Neuroscience Vol. 54, No. 4, pp. 1091–1101, 1993
Printed in Great Britain

0306-4522/93 $6.00 + 0.00
Pergamon Press Ltd

LAMINAR ORGANIZATION OF ACETYLCHOLINESTERASE AND CYTOCHROME OXIDASE IN THE LATERAL GENICULATE NUCLEUS OF PROSIMIANS

C. T. McDonald, E. R. McGuinness and J. M. Allman*
Division of Biology, California Institute of Technology, Pasadena, CA 91125, U.S.A.

Abstract—Hess and Rockland [Hess and Rockland (1983) *Brain Res.* **289**, 322–325] proposed that the distribution of acetylcholinesterase within the lateral geniculate nucleus might correlate with the daily activity patterns shown by primates. In diurnal primates, the magnocellular laminae show a greater acetylcholinesterase reaction product. In nocturnal primates, the parvocellular laminae are more heavily stained. We have examined the laminar distribution of acetylcholinesterase and cytochrome oxidase in the lateral geniculate nucleus of a series of rare prosimian primates. In all prosimians examined, the most dense acetylcholinesterase reaction product is seen in the parvocellular layers of the lateral geniculate nucleus. Heavy cytochrome oxidase activity is seen in both the magnocellular and parvocellular layers, but not the koniocellular layers of the prosimian lateral geniculate nucleus. We have also employed a polyclonal antibody to choline acetyltransferase to examine the laminar organization or cholinergic activity in the *Galago* (Bushbaby) lateral geniculate nucleus. We report that choline acetyltransferase immunoreactivity does not correlate with acetylcholinesterase activity in the prosimian lateral geniculate nucleus. Although the lateral geniculate nucleus is more immunoreactive than most other thalamic structures and although the intercalated koniocellular laminae demonstrate somewhat lighter choline acetyltransferase immunoreactivity, no great difference in staining intensity is seen between the parvocellular and magnocellular laminae.

In addition, we examined the phenotype of known inputs to assess the laminar specificity of cholinergic projections to the bushbaby lateral geniculate nucleus. Layer VI of primary visual cortex, which is known to be a source of acetylcholinesterase in the parvocellular layers, does not contain cholinergic cells, nor does the pretectal nucleus, which projects mainly to the parvocellular layers. The parabigeminal nucleus is cholinergic; however, this nucleus is known to project to the koniocellular layers, along with the non-cholinergic superior colliculus. Finally, the pedunculopontine tegmental nucleus, which provides a strong input to many regions of the thalamus, including the lateral geniculate nucleus, is cholinergic. The laminar organization of its input to the lateral geniculate nucleus is not known.

Increased acetylcholinesterase reaction product within the parvocellular layers of the lateral geniculate nucleus is common to all strepsirhine primates. The pattern is also seen in the only two nocturnal haplorhine primates, *Tarsius* and *Aotus* (owl monkey). The relation of this increased acetylcholinesterase activity to cholinergic function remains unclear. We note exceptions to the suggested correlation between nocturnality and increased density of acetylcholinesterase within the parvocellular layers. We hypothesize that rather than reflecting nocturnal/diurnal lifestyles, the acetylcholinesterase staining pattern may be a primitive feature of geniculate organization in primates.

The lateral geniculate nucleus (LGN) is a distinctly laminated thalamic structure which serves to relay visual information from the ganglion cells of the retina to the primary visual cortex (striate cortex; area 17; V1). Information from the two eyes is carried separately within different layers of the LGN and this information is further specified into functional "streams" which are maintained throughout the primate visual system (for review, see Refs 1, 8, 35).

Several histochemical studies have examined the laminar distribution of acetylcholinesterase (AChE) and cytochrome oxidase (CO) within the LGN of various primate species, including the macaque monkey,[6,21] the owl monkey and the prosimian bushbaby,[11,12] and the squirrel monkey.[17,21] Hess and Rockland[17] were the first to suggest that the distribution of AChE within the LGN might correlate with the daily activity patterns shown by the primates. In diurnal primates such as the macaque and the squirrel monkey, the magnocellular laminae show a greater AChE reaction product. In nocturnal primates such as the owl monkey and the bushbaby, the parvocellular laminae are more heavily stained with AChE reaction product.

We have sought to further these observations by examining the distribution of AChE and CO within the LGN of a series of rare prosimian primates that had died of natural causes. In an effort to account for increased AChE reaction product in the parvocellular layers of the prosimian *Galago senegalensis* (Lesser bushbaby), we have also employed choline acetyltransferase (ChAT) immunohistochemistry to explore the

*To whom correspondence should be addressed at: 216–76, California Institute of Technology, Pasadena, CA 91125, U.S.A.

Abbreviations: AChE, acetylcholinesterase; ChAT, choline acetyltransferase; CO, cytochrome oxidase; LGN, lateral geniculate nucleus; NRS, normal rabbit serum; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.

phenotype of projections to the LGN. Some of these results have been described in preliminary reports.[26,27]

EXPERIMENTAL PROCEDURES

Most of the animals used in this study were rare prosimian primates from the Duke University Primate Center that had died of natural causes. Animals obtained from the Primate Center included *Microcebus* (Mouse lemur, one animal); *Cheirogaleus* (Dwarf lemur, one); *Hapalemur griseus* (Grey gentle lemur, two); *Lemur fulvus* (Brown lemur, two) and *Lemur macaco* (Black lemur, one); *Propithecus* (Sifaka, one); *Loris tardigradus* (Slender loris, one); *Nycticebus coucang* (Slow loris, one); *Perodicticus potto* (Potto, one); *Tarsius bancanus* (Horsfield's tarsier, two); and *Tarsius syrichta* (Phillipine tarsier, two). Soon after an animal's death (usually, within 2 h), the head was frozen to −80°C and shipped in dry-ice by overnight carrier. The brain was removed from the skull and thawed in a cold bath of 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was then separated right from left hemisphere by a midline sagittal incision through the corpus callosum and the third and fourth ventricles.

The hemisphere designated for CO histochemistry (method of Wong-Riley[36]) was fixed by immersion at 4°C with 4% glutaraldehyde in 0.1 M phosphate buffer. Alternate sections from this hemisphere were stained for Cresyl Violet and in some cases AChE. The second hemisphere was fixed by immersion at 4°C with 4% paraformaldehyde in 0.1 M phosphate buffer. Alternate sections from this hemisphere were stained for AChE, Cresyl Violet, and in some cases the Gallyas fiber stain.[14] The hemispheres were cryoprotected by adding increasing concentrations of sucrose to the fixative (up to 30%). When the hemisphere had become sunken (one to two days depending on size), it was sectioned in either the coronal or parasagittal plane at 40 μm on a freezing microtome (American Optical).

The AChE histochemistry protocol was that of Rockland (1984, personal communication). Free-floating sections were incubated in 500 ml of distilled water containing 500 mg of acetylthiocholine iodide, 375 mg of glycine, 250 mg of cupric sulfate, and 2140 mg of anhydrous sodium acetate, titrated with acetic acid to pH 5.2. Ethopropazine (1 mM) served as inhibitor of non-AChE cholinesterases. Sections were incubated at 4°C until AChE-rich structures (e.g. caudate nucleus) turned chalky white. Reaction product was visualized using 10% potassium ferricyanide in distilled water at room temperature (5–10 min). Finally, sections were rinsed in three changes of 0.9% saline and mounted on to glass slides. Control sections were incubated in the presence of 1 mM BW248c51 (Buroughs–Wellcome), an inhibitor of AChE.

For the ChAT immunohistochemistry, two *Galago senegalensis* (Lesser bushbabies) were deeply anesthetized (sodium pentobarbitol, 30 mg/kg body weight, Abbott) and perfused through the heart with cold 0.05 M phosphate-buffered saline (PBS, 9% NaCl, pH 7.4) for 2 min, followed by 4% paraformaldehyde in PBS (15 min), and 4% paraformaldehyde + 10% sucrose in PBS (10 min) using a Masterflex perfusion apparatus (150 ml/min, Cole–Palmer). The brains were removed and allowed to sink overnight at 4°C in 4% paraformaldehyde + 30% sucrose in PBS, and sectioned in the coronal plane at 40 μm on a freezing microtome.

The primary antibody to ChAT was kindly supplied by Dr Felix Eckenstein (see Refs 9, 10 for a discussion on the production and specificity of the polyclonal antibody). With the exception of the above-mentioned fixation protocol, the immunohistochemical staining protocol of Amaral and Kurz[2] was employed. Free-floating sections were incubated for 15 min in a 0.4% Triton X-100 (TX-100) solution in 0.05 M Tris-buffered saline (TBS, pH 7.2–7.4), and then for 2 h in TBS with 3% normal rabbit serum (NRS) and 0.1% Triton X-100. Tissue was then incubated overnight in primary ChAT antibody at a 1:4 dilution (in TBS with 1% NRS and 0.1% Triton X-100) at room temperature. Sections were washed in three changes of TBS over a period of 30 min and incubated for 2 h in a 1:50 dilution of rabbit anti-rat IgG secondary (Accurate Scientific, Westbury, NY) in TBS containing 1% NRS and 0.1% Triton X-100. Sections were again washed and then incubated for 2 h in a 1:60 dilution of peroxidase–antiperoxidase (Sternberger and Meyer, Jarretsville, MD) in TBS containing 1% NRS, washed again, and treated with a 0.05% diaminobenzidine hydrochloride solution containing 0.015% hydrogen peroxide for 10–15 min. The reaction was stopped by washing the sections in TBS. The sections were washed in PBS, mounted, allowed to dry overnight, and rapidly dehydrated and coverslipped the following day. Alternate sections were processed for AChE histochemistry and for Cresyl Violet stain.

RESULTS

The laminar organization of the primate LGN has been described previously.[18,20] The LGN of the tarsier, the only haplorhine prosimian, contains four cell layers: two ventral magnocellular layers and two dorsal parvocellular layers. The LGN of strepsirhine primates (all prosimians except tarsiers) contains six

Fig. 1. (A) Parasagittal section through the LGN of the *Lemur fulvus* stained for Cresyl Violet. Anterior is to the left. The strepsirhine LGN contains six cell layers; two ventral magnocellular layers (ME, MI) and two koniocellular layers (KE, KI) intercalated between the two dorsal parvocellular layers (PE, PI). The arrow points to the "representation" of the optic disc. (B) Adjacent section stained for AChE. The two parvocellular layers (PE, PI) are more heavily stained for AChE reaction product than are the intercalated koniocellular and ventral magnocellular layers. Scale bar for A and B = 500 μm. (C) Coronal section through the LGN of the *Tarsius syrichta* stained for Cresyl Violet. Medial is to the left, dorsal to the top. The parvocellular and magnocellular laminae are separated by a prominent interlaminar fiber bundle (IL). (D) Adjacent section stained for AChE. Dense AChE reaction product is seen in the two dorsal parvocellular layers (PC), but relatively little reaction product is seen in the two ventral magnocellular layers (MC) or in the interlaminar fiber bundle. Scale bar for C and D = 500 μm. (E) Coronal section through the LGN of the *Microcebus* (Mouse lemur) stained for Cresyl Violet. Medial is to the left, dorsal to the top. (F) Adjacent section stained for AChE. Note that while the cellular lamination of the LGN is less distinct in Cresyl Violet-stained sections, the two parvocellular layers (PE, PI) contain the densest AChE reaction product. Scale bar for E and F = 500 μm. (G) Parasagittal section through the LGN of the *Propithecus* (Sifika) stained for Cresyl Violet. Anterior is to the left. (H) Adjacent section stained for AChE showing dense staining of the two parvocellular layers (PE, PI). Note the "representation" of the optic disc can be seen as a discontinuity in the parvocellular external layer (arrow). Scale bar for G and H = 500 μm.

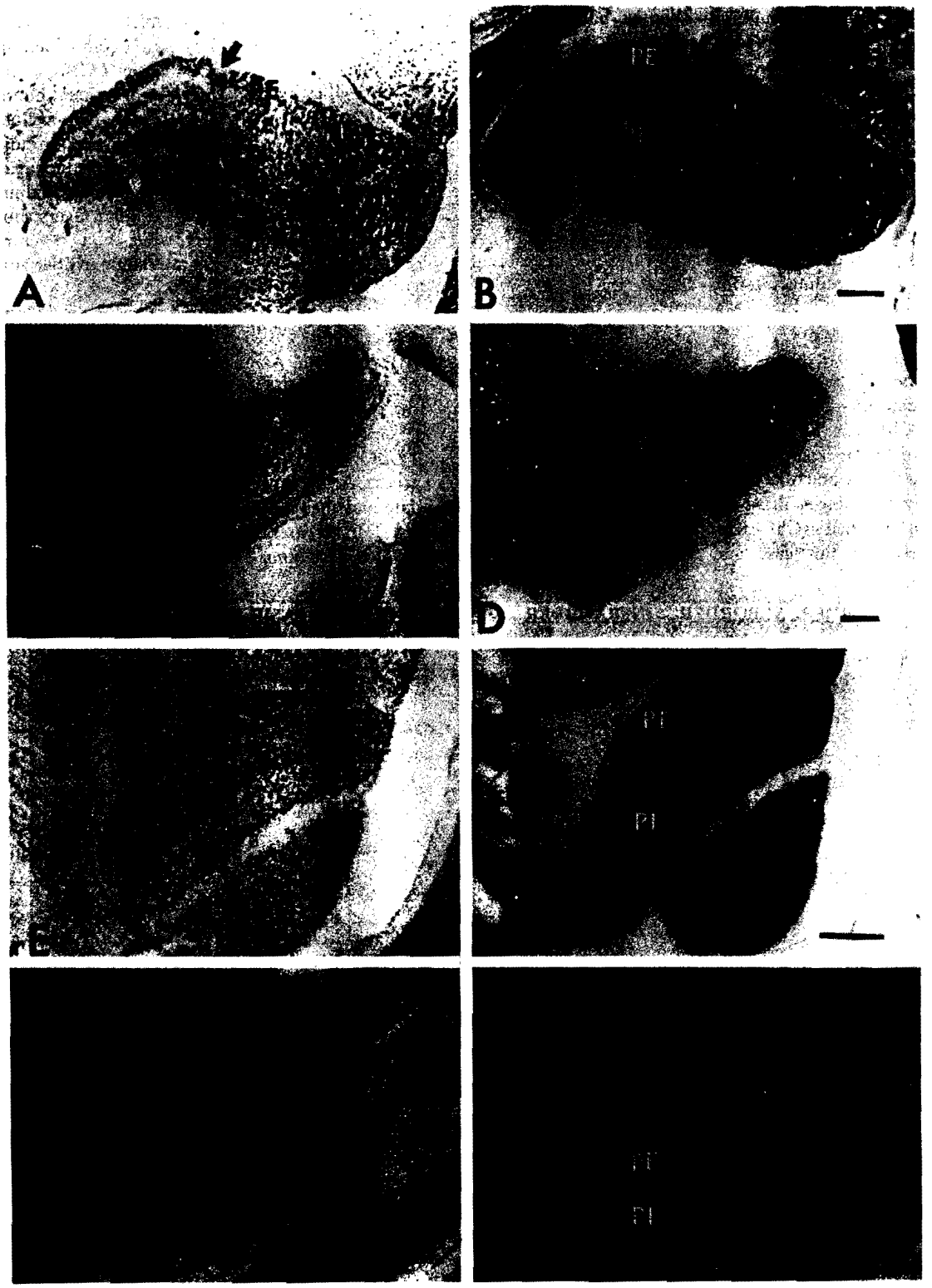

Fig. 1.

cell layers, numbered from ventral to dorsal. Layers 1 and 2 contain large neurons and are termed the magnocellular layers. Layers 3 and 6 (parvocellular layers) contain small, more densely packed neurons. There are two neuronal layers (koniocellular layers) interposed between the dorsal parvocellular layers and these contain smaller neurons that are more palely stained with Cresyl Violet.

A Cresyl Violet-stained parasagittal section of the LGN of a strepsirhine primate, *Lemur fulvus* (Brown lemur), can be seen in Fig. 1A. The central visual field is posterior. The magnocellular, koniocellular, and parvocellular external layers receive input from the contralateral retina. The portions of the external layers extending anteriorly beyond the internal layers correspond to the monocular representation of the peripheral visual field. The "representation" of the optic disc can be seen as a discontinuity in the external parvocellular layer.[19] Figure 1B presents an adjacent section through the lemur LGN stained for AChE reaction product. The two parvocellular layers are AChE-rich, while the two interposed koniocellular layers and the two ventral magnocellular layers are relatively AChE-poor. It is important to note that all expected AChE-rich structures (e.g. caudate nucleus) in this case and all others showed dense reaction product demonstrating that our freezing, thawing, and fixation protocols did not have an adverse effect on AChE histochemistry.

Figure 1C shows a Cresyl Violet-stained section through the LGN of the *Tarsius syrichta* (Phillipine tarsier). As discussed above, the tarsier LGN contains only four cell layers. There exists a prominent bundle of interlaminar fibers between the parvocellular internal and magnocellular internal layers. An adjacent section stained for AChE reaction product again demonstrates the characteristic prosimian staining pattern (Fig. 1D) The two parvocellular layers show dense AChE reaction product, while the two ventral magnocellular layers and the interlaminar fiber zone are relatively lightly stained.

The notion of a characteristic prosimian staining pattern is reinforced by adjacent sections stained for Cresyl Violet and AChE reaction product from two other strepsirhine species in our collection [*Microcebus* (Mouse lemur), Fig. 1E, F; *Propithecus* (Sifika), Fig. 1G, H]. We have now catalogued AChE staining in the LGN of 12 species of prosimians, representing 10 genera. A listing of the prosimian genera examined to date and their phylogenetic relationships is given in Fig. 2. In all cases, the parvocellular laminae show a heightened staining of AChE reaction product.

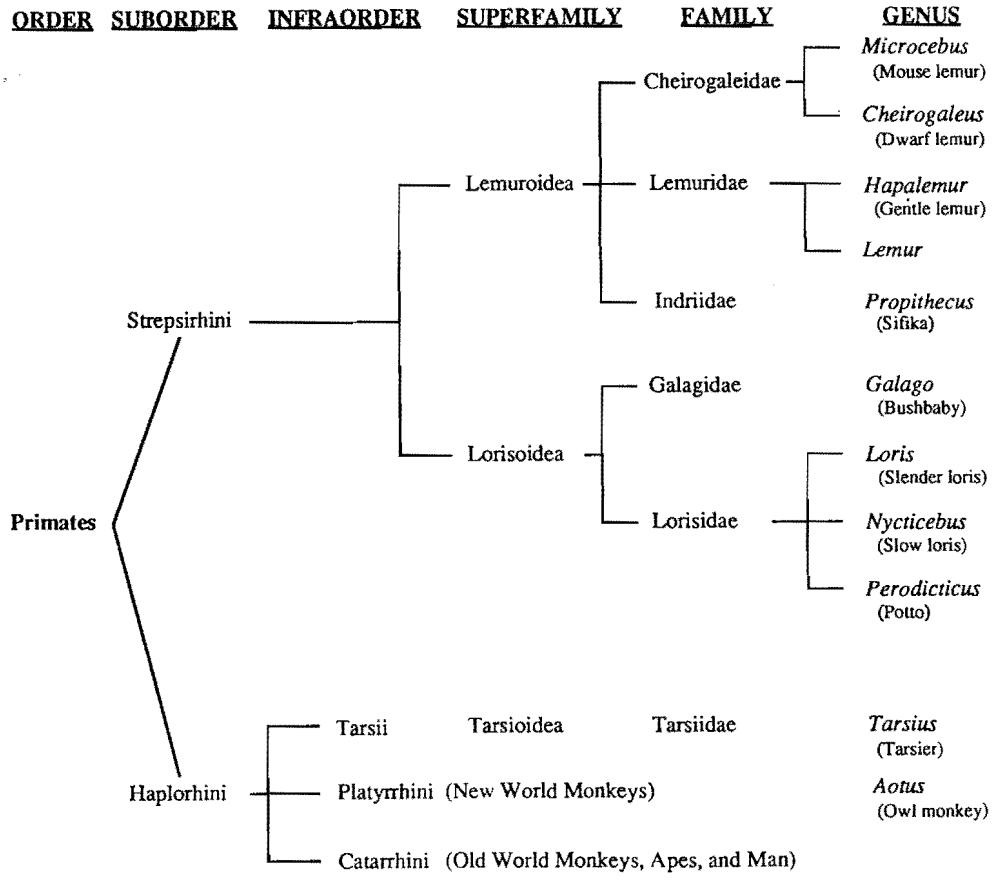

Fig. 2. Chart of the prosimians examined to date and their phylogenetic relationships. All 12 species (representing 10 genera) show dense AChE in the parvocellular layers of the LGN. Two species of haplorhine prosimians, *Tarsius bancanus* and *Tarsius syrichta* are included in our study.

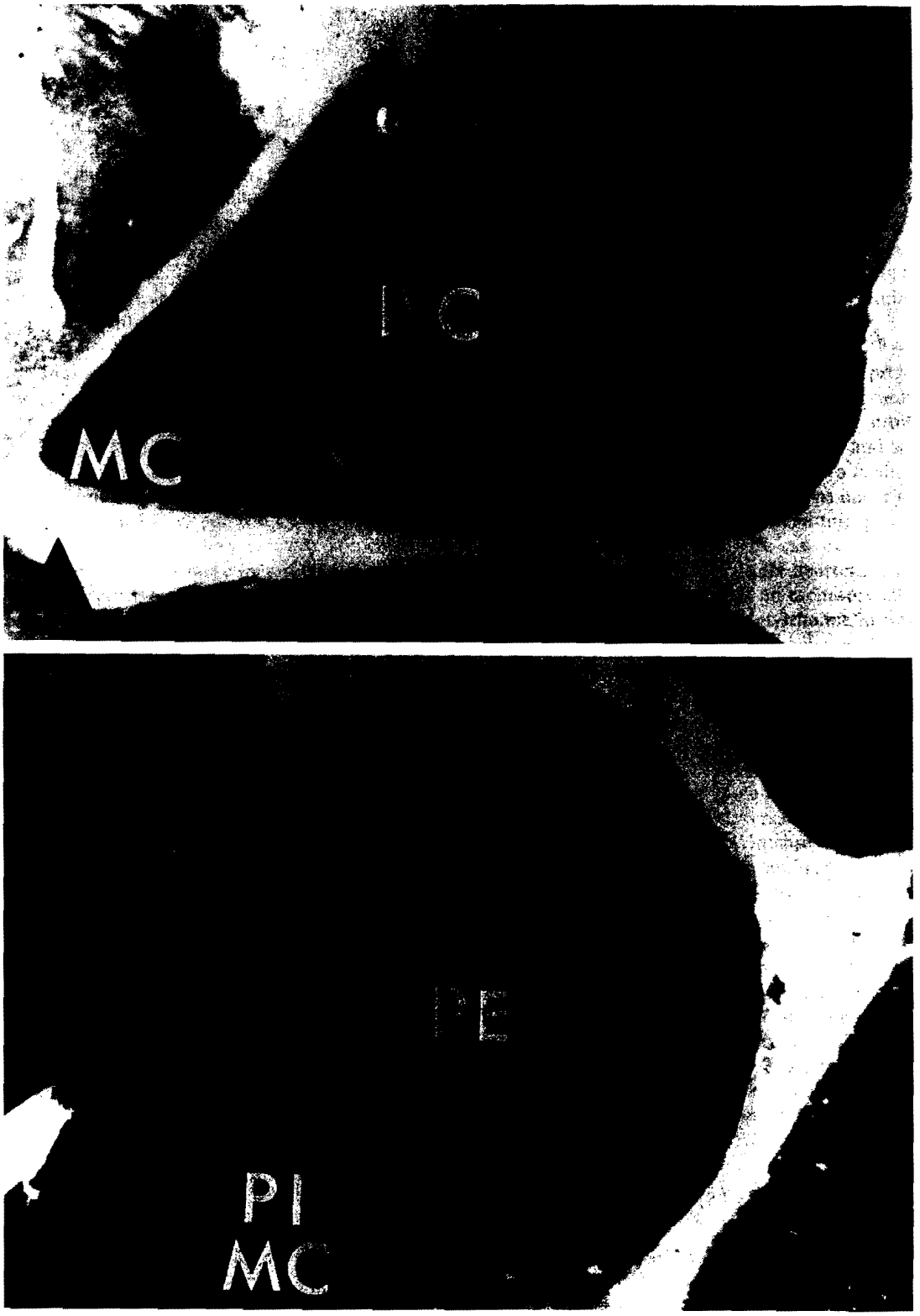

Fig. 3. (A) Coronal section through the LGN of the *Tarsius syrichta* stained for CO activity. Medial is to the left. Heavy CO activity is seen in both the ventral magnocellular (MC) and the dorsal parvocellular laminae (PC), but not the interlaminar fiber bundle (IL). (B) Parasagittal section through the LGN of the *Nycticebus coucang* (Slow loris) stained for CO activity. Anterior is to the left. Heavy CO activity is seen in the parvocellular (PE, PI) and magnocellular (ME, MI) laminae, but not the two koniocellular layers (KE, KI) nor the interlaminar regions. Scale bar = 500 μm.

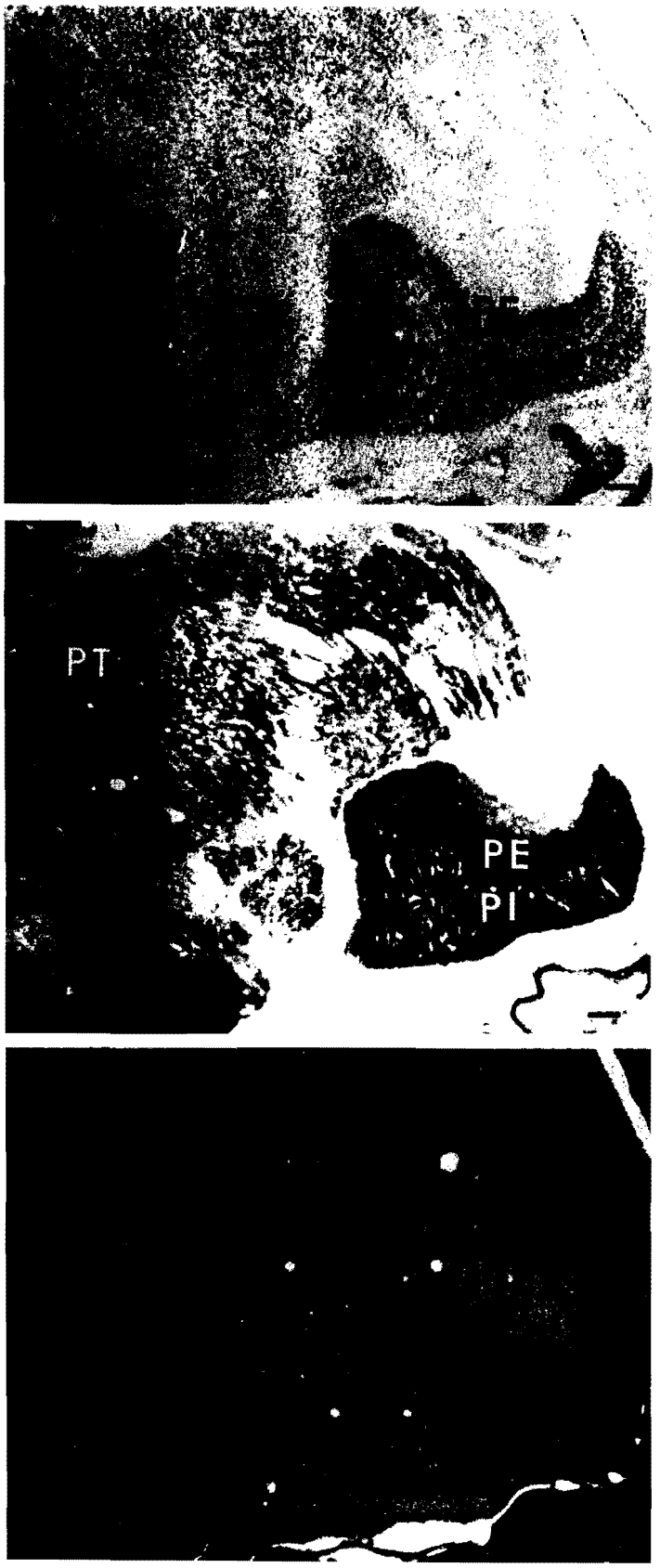

Fig. 4.

We have also examined the distribution of CO activity in the LGN of prosimian primates. In Fig. 3A, the LGN of the haplorhine *Tarsius syrichta* shows relatively uniform and dense CO activity in both the parvocellular and magnocellular laminae. The interlaminar fiber zone is free of dense CO activity. In all strepsirhine primates, such as the *Nycticebus coucang* (Slow loris), heavy CO activity is seen in the parvocellular and magnocellular layers, but little CO activity is seen in the two koniocellular layers (Fig. 3B).

We employed a polyclonal antibody to ChAT to examine the laminar organization of cholinergic activity in the LGN of the *Galago senegalensis* (Lesser bushbaby). Figure 4A presents a coronal section through the bushbaby LGN stained for Cresyl Violet. An adjacent section stained for AChE again demonstrates the expected increased AChE reaction product in the parvocellular layers of the LGN as well as in the pretectal nucleus (Fig. 4B). An adjacent section stained for ChAT immunoreactivity is presented in Fig. 4C. Both the LGN and pretectal nucleus are ChAT-immunoreactive. Immunoreactive profiles were seen in the LGN, but not ChAT-positive cell bodies. Although the intercalated koniocellular laminae are somewhat more lightly ChAT-immunoreactive, no great difference in staining intensity is seen between the parvocellular and magnocellular laminae.

In addition, we have employed ChAT immunostaining to examine the phenotype of known inputs to the bushbaby lateral geniculate nucleus. The pretectal nucleus contains many ChAT-immunoreactive profiles but the neurons themselves are not ChAT-positive (Fig. 5A). Likewise, the superior colliculus contains many ChAT-immunoreactive profiles but no ChAT-positive neurons (data not shown). However, neurons in the parabigeminal nucleus (Fig. 5B) and the pedunculopontine tegmental nucleus (Fig. 5C) did contain many AChE- and ChAT-positive neurons. Finally, layer VI of striate cortex contains AChE-positive neurons but no ChAT-positive neurons (data not shown).

DISCUSSION

In all prosimian primate brains examined thus far, the parvocellular laminae of the LGN stain more densely with AChE reaction product than do the magnocellular laminae. As noted, Hess and Rockland[17] were the first to suggest that AChE staining of the LGN might correlate with a primate's daily activity pattern and that heightened staining of the parvocellular laminae might reflect an adaptation to a nocturnal visual habitat.

Most prosimians are known to be nocturnal and so the correlation between daily activity pattern and AChE staining of the LGN might seem to hold. However, there are exceptions. The *Propithecus* (Sifika) from Madagascar exhibits a diurnal activity pattern,[24] yet there is a dense AChE reaction product in the parvocellular laminae of the LGN (see Fig. 1G, H). *Lemur fulvus* (Brown lemur) exhibits a diel activity pattern, being most active under the low light conditions of dusk and dawn,[4] and once again, AChE staining is densest within the parvocellular laminae (see Fig. 1A, B).

The increased AChE reaction product initially suggested that there may be a denser cholinergic projection to the parvocellular laminae of the prosimian LGN. There is less spatial convergence by retinal ganglion cells on to parvocellular neurons, and so a denser cholinergic projection might serve to facilitate transmission via the parvocellular neurons under low light conditions. Indeed, there is mounting physiological evidence that acetylcholine can increase the responsiveness of mammalian LGN neurons. Sillito *et al.*[33] and others[5,31] have shown an excitatory effect through *in vivo* application of acetylcholine into the cat LGN. McCormick and Prince[25] have used an *in vitro* slice preparation of the cat LGN to demonstrate that activation of nicotinic acetylcholine receptors induces a fast depolarization in nearly all cells examined. However, our study fails to demonstrate an increased cholinergic input to the parvocellular layers of the prosimian LGN. Although AChE reactivity is increased in the parvocellular layers, ChAT-immunoreactivity appears of near equal intensity in both the parvocellular and magnocellular laminae.

In addition to an increased cholinergic input, we have failed to demonstrate a specific cholinergic input to the parvocellular layers of the prosimian LGN. The pretectal nucleus projects primarily to the AChE-rich layers of the prosimian LGN.[16] However, although this nucleus contains many ChAT-immunoreactive profiles, the neurons themselves are not ChAT-positive (see Fig. 5A). While neurons in the parabigeminal nucleus (see Fig. 5B) do exhibit a cholinergic phenotype, the nucleus has been shown to project mainly to the koniocellular layers.[16] The superior colliculus does not contain cholinergic cells and furthermore has also been shown to project mainly to the koniocellular layers.[16] Finally, future experiments will be

Fig. 4. (A) Coronal section through the LGN and pretectal nucleus (PT) of the *Galago senegalensis* (Lesser bushbaby) stained for Cresyl Violet. The medial geniculate (MG) is to the left of the LGN. Dorsal is to the top. (B) Adjacent section stained for AChE. Increased AChE reaction product is seen in the pretectal nucleus as well as the parvocellular layers (PE, PI) but not the koniocellular nor magnocellular layers (MC) of the LGN. (C) Adjacent section stained for ChAT immunoreactivity. The LGN and the pretectal nucleus are both ChAT-immunoreactive. Although the two koniocellular layers (KC) show comparatively less ChAT-immunoreactivity, there does not appear to be a great difference in staining intensity between the parvocellular (PE, PI) and magnocellular (MC) laminae. Scale bar = 500 μm.

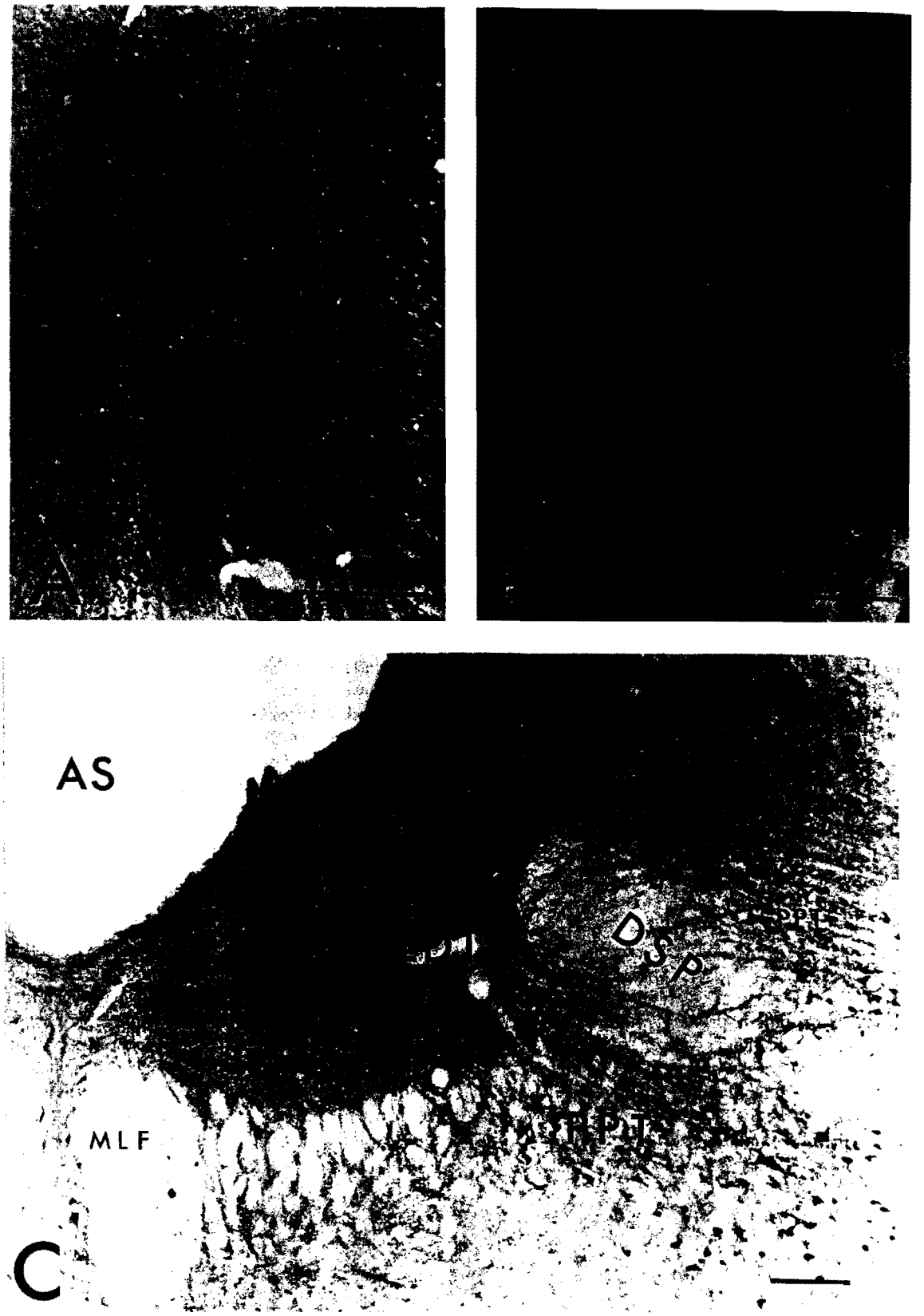

Fig. 5.

needed to determine if the cholinergic neurons of the pedunculopontine tegmental nucleus (see Fig. 5C) provide a specific input to the parvocellular laminae of the prosimian LGN.

As a side note, there has been some disagreement regarding the cholinergic phenotype of neurons in the parabigeminal nucleus. ChAT-immunoreactive cells have been found in the parabigeminal nucleus of the mouse,[29] the tree shrew, and the cat.[13] However, cholinergic neurons were not found in the the parabigeminal nucleus of the rat,[3] nor were they seen in a separate study of the cat.[7] We confirm the observation of Fitzpatrick *et al.*[13] that the parabigeminal neurons, while clearly cholinergic, do not stain as intensely as the ChAT-immunoreactive cells in the pedunculopontine tegmental nucleus.

It is known that layer VI of striate cortex projects back to the LGN in all primates,[23] including the *Galago*.[32] In an elegant series of experiments, Fitzpatrick and Diamond[12] demonstrated that kainic acid lesions of the striate cortex of the *Galago senegalensis* eliminated the dense AChE staining in the visuotopically corresponding parts of the parvocellular layers of the LGN. However, this dense parvocellular staining was not reduced by eliminating the retinal input nor by injections of kainic acid directly into the LGN. These results strongly suggest that layer VI of striate cortex is the source of the dense AChE reaction product found in the parvocellular laminae of the *Galago* LGN.

Conversely, Hess and Rockland[17] found AChE staining to be denser in the magnocellular layers of the LGN in the diurnal squirrel monkey (*Saimiri sciureus*). However, lesions within the striate cortex of the squirrel monkey and the macaque, also a diurnal primate, did not result in a decrease in AChE activity in the magnocellular laminae of the LGN. Taken together, these results indicate that in prosimian primates there is an AChE-rich projection feeding back from the primary visual cortex to the parvocellular laminae of the LGN that is not present in diurnal primates.

In the *Galago senegalensis*, AChE-positive cells are found within layer VI of primary visual cortex.[12] Although ChAT immunohistochemistry reveals all expected cholinergic structures, including ChAT-positive profiles within the *Galago* primary visual cortex, ChAT-immunoreactive cells are not seen within layer VI (data not shown). A similar observation has been made in the cortex of the macaque monkey.[28] Thus, while layer VI of the *Galago* primary visual cortex appears to be a specific source of AChE to the parvocellular layers of the LGN, it does not appear to be a source of cholinergic input to the nucleus.

In the prosimian LGN, both the magnocellular layers (1 and 2) and the parvocellular layers (3 and 6) stain more intensely for CO than do the koniocellular layers (4 and 5) (see Fig. 4A, B). Norton and Cassagrande[30] have mapped the receptive field properties in the LGN of the *Galago crassicaudatus*. The magnocellular layers contain cells that exhibit Y-like properties (short latency, transient responses to standing contrast and rapid movement). The parvocellular layers contain X-like cells (medium latency, sustained responses to standing contrast). Also, the koniocellular layers contain many W-like cells (long latency, sluggish responses to visual stimuli). Kageyama and Wong-Riley[21] have previously noted in a study of the LGN of the ferret, cat and diurnal monkeys (macaque and squirrel monkey) that large (presumed Y-like) and medium (presumed X-like) cells were more darkly stained for CO than small (presumed W-like) cells. Lachica *et al.*[22] have also shown in the LGN of the tree shrew (*Tupaia belangeri*) that W-like cells are more lightly stained for CO than are other relay cells in the nucleus. Thus, CO staining of the LGN in prosimian primates is in agreement with findings in other species.

CONCLUSIONS

The relation of increased AChE activity to cholinergic function in the parvocellular layers of the prosimian LGN remains unclear. We fail to demonstrate either a specific or an increased cholinergic input to the parvocellular layers. Instead, increased AChE may be serving a non-cholinergic function in the prosimian LGN. Greenfield[15] has recently outlined several of these possible functions for AChE including hydrolysing an as yet unidentified substrate found in increased quantities in the parvocellular layers, direct modulation of acetylcholine receptors, modulation of non-cholinergic receptors, or direct modulation of the cell membrane. Yet another possibility is that the increased AChE serves an anti-cholinergic function in the prosimian LGN and decreases the effect of any cholinergic input to the parvocellular layers. McCormick and Prince[25] have shown that the physiological responses of LGN

Fig. 5. Coronal sections through the brain of the *Galago* stained for ChAT immunoreactivity. Medial is to the left. (A) The pretectal nucleus seen at a higher power than Fig. 4C. The pretectal nucleus (PT) contains ChAT-positive profiles, but not ChAT-positive cell bodies. Scale bar = 200 μm. (B) The parabigeminal nucleus contains neuronal cell bodies with a cholinergic phenotype (black arrows). Note also the ChAT-positive terminal profiles which appear to ascend toward a weakly stained neuronal cell body (white arrow). Scale bar = 100 μm. (C) The pedunculopontine tegmental nucleus (PPT) and the lateral dorsal tegmental nucleus (LDT) contain neurons which are strongly ChAT-immunoreactive. AS, aqueduct of Sylvius; DSP, decussation of the superior cerebellar peduncle; MLF, medial longitudinal fasciculus. Scale bar = 200 μm.

neurons are complex in the face of cholinergic activation. Some cells demonstrate delayed hyperpolarization and it may be this effect that increased AChE serves to decrease.

Although its exact function remains to be elucidated, AChE reaction product is concentrated in the parvocellular laminae of all strepsirhine prosimians. It is also concentrated in the parvocellular laminae of the only two nocturnal haplorhine primates, *Tarsius* and *Aotus* (owl monkey).[11,12] Our results indicate that the correlation between daily activity pattern and AChE distribution in the primate LGN is not inviolate. In the diurnal prosimian species, *Propithecus*, an increased AChE reaction product is still seen in the parvocellular layers of the LGN. Thus, rather than reflecting diurnal/nocturnal lifestyles, we hypothesize that dense staining of the parvocellular laminae may be a primitive feature of lateral geniculate organization. Both haplorhines that have this feature, *Tarsius* and *Aotus*, are related to ancient fossils. Tarsiformes are amongst the oldest fossil primates, dating from the Eocene.[34] Finally, Szalay and Delson[34] have noted the several similarities, including enlarged orbits, between the skulls of the owl monkey and *Tremacebus*, a late Oligocene primate, suggesting a primitive status for *Aotus* as well.

Acknowledgements—We are indebted to Drs Elwyn Simons and Patricia Wright of the Duke University Primate Center for providing the rare prosimian brains used in this study. We also gratefully acknowledge the assistance of Dr David Amaral of the Salk Institute in providing the services of his laboratory during the immunohistochemical portion of this study. Finally, we wish to thank Josephine Macenka of Caltech for expert technical assistance during many phases of this project. This work was supported by the Hixon Professorship of Psychobiology at Caltech, a Lucille B. Markey Charitable Trust Foundation grant, and a NRSA T32 GM07737 to C.T.M.

REFERENCES

1. Allman J. and McGuinness E. M. (1988) Visual cortex in primates. *Comp. Primate Biol.* **4,** 279–326.
2. Amaral D. G. and Kurz J. (1985) An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. *J. comp. Neurol.* **240,** 37–59.
3. Beninato M. and Spencer R. F. (1986) A cholinergic projection to the rat superior colliculus demonstrated by retrograde transport of horseradish peroxidase and choline acetyltransferase immunohistochemistry. *J. comp. Neurol.* **253,** 525–538.
4. Conley J. M. (1975) Notes on the activity pattern of *Lemur fulvus*. *J. Mammal* **56,** 712–715.
5. Curtis D. R. and Davis R. (1963) Excitation of lateral geniculate neurones by quaternary ammonium derivatives. *J. Physiol.* **165,** 62–82.
6. Dean A. F., Bunch S. T., Tolhurst D. J. and Lewis P. R. (1982) The distribution of acetylcholinesterase in the lateral geniculate nucleus of the cat and monkey. *Brain Res.* **244,** 123–134.
7. deLima A. and Singer W. (1987) The brainstem projection to the lateral geniculate nucleus in the cat: identification of cholinergic and monoaminergic elements. *J. comp. Neurol.* **259,** 92–121.
8. DeYoe E. A. and Van Essen D. C. (1988) Concurrent processing streams in monkey visual cortex. *Trends Neurosci.* **11,** 219–226.
9. Eckenstein F., Barde Y. A. and Thoenen H. (1981) Production of specific antibodies to choline acetyl transferase purified from pig brain. *Neuroscience* **6,** 993–1000.
10. Eckenstein F. and Thoenen H. (1982) Production of specific antisera and monoclonal antibodies to choline acetyl transferase: characterization and use for identification of cholinergic neurons. *Eur. molec. Biol. Org. J.* **1,** 363–369.
11. Fitzpatrick D. and Diamond I. T. (1979) The laminar organization of the lateral geniculate body in *Galago senegalensis*: a pair of layers identified by acetylcholinesterase activity. *Brain Res.* **170,** 538–542.
12. Fitzpatrick D. and Diamond I. T. (1980) Distribution of acetylcholinesterase in the geniculostriate system of *Galago senegalensis* and *Aotus trivirgatus*: evidence for the origin of the reaction product in the lateral geniculate body. *J. comp. Neurol.* **194,** 703–719.
13. Fitzpatrick D., Conley M., Luppino G., Matelli M. and Diamond I. T. (1988) Cholinergic projections from the midbrain reticular formation and the parabigeminal nucleus to the lateral geniculate nucleus in the tree shrew. *J. comp. Neurol.* **272,** 43–67.
14. Gallyas F. (1979) Silver staining of myelin by means of physical development. *Neurol. Res.* **1,** 203–209.
15. Greenfield S. A. (1991) A non-cholinergic action of acetylcholinesterase (AChE) in the brain: from neuronal secretion to the generation of movement. *Cell. molec. Neurobiol.* **11,** 55–77.
16. Harting J. K., Hashikawa T. and Van Lieshout D. (1986) Laminar distribution of tectal, parabigeminal and pretectal inputs to the primate dorsal lateral geniculate nucleus: connectional studies in *Galago crassicaudatus*. *Brain Res.* **366,** 358–363.
17. Hess D. T. and Rockland K. S. (1983) The distribution of cholinesterase and cytochrome oxidase within the dorsal lateral geniculate nucleus of the squirrel monkey. *Brain Res.* **289,** 322–325.
18. Kaas J. H., Guillery R. W. and Allman J. M. (1972) Some principles of the organization in the dorsal lateral geniculate nucleus. *Brain Behav. Evol.* **6,** 253–299.
19. Kaas J. H., Guillery R. W. and Allman J. M. (1972) Discontinuities in the dorsal level geniculate nucleus corresponding to the optic disc: a comparative study. *J. comp. Neurol.* **147,** 163–180.
20. Kaas J., Heurta M. F., Weber J. T. and Harting J. K. (1978) Patterns of retinal terminations and laminar organization of the lateral geniculate nucleus of primates. *J. comp. Neurol.* **182,** 517–554.
21. Kageyama G. H. and Wong-Riley M. T. T. (1984) The histochemical localization of cytochrome oxidase in the retina and lateral geniculate nucleus of the ferret, cat, and monkey, with particular reference to retinal mosaics and ON/OFF-center channels. *J. Neurosci.* **4,** 2445–2459.

22. Lachica E. A., Condo G. J. and Casagrande V. A. (1987) Development of cytochrome oxidase staining in the retina and lateral geniculate nucleus: a possible correlate of ON- and OFF-center channel maturation. *Devl Brain Res.* **34,** 298–302.
23. Lund J. S., Lund R. D., Hendrickson A. E., Bunt A. H. and Fuchs A. F. (1975) The origin of efferent pathways from the primary visual cortex, area 17, of the macaque monkey as shown by retrograde transport of horseradish peroxidase. *J. comp. Neurol.* **164,** 287–304.
24. Martin R. D. (1990) *Primate Origins and Evolution*, pp. 11 and 664. Chapman and Hall, London.
25. 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.* **392,** 147–165.
26. McDonald C., McGuinness E. and Allman J. (1985) Laminar distribution of acetylcholinesterase and cytochrome oxidase in the dorsal lateral geniculate nucleus of *Lemur fulvus*. *Invest. Ophthal.* **26,** 163.
27. McGuinness E. and Allman J. (1985) Organization of the visual system in tarsiers. *Am. J. Phys. Anthropol.* **66,** 200.
28. Mesulam M.-M., Mufson E. J., Levey A. I. and Wainer B. H. (1984) Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. *Neuroscience* **12,** 669–686.
29. Mufson E. J., Martin T. L., Mash D. C., Wainer B. H. and Mesulam M.-M. (1986) Cholinergic projections from the parabigeminal nucleus (Ch8) to the superior colliculus in the mouse: a combined analysis of horseradish peroxidase transport and choline acetyltransferase immunohistochemistry. *Brain Res.* **370,** 144–148.
30. Norton T. T. and Casagrande V. A. (1982) Laminar organization of receptive-field properties in lateral geniculate nucleus of bush baby (*Galago crassicaudatus*). *J. Neurophysiol.* **47,** 715–741.
31. Phillis J. W., Tebecis A. K. and York D. H. (1967) A study of cholinoceptive cells in the lateral geniculate nucleus. *J. Physiol.* **192,** 695–713.
32. Raczkowski D. and Diamond I. T. (1978) Connections of the striate cortex in *Galago*. *Brain Res.* **144,** 383–388.
33. Sillito A. M., Kemp J. A. and Berardi N. (1983) The cholinergic influence on the function of the cat dorsal lateral geniculate nucleus (dLGN). *Brain Res.* **280,** 299–307.
34. Szalay F. S. and Delson E. (1979) *Evolutionary History of the Primates*, p. 299. Academic Press, New York.
35. Van Essen D. C. and Maunsell J. H. R. (1983) Hierarchical organization and functional streams in the visual cortex. *Trends Neurosci.* **6,** 370–375.
36. Wong-Riley M. T. T. (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Res.* **171,** 11–28.

(*Accepted* 9 *December* 1992)