cells of the PPT. In addition, unlike the intermingling of cholinergic and noradrenergic cells that is found in the parabrachial region of the cat (Jones and Beaudet, 1987; Reiner and Vincent, 1987; Bickford et al., 1993), we found that these cell groups are adjacent, but well segregated, in the monkey brainstem.

In the monkey, the thalamic projections of the noradrenergic cells appear to be much more limited to the midline thalamic nuclei (Rico and Cavad, 1998) than in species such as the rat (Papadopoulos and Parnavelas, 1990) and cat (Fitzpatrick et al., 1989). This appears to be the main difference in the innervation of the monkey LGN and the LGN of other species. In fact, it appears that moving up the evolutionary scale, there is a decreasing density of noradrenergic innervation of the LGN. In contrast, the cholinergic/nitric projections to the monkey LGN are robust, perhaps emphasizing the importance of the projections from the PPT in visual processing.

In summary, we have shown that the organization of neurotransmitter inputs to the monkey LGN is strikingly similar to the patterns occurring in the cat, with the notable exception of the absence of a noradrenergic brainstem input. Our findings, coupled with recent data showing direct parallels in the intrinsic membrane properties of primate thalamic neurons compared with cats (Ramcharan et al., 1999), suggest that studies of basic mechanisms underlying the control of visual information flow through the LGN of the cat may relate directly to similar issues in primates, and ultimately, humans.

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