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Human Reticular Formation: Cholinergic Neurons of the Pedunculopontine and Laterodorsal Tegmental Nuclei and Some Cytochemical Comparisons to Forebrain Cholinergic Neurons

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ABSTRACT

Choline acetyltransferase immunohistochemistry showed that the human rostral brainstem contained cholinergic neurons in the oculomotor, trochlear, and parabigeminal nuclei as well as within the reticular formation. The cholinergic neurons of the reticular formation were the most numerous and formed two intersecting constellations. One of these, designated Ch5, reached its peak density within the compact pedunculopontine nucleus but also extended into the regions through which the superior cerebellar peduncle and central tegmental tract course. The second constellation, designated Ch6, was centered around the laterodorsal tegmental nucleus and spread into the central gray and medial longitudinal fasciculus. There was considerable transmitter-related heterogeneity within the regions containing Ch5 and Ch6. In particular, Ch6 neurons were intermingled with catecholaminergic neurons belonging to the locus coeruleus complex. The lack of confinement within specifiable cytoarchitectonic boundaries and the transmitter heterogeneity justified the transmitter-specific Ch5 and Ch6 nomenclature for these two groups of cholinergic neurons.

The cholinergic neurons in the nucleus basalis (Ch4) and those of the Ch5-Ch6 complex were both characterized by perikaryal heteromorphism and isodendritic arborizations. In addition to choline acetyltransferase, the cell bodies in both complexes also had high levels of acetylcholinesterase activity and nonphosphorylated neurofilament protein. However, there were also marked differences in cytochemical signature. For example, the Ch5-Ch6 neurons had high levels of NADPHd activity, whereas Ch4 neurons did not. On the other hand, the Ch4 neurons had high levels of NGF receptor protein, whereas those of Ch5-Ch6 did not.

On the basis of animal experiments, it can be assumed that the Ch5 and Ch6 neurons provide the major cholinergic innervation of the human thalamus and that they participate in the neural circuitry of the reticular activating, limbic, and perhaps also extrapyramidal systems.

Key words: human brain, reticular formation, basal forebrain, colinergic, pedunculopontine nucleus, laterodorsal tegmental nucleus, parabigeminal nucleus

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The cerebral cortex and thalamic nuclei of the mammalian brain contain an extensive network of cholinergic fibers and receptors. The cholinergic innervation for the cerebral cortex originates within the basal forebrain while that of thalamic nuclei comes predominantly from two groups of brainstem neurons embedded within the pontomesencephalic reticular formation (See Mesulam '88 for review).

Considerable attention has been devoted to the neuroanatomical characteristics of the cholinergic brainstem neurons in the rat, cat, dog, monkey, and baboon (Kimura et al, '81; Armstrong et al., '83; Mesulam et al., '83; '84; Satoh and Fibiger, '85; '86; Sofroniew et al., '85; Isaacson and Tanaka, '86; Woolf and Butcher '86; Hallanger et al '87; Jones and Beaudet, '87). Information on this aspect of the human brain is relatively limited but consistent with the existence of an equally extensive neuronal system (Mizukawa et al., '86). One purpose of this report is to provide a detailed anatomical description of these neurons in the human brain. Another is to provide a cytochemical comparison with the cholinergic neurons in the forebrain.

METHODS

The observations in this report are based on six brains from individuals without prior neurological disease and spanning an age range from 22 to 91 years. One of the brains was embedded in celloidon, cut at 35 micron-thick sections, and stained with cresyl violet and myelin stains. Blocks from the other five brains (autolysis interval from 5 to 18 hours) were fixed by immersion and cut in the coronal plane as described previously (Mesulam and Geula, '88).

Matching series of sections through the rostral brainstem and the basal forebrain were processed according to four groups of methods.

1) Cresyl violet, thionin, and hematoxylin-eosin stains were used to reveal cytological detail.

2) Immunohistochemistry was done using well characterized antibodies to *choline acetyltransferase* (German et al., '85), *tyrosine hydroxylase*—purchased from Eugene Tech, Allendale, New Jersey—(Joh and Ross, '83), *nerve growth factor receptors* (Marano et al '87) and *nonphosphorylated neurofilament protein,* antibody SMI-32, purchased from Sternberger-Meyer Immunocytochemicals, Jarrettsville, MD (Sternberger, '86; Morrison et al., '87). The peroxidase-antiperoxidase method of Sternberger ('86) was used for all immunohistochemistry. Control sections were prepared by using equivalent concentrations of an irrelevant IgG of the same origin as the antibody. Only staining that did not appear in control material was accepted as evidence for positive immunostaining. A number of sections processed for histochemistry were also counterstained with cresyl violet or thionin.

3) Acetylcholinesterase (AChE) histochemistry was done according to a modification of the copper-thiocholine Koelle-Friedenwald method (Method A in Mesulam and Geula, '88). Ethopropazine was used to inhibit nonacetylcholinesterases, and control sections were obtained using butyrylthiocholine as the substrate instead of acetylthiocholine. Sections were also prepared for the concurrent demonstration of AChE with choline acetyltransferase (ChAT) and SMI-32 immunohistochemistry. The method for the concurrent demonstration of AChE and immunostaining has been described previously (Levey et al., '83).

4) Additional sections were prepared for the demonstration of nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) activity, based on the reduction (in the presence of NADPH) of a tetrazolium dye into a dark blue formazan precipitate (Thomas and Pearse, '61; Ellison et al., '87). Control sections were prepared by processing sections in an identical incubation medium but without the NADPH substrate. In some sections, NADPHd activity was demonstrated concurrently with ChAT, tyrosine hydroxylase (TH), and SMI-32 immunohistochemistry. This was achieved by successively processing the tissue, first for immunohistochemistry and then for NADPHd (Vincent et al., '86).

The ChAT-positive neurons were charted with a plotter electronically coupled to the stage of a compound microscope. These neurons were also analyzed with an image processor (Imaging Research, Inc.) for the determination of perikaryal area and maximum diameter. The methodology and considerations pertinent to the morphometric analysis of cholinergic neurons has been discussed elsewhere (Mesulam et al., '87). The analysis was done with a 20× objective. The image was digitized, the perikaryal outline was manually drawn with a cursor, and the area and diameters were computed by the image processor. Only perikarya with clearly definable boundaries and recognizable neuronal shapes were included. Overlapping neurons and fragments were excluded. In each pertinent area, 100 representative neurons were selected for morphometric analysis.

Abbreviations

a	cerebral aqueduct
ac	anterior commissure
cg	central gray
Ch4	fourth cholinergic cell group
Ch5	fifth cholinergic cell group
Ch5c	compact sector of Ch5
Ch5d	diffuse-interstitial sector of Ch5
Ch6	sixth cholinergic cell group
Ch8	eighth cholinergic cell group
ci	capsula interna
cp	cerebral peduncle
ctt	central tegmental tract
dr	dorsal raphe nucleus
dsc	decussation of the superior cerebellar peduncle
dt	dorsal tegmental nucleus
ew	Edinger-Westphal nucleus
gp	globus pallidus
gpv	globus pallidus, ventral part
ic	inferior colliculus
ldt	laterodorsal tegmental nucleus
LL	lateral lemniscus
ml	medial lemniscus
mlf	medial longitudinal fasciculus
nb	nucleus basalis
nl	nucleus locus coeruleus
nc	nucleus cuneiformis
on	oculomotor nucleus
pb	parabigeminal nucleus
pg	pontine gray
pn	Perlia's nucleus
pp_c	compact sector of pedunculopontine nucleus
rn	red nucleus
scp	superior cerebellar peduncle
tn	trochlear nucleus
v IV	fourth ventricle

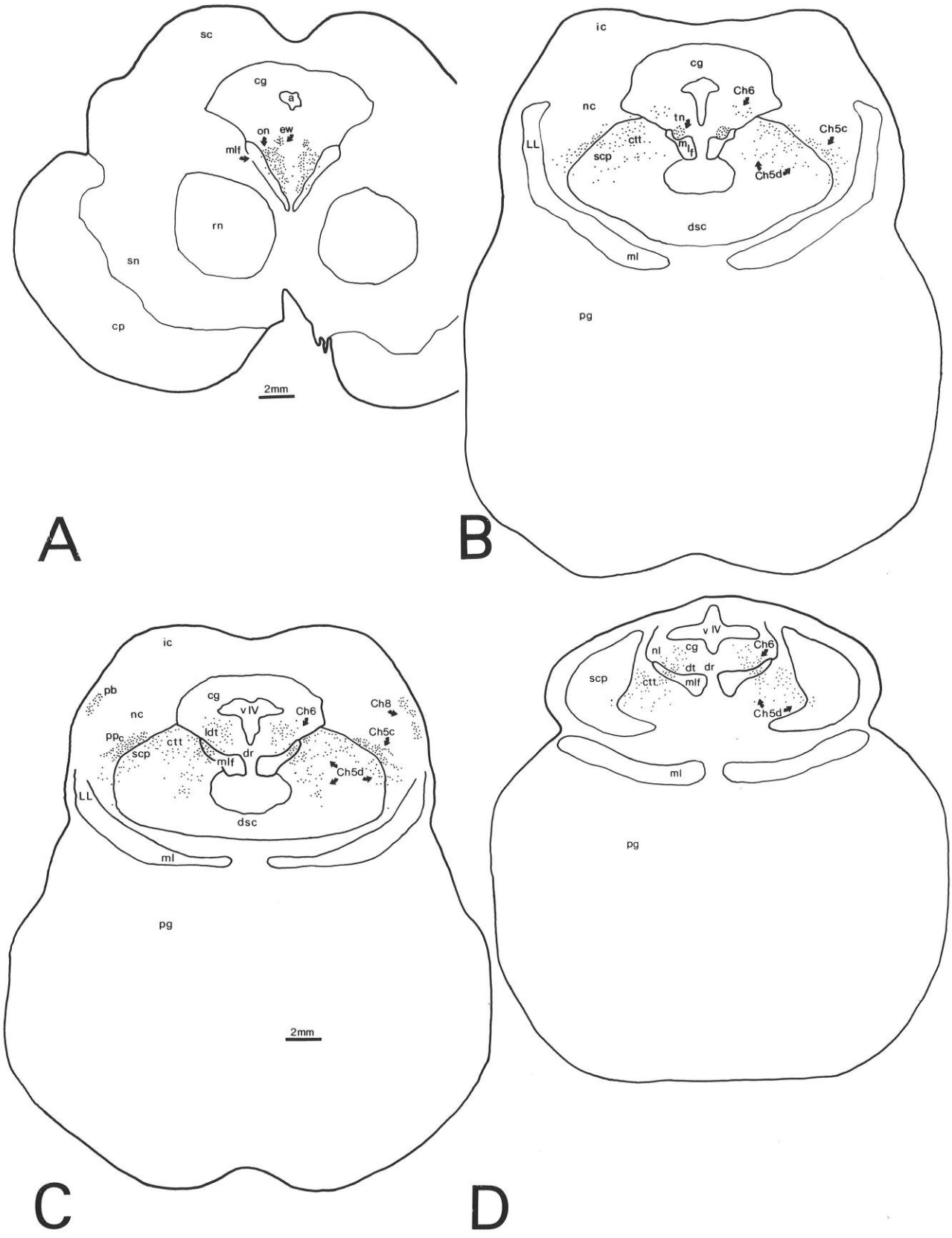

Fig. 1. Plotter chartings of ChAT-positive neurons in the human brainstem.

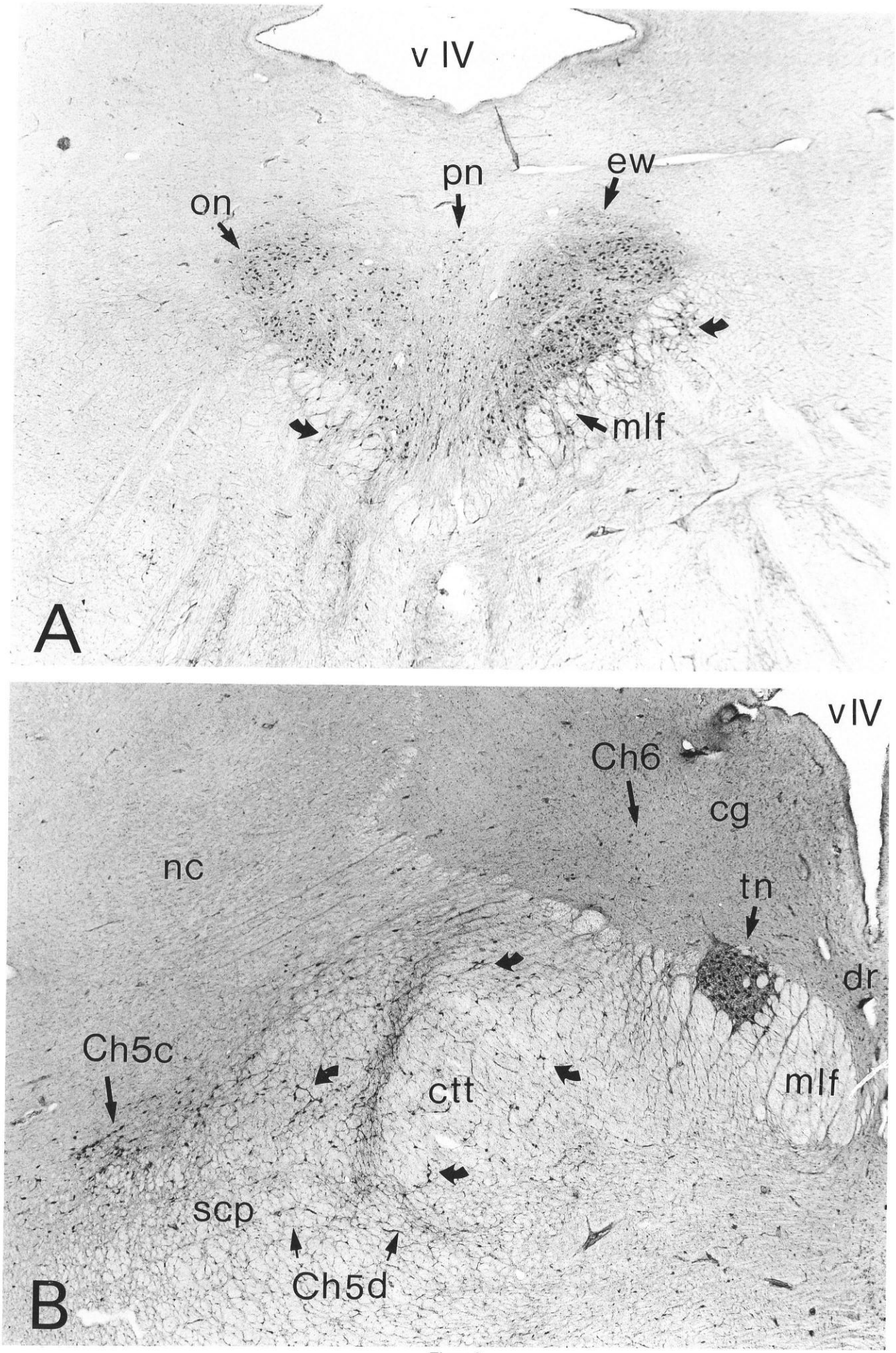

Figure 2

RESULTS

Choline acetyltransferase immunohistochemistry

In the rostral brainstem, immunohistochemistry with the ChAT antibody demonstrated positive staining in the third and fourth nerve nuclei, the parabigeminal nucleus, the central gray, and the pontomesencephalic tegmentum (Figs. 1–6). We also examined forebrain sections that contained ChAT-positive neurons in the nucleus basalis (the Ch4 cell group) and in the striatum. These forebrain neurons have been described elsewhere (Mesulam and Geula, '88) and will be mentioned in this report only for the purpose of cytochemical comparisons to the cholinergic neurons of the rostral brainstem.

Third and fourth cranial nerve nuclei. Sections counterstained for Nissl substance showed that all of the large neurons in the oculomotor and trochlear nuclei were intensely ChAT positive (Figs. 1A, 1B, 2A, 2B). The principal oculomotor nucleus was subdivided by relatively cell-poor zones into several neuronal clusters (Fig. 2A). In addition to these principal motoneuron pools, it was also possible to identify a collection of ChAT-positive midline cells that corresponded to Perlia's nucleus and a dorsomedially situated collection of slightly smaller and less intensely stained neurons that corresponded to the Edinger-Westphal nucleus (Figs. 1A, 2A). Perlia's nucleus is thought to coordinate ocular convergence, whereas the Edinger-Westphal nucleus contains the preganglionic parasympathetic neurons innervating the pupil. Clusters of intensely stained neurons extended from the principal oculomotor nucleus into the medial longitudinal fasciculus, within which they were embedded (curved arrow in Fig. 2A). Tracer experiments in baboons suggest that these neurons are part of the principal oculomotor complex and that they innervate the ipsilateral medial rectus muscle (Augustine et al., '81).

In the oculomotor and, especially, trochlear nuclei, dendrites and cell bodies remained confined to cytoarchitectonically definable nuclear boundaries (Fig. 2A, 2B). There was relatively little variation in cell size, shape, and dendritic branching patterns. The axons formed bundles that exited the nuclei in the form of intensely ChAT-positive rootlets.

Parabigeminal nucleus. On Nissl stains, the parabigeminal nucleus can be identified as a lenticular collection of relatively small and hypochromic neurons situated on the lateral surface of the pontomesencephalic region, flanked medially by the lateral lemniscus and dorsally by the inferior colliculus. Immunohistochemical preparations counterstained with cresyl violet showed that 80–90% of these neurons give a ChAT-positive reaction of moderate intensity. In a few sections, clusters of these neurons seemed to extend into the boundaries of the inferior colliculus. The vast majority of the cholinergic neurons in this region, however, remain confined within the cytoarchitectonic limits of the parabigeminal nucleus (Figs. 1C, 3A, 3B). The cholinergic neurons of the parabigeminal nucleus are designated as the Ch8 cell group (Mufson et al., '86).

The cholinergic neurons of the tegmentum and central gray. In contrast to the oculomotor, trochlear, and parabigeminal neurons, this third type of cholinergic neuron, by far the most numerous, did not remain confined within any nuclear boundary and extended from the retrorubral area in the mid-mesencephalon to the parabrachial region in the mid-pons (Figs. 1B–D, 2B, 3A, 4A–B, 6). These neurons appear to form two major constellations, one focused around the compact portion of the pedunculopontine nucleus (Olszewski and Baxter, '56), and the second around the laterodorsal tegmental nucleus (Crosby and Woodburne, '43). Within the vast territory covered by these two constellations of neurons, sections prepared for the concurrent demonstration of ChAT and Nissl substance indicated that the ratio of cholinergic to noncholinergic cells varied greatly. Based on a similar analysis in rodents and monkeys (Mesulam et al., '83; '84), we chose to designate the constellation of cholinergic neurons clustered around the pedunculopontine nucleus as Ch5 and that focused around the laterodorsal tegmental nucleus as Ch6.

The Ch5 complex. The most rostral neurons that can be attributed to the Ch5 complex make their appearance just caudal to the red nucleus and dorsal to the substantia nigra in the lateroventral mesencephalic tegmentum. Appreciable clusters of these neurons appear more caudally, at the level of the trochlear nucleus. At this level, the densest concentrations of Ch5 neurons were located within the pedunculopontine nucleus (pars compacta) of Olszewski and Baxter ('56), one of the few "nuclei" in the reticular formation with relatively identifiable boundaries (Figs. 1B–C, 2B, 3A, 4A, 7A). Sections counterstained with cresyl violet indicated that 80–90% of the large cells within the compact pedunculopontine nucleus are intensely ChAT-positive. These neurons are much larger and more intensely ChAT-positive than the adjacent neurons of the parabigeminal nucleus (Figs. 3, 8), and form the *compact* sector of Ch5 (Ch5c).

In addition to Ch5c, there is also a more *diffuse* sector of Ch5 (Ch5d) consisting of ChAT-positive interstitial neurons embedded within the lateral lemniscus, superior cerebellar peduncle, and especially the central tegmental tract (Figs. 1B–D, 2B, 3A, 4A, 4B, 5, 6). A few isolated Ch5d neurons also extended into the traditional boundaries of the cuneiform and parabrachial nuclei, as well as into the subcoeruleus zone. In general, the Ch5d neurons tended to be smaller and more elongated than those of Ch5c (Figs. 5, 8). The ratio of ChAT-positive to ChAT-negative neurons showed considerable regional variations within the area covered by Ch5d and varied from 75% to 25%. The cytological appearance of ChAT-negative neurons was not distinctive—of two adjacent neurons with identical morphology on cresyl violet staining, one could be ChAT-positive and the other not. The Ch5 neurons were highly heterogeneous in shape (ranging from pyramidal to fusiform and multipolar), did not have a common orientation of dendrites or axons, and displayed overlapping isodendritic arborizations (Figs. 3C, 5).

The Ch6 complex. The constellation of cholinergic neurons designated Ch6 is focused around the laterodorsal tegmental nucleus of the rostral pontine central gray (Figs. 1B–D, 4A–B, 6). Just caudal to the trochlear nucleus, the central gray becomes an exceedingly complex region with several intercalated cell groups (Fig. 7B). This region is bounded ventrally by the medial longitudinal fasciculus and dorsally

Fig. 2. ChAT immunohistochemistry. **A.** ChAT-positive neurons are seen throughout the oculomotor complex, including the principal motoneuron pools (on), Perlia's nucleus (pn) and the Edinger-Westphal nucleus (ew). The curved arrows point to oculomotor neurons embedded within the medial longitudinal fasciculus (mlf). Dorsal is towards the top. Magnification ×11. **B.** Anterior part of Ch5 at the level of the trochlear nucleus (tn). The level is just rostral to that shown in Figure 1B. The curved arrows point to some of the interstitial ChAT-positive neurons in the diffuse part of Ch5 (Ch5d). The Ch6 group is making its most rostral appearance at this level. Dorsal is towards the top, medial towards the right. Magnification ×12.

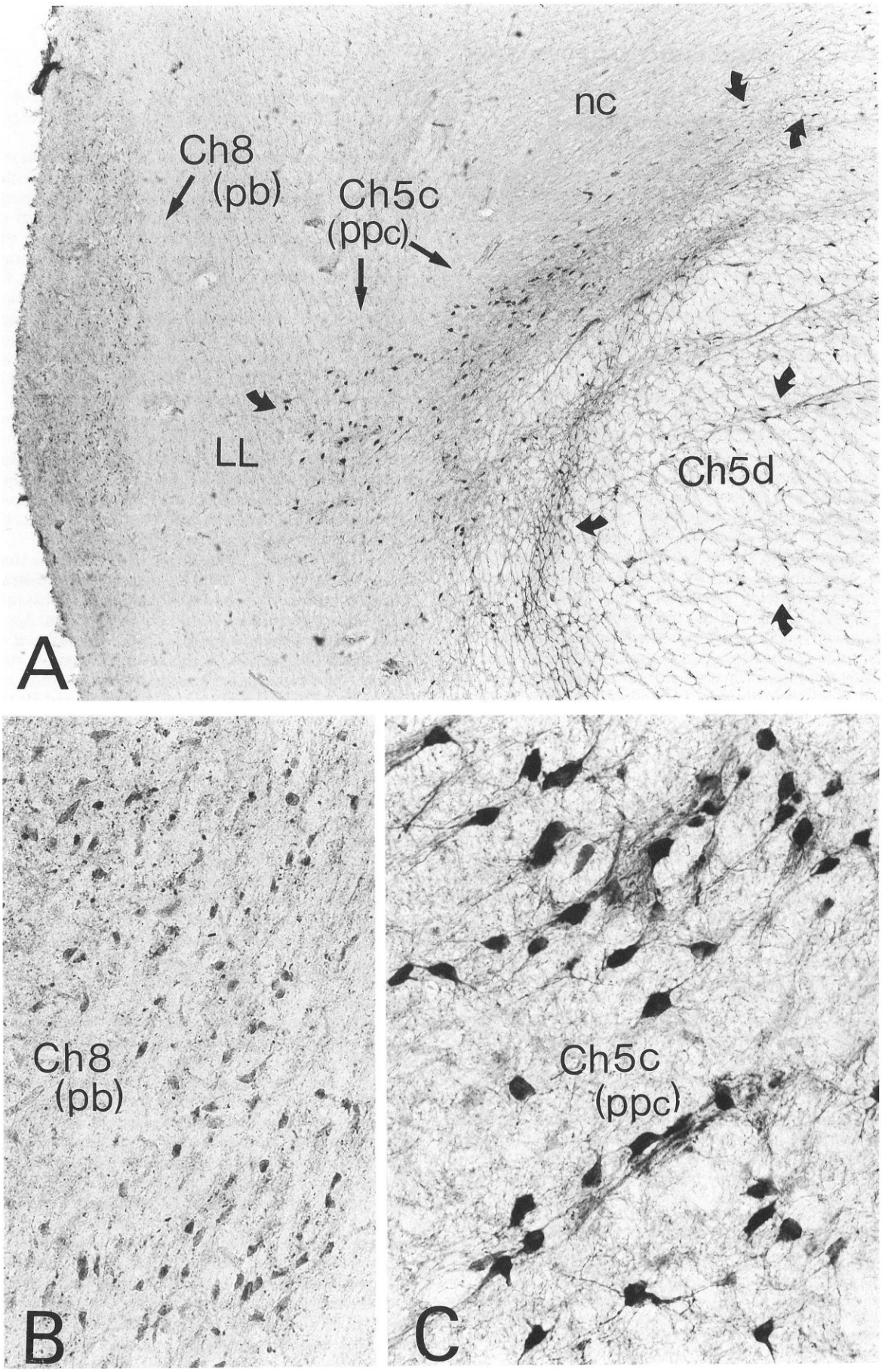

Figure 3

by the fourth ventricle. The dorsal raphe (dr in Fig. 7B), a collection of medium-sized, hyperchromic neurons, constitutes the most medial cell group in this region. The dorsal tegmental nucleus (dt in Fig. 7B), a relatively circular collection of hypochromic neurons, is situated more laterally. The medium-sized hyperchromic neurons of the laterodorsal tegmental nucleus (ldt in Fig. 7B) remain just lateral to the dorsal tegmental nucleus. Still more laterally are the heavily pigmented, large and oval-to-round neurons of the nucleus locus coeruleus (nl in Fig. 7B). These boundaries are considerably blurred in the more dorsal aspects of the central gray. Even ventrally, a good deal of interdigitation occurs so that the pigmented neurons of the locus coeruleus and the neurons of the laterodorsal tegmental nucleus show appreciable intermingling (Fig. 6).

Immunohistochemical preparations indicate that 80–90% of the neurons within the laterodorsal tegmental nucleus are ChAT-positive. These constitute the focus of the Ch6 constellation. Additional cholinergic Ch6 neurons extend into the surrounding regions of the central gray, especially dorsally and laterally, extending into the traditional boundaries of the nucleus locus coeruleus. The ratio of cholinergic to non-cholinergic neurons was considerably reduced in these outer reaches of Ch6. In immunohistochemical preparations, the black and granular melanin of locus coeruleus neurons is easily distinguished from the brown ChAT reaction-product of the Ch6 neurons. A large number of ChAT-positive neurons are embedded within the lateral part of the medial longitudinal fasciculus and should probably also be included in Ch6 (Figs. 6, 7B). The Ch6 neurons also display perikaryal heterogeneity and an isodendritic pattern of branching.

The medial longitudinal fasciculus provides a relative delineation between Ch5 and Ch6, even though there are no precise boundaries between the two groups. In general, the Ch6 perikarya are slightly larger than those of Ch5 (Fig. 8). The Ch5 and Ch6 neurons occasionally appear to form septae that divide the fiber bundles within which they are embedded (such as the superior cerebellar peduncle, the central tegmental tract, or the medial longitudinal fasciculus) into fascicular compartments (Figs. 2B, 3A, 6). Other ChAT-positive neurons of Ch5 and Ch6, on the other hand, appear to be scattered almost randomly within these fiber bundles (Figs. 4A, 4B).

Cytochemical comparison of pontomesencephalic cholinergic neurons to those of the forebrain

Shape and size. The Ch5-Ch6 neurons as well as those of Ch4 (nucleus basalis) shared several morphological properties such as perikaryal heteromorphism, isodendritic arborizations, lack of common orientation, a failure to be contained within single cytoarchitectonic boundaries, and frequent excursions into the territories of adjacent fiber bundles. In contrast, the cholinergic neurons of the putamen, parabigeminal nucleus, oculomotor nucleus, and trochlear nucleus displayed greater perikaryal uniformity, specialized dendritic aborizations, and confinement within identifiable nuclear boundaries.

With respect to size, the Ch4 neurons are the largest, being followed by those of Ch6 and Ch5c (Fig. 8). The neurons of Ch5d are slightly smaller than those of Ch5c. The cholinergic neurons of the parabigeminal nucleus are by far the smallest and most uniform in shape and size (Fig. 8).

NADPHd. The NADPHd procedure yielded a dark blue reaction product that helped to visualize not only the perikaryon but also a considerable extent of the dendrites and axons. Control material obtained by omitting the NADPH did not contain this type of perikaryal staining in the areas under investigation. In sections processed for the concurrent demonstration of NADPHd and ChAT, some neurons were labeled only with one of the reaction products while others were labeled with both. In the latter case, the ChAT reaction product was brown to reddish-brown in color, whereas the NADPHd reaction product displayed a purplish hue.

In the brainstem, a comparison of sections prepared for ChAT immunohistochemistry with matching sections prepared for NADPHd histochemistry showed that all areas containing the ChAT-positive neurons of Ch5-Ch6 also contained NADPHd-rich neurons in an identical distribution (Figs. 9A–B). Sections prepared for the concurrent visualization of both reaction products showed that virtually all Ch5 and Ch6 neurons were also intensely NADPHd-rich (Fig. 10A). However, approximately 10% of NADPHd-rich neurons in the general region of Ch5-Ch6 were ChAT negative. This percentage was even higher in the diffuse sector of Ch5 (Ch5d) and in the dorsal part of the central gray. All Ch5-Ch6 cholinergic neurons were thus NADPHd-rich but were intermixed with a small number of NADPHd-rich neurons that appeared to be non-cholinergic. Except for some neurons near and within the raphe region, the Ch5 and Ch6 zones contained the only major concentration of NADPHd-rich neurons in the upper brainstem. Thus, NADPHd histochemistry in the rostral brainstem provides an excellent first approximation for the cholinergic Ch5-Ch6 neurons. The cholinergic neurons of the third and fourth cranial nerve nuclei and those of the parabigeminal nucleus were NADPHd-negative (Table 1). These results are in agreement with the recent descriptions of NADPHd staining in the human brainstem (Kowall and Mueller, '88; Nakamura et al., '88).

In both the striatum and the nucleus basalis of Meynert (Ch4) we observed ChAT-positive as well as NADPHd-rich neurons. However, these constituted entirely non-overlapping populations, each with a distinctly different perikaryal morphology (Fig. 9C). Thus, the cholinergic neurons of Ch5 and Ch6 appeared to be unique among all other cholinergic neurons of the rostral brainstem and forebrain in containing high levels of NADPHd activity.

The AChE reaction. The AChE reaction product was black and granular. In sections stained for the concurrent demonstration of AChE and ChAT, the black AChE reaction product was easily distinguished from the brown ChAT reaction product (Fig. 10B). When butyrylthiocholine was used instead of acetylthiocholine (in the presence of ethopropazine) no AChE staining was seen in the areas of interest. A comparison of ChAT immunohistochemical preparations with the AChE stain showed that the distribution of

Fig. 3. ChAT immunohistochemistry. **A.** Curved arrows point to interstitial elements of Ch5d. Some of these neurons extend into the lateral lemniscus (LL) and the cuneiform nucleus (nc). Note the considerably smaller ChAT-positive Ch8 neurons of the parabigeminal nucleus (pb). The level is approximately equivalent to that of Figure 1C. Dorsal is towards the top, medial towards the right. Magnification ×22. **B** and **C.** Detail of Ch8 and Ch5c. The magnification is ×150 for both. The ChAT staining in Ch8 is of lesser intensity than in Ch5 and the neuronal sizes are markedly different. Note the cytological heterogeneity and lack of common dendritic orientation in Ch5c.

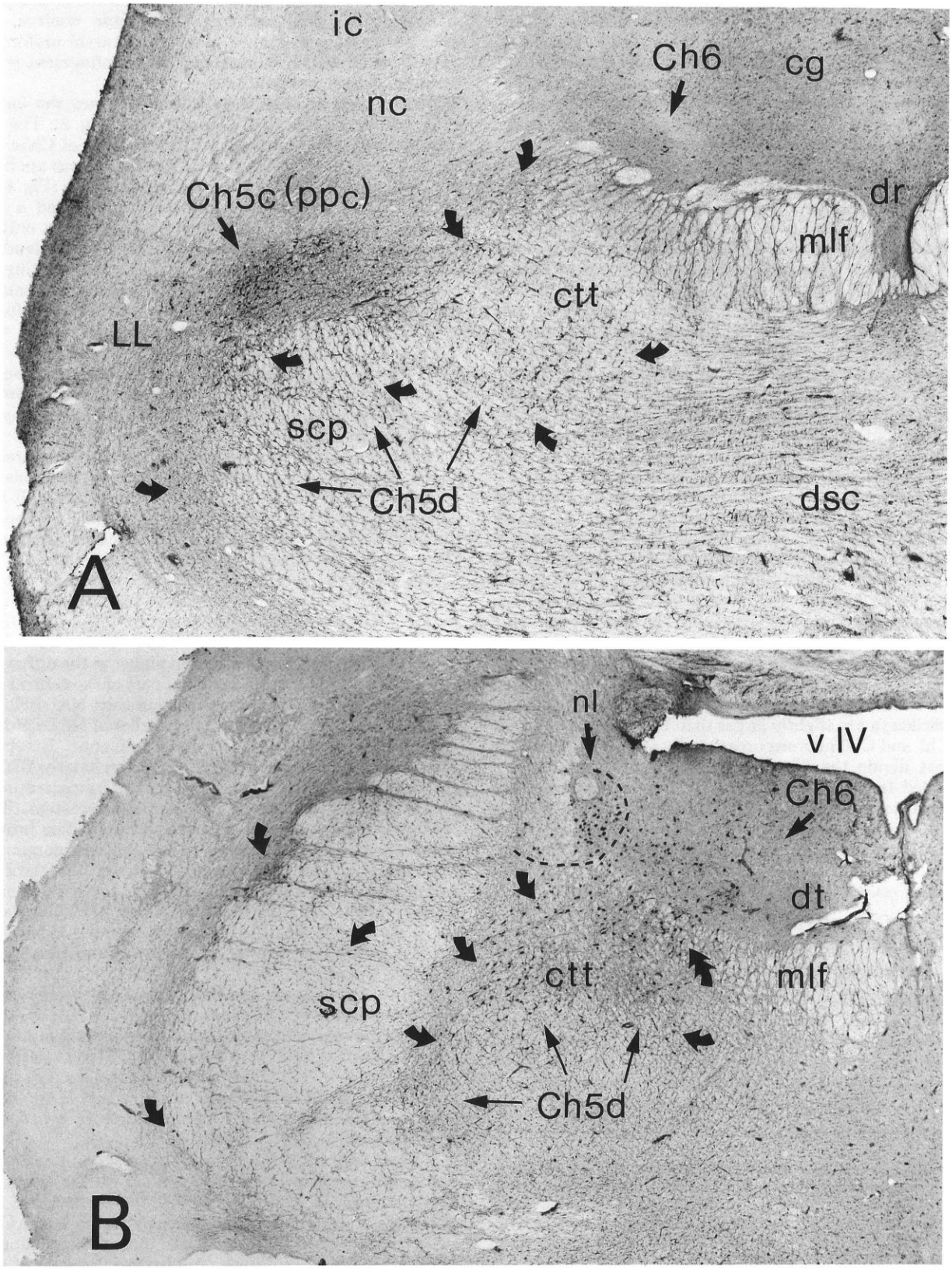

Fig. 4. **A** and **B**: ChAT immunohistochemistry corresponding to levels slightly more caudal than that of Figure 1C(A) and very close to that of 1D(B). Single curved arrows point to some of the interstitial ChAT-positive neurons of Ch5d. The double-headed curved arrow in B points to the interstitial Ch6 neurons embedded within the lateral part of the medial longitudinal fasciculus (mlf). The broken line in B provides an arbitrary boundary for the nucleus locus coeruleus (nl). Although considerable intermingling does exist, the black color of the melanin-laden locus coeruleus neurons is easily differentiated from the brown ChAT reaction product of the Ch6 neurons. Dorsal is towards the top, medial towards the right. Note that Ch5d extends further caudally than Ch5c. Magnification ×23.

ChAT-positive neurons in Ch5 and Ch6 overlapped almost exactly with the distribution of intensely AChE-rich neurons (Fig. 11). Sections stained concurrently for ChAT and AChE showed that nearly all (95% to 99%) of ChAT neurons in Ch5 and Ch6 were also AChE-rich (Fig. 10B). Within the compact part of Ch5 and in the laterodorsal tegmental region, AChE-rich but ChAT-negative neurons were rare. However, this type of neuron became more frequent (up to 10–25% of all AChE-rich perikarya) in the interstitial part of Ch5 (Ch5d) and in parts of the central gray surrounding the laterodorsal tegmental nucleus. Furthermore, the substantia nigra, ventral tegmental area, raphe nuclei, and the nucleus locus coeruleus also contained somewhat less intensely stained but strongly AChE-positive neurons (Fig. 11A). Thus, all Ch5-Ch6 neurons contain AChE but this enzyme is not specific to cholinergic neurons.

The AChE-rich staining pattern is not unique to Ch5-Ch6 and was a universal feature of all cholinergic cells that we examined, including those of the parabigeminal nucleus, the oculomotor and trochlear nuclei, the striatal ChAT-positive neurons, and the Ch4 neurons of the nucleus basalis (Table 1).

Nerve growth factor receptors (NGFr). There was a perfect topographic overlap between the distribution of ChAT-positive and NGFr-positive neurons in the nucleus basalis-Ch4 complex (Figs. 12, 13). Sections counterstained for Nissl substance indicated that 90% of the magnocellular perikarya in the nucleus basalis were ChAT-positive. An identical ratio of immunostaining was obtained when sections stained for NGFr and counterstained for Nissl substance were examined. Sections processed for the concurrent demonstration of NGFr with NADPHd showed a complete lack of overlap between the two reaction products in a pattern that was identical to that seen when ChAT and NADPHd were demonstrated concurrently. These observations led to the conclusion that most, if not all, ChAT-positive Ch4 neurons also contain NGFr.

This type of NGFr immunoreactivity could not be discerned in the striatum, oculomotor-trochlear nuclei, the parabigeminal nucleus or in Ch5-Ch6 (Fig. 14). Although the failure to obtain immunostaining cannot prove the absence of NGFr, it is clear that the cholinergic neurons of Ch4 have a uniquely high concentration of NGFr (Table 1).

Nonphosphorylated neurofilament protein. An antibody to nonphosphorylated neurofilament protein (SMI-32) yielded intense immunopositivity throughout the perikaryon, dendrites, and axon of many neuronal subsets. The staining in such intensely immunopositive neurons had a fibrillary rather than a diffuse cytoplasmic distribution. Most of the neurons in the nucleus basalis (Ch4), the oculomotor nucleus, the trochlear nucleus, the laterodorsal tegmental nucleus (Ch6), and the pedunculopontine nucleus (Ch5) were intensely SMI-32-positive (Fig. 15). Such intense staining could not be detected within cholinergic neurons of the striatum or of the parabigeminal nucleus (Table 1). The intense staining pattern with SMI-32 was also present in several other cell groups of the forebrain and brainstem and is therefore not confined to cholinergic perikarya.

Tyrosine hydroxylase immunohistochemistry

Tyrosine hydroxylase (TH) immunostaining was performed to study the extent of intermingling between catecholaminergic and cholinergic neurons within the regions containing the Ch5 and Ch6 constellations. We used NADPHd histochemistry as a marker for the cholinergic Ch5-Ch6 neurons and processed tissue sections for the concurrent demonstration of TH and NADPHd.

As expected, the greatest concentrations of TH-positive neurons were detected in the substantia nigra, ventral tegmental area, the nucleus locus coeruleus, and the subcoeruleus nuclei (Pearson et al., '83; DeLima and Singer, '87; Jones and Beaudet, '87; Kitahama et al., '88). The overlap between catecholaminergic TH-positive neurons and NADPHd-rich neurons was relatively minor in Ch5, even though there were individual TH-positive neurons embedded within clusters of NADPHd-rich neurons. The overlap between these two types of neurons was much more extensive in the regions containing the Ch6 complex (Fig. 10C). In general, the TH-positive neurons were larger than the NADPHd-rich (presumably also ChAT-positive) neurons but this was not consistent. No double labeled TH-positive and NADPHd-rich neurons were encountered. These observations provide additional evidence for considerable transmitter-related heterogeneity in the regions under study and further justify the use of the transmitter-specific Ch5 and Ch6 designations.

DISCUSSION

Choline acetyltransferase (ChAT) immunohistochemistry, revealed three types of cholinergic neurons in the human rostral brainstem: 1) The autonomic and skeletal motoneurons of the third and fourth cranial nerve nuclei; 2) small neurons of the parabigeminal nucleus (the Ch8 cell group); 3) two intersecting constellations of moderately large neurons that we have designated Ch5 and Ch6. The Ch5-Ch6 neurons, by far the most numerous of cholinergic neurons in the rostral brainstem, constitute the focus of this report.

The Ch5 and Ch6 neurons

The Ch5 complex, extending from mid-mesencephalic to mid-pontine levels, has its center of gravity within the pedunculopontine nucleus. Nearly all of the hyperchromic neurons in the pedunculopontine nucleus (pars compacta) are intensely ChAT-positive and constitute the compact sector of Ch5 (Ch5c). The Ch5c component is surrounded by a diffuse-interstitial component (Ch5d) containing slightly smaller ChAT-positive neurons embedded within passing fiber systems such as the superior cerebellar peduncle and especially the central tegmental tract. A few isolated Ch5d neurons were also seen in the traditional boundaries of the cuneiform nucleus, the parabrachial nuclei, the subcoeruleus zone, and the lateral lemniscus. The interstitial Ch5 neurons are at least as numerous as those within the compact pedunculopontine region.

Heterogeneity of primary transmitter appears to be the rule. Approximately 10% of neurons within the compact sector of the pedunculopontine nucleus fail to give a detectable ChAT reaction, even though they stain no differently with Nissl preparations. The frequency of noncholinergic neurons can be as high as 75% in regions containing the interstitial components of Ch5. Some of the noncholinergic neurons intermingled with Ch5 are catecholaminergic, but the transmitter identity of most remains unknown.

The Ch6 complex has its center of gravity within the laterodorsal tegmental nucleus, but its neurons spread into the adjacent parts of the central gray, the medial longitudinal fasciculus and even into the region of the nucleus locus coeruleus. The Ch6 complex reaches peak density at a level

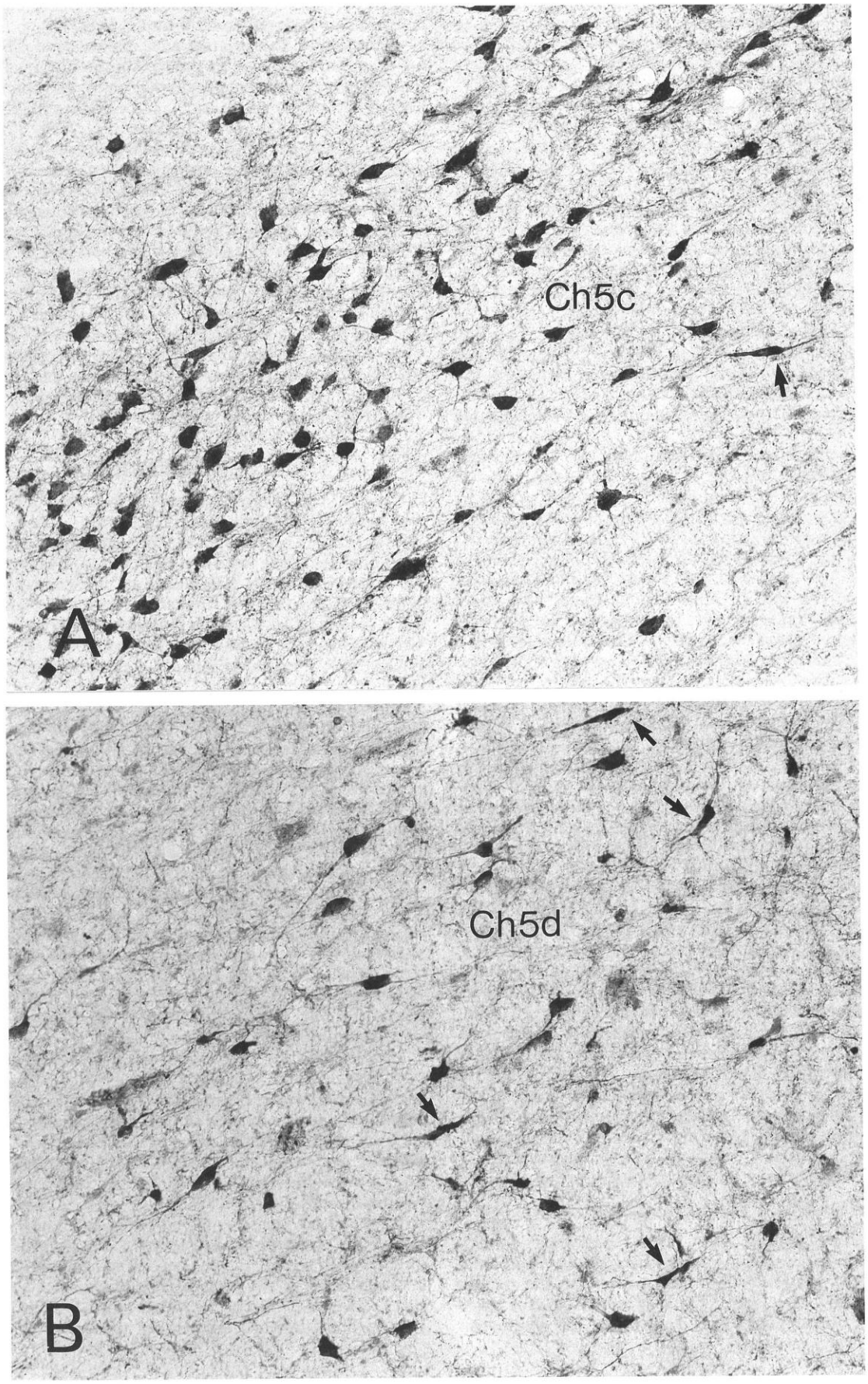

Figure 5

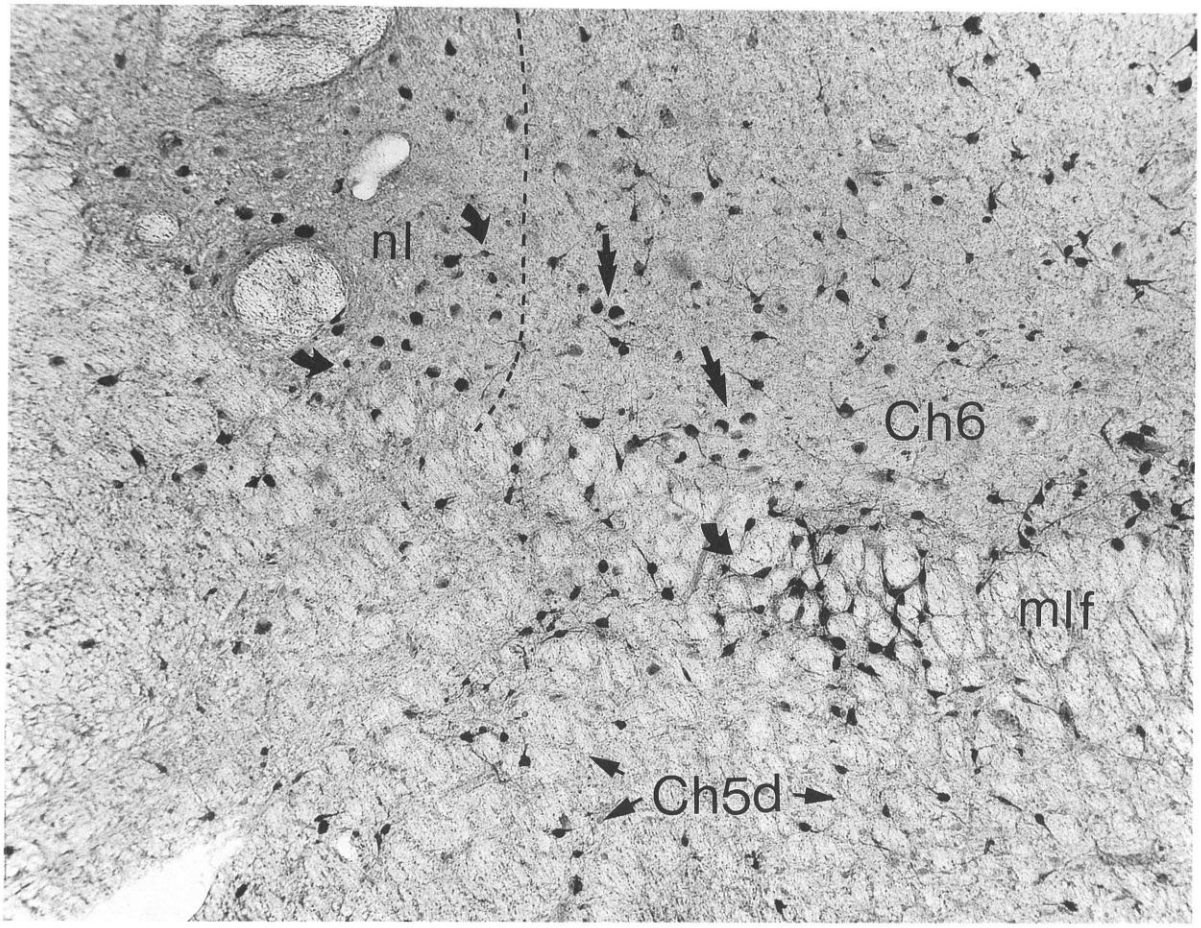

Fig. 6. ChAT immunohistochemistry of Ch6. Dorsal is towards the top, medial towards the right. Some interstitial elements of Ch6, embedded within the medial longitudinal fasciculus (mlf), are indicated by the curved arrow on the right. The broken line provides an arbitrary boundary for the nucleus locus coeruleus (nl). Under microscopic examination, the black color of melanin in the nl neurons can be differentiated readily from the brown ChAT reaction product of the Ch6 neurons. Clusters of nl neurons (double arrows) are present in the area of the lateral tegmental nucleus while isolated Ch6 neurons (the curved arrows on the left) are seen among the nl neurons. The coronal level is equivalent to that of Figure 1D. Magnification ×53.

somewhat more caudal than the region of peak density for Ch5. The Ch6 neurons are slightly smaller than those of Ch5c. Transmitter heterogeneity is also conspicuous. The laterodorsal tegmental nucleus contains a relatively pure population of cholinergic neurons. However, the surrounding zones of the central gray show extensive intermingling of cholinergic Ch6 neurons with tyrosine hydroxylase-positive catecholaminergic neurons. This intercalation of catecholaminergic and cholinergic neurons is also a feature of the feline brainstem (DeLima and Singer, '87; Jones and Beaudet, '87).

Fig. 5. **A** and **B**: ChAT immunohistochemistry of the compact (Ch5c) and diffuse (Ch5d) components of Ch5. Note cytological heterogeneity and diversity of dendritic branching patterns. Markedly elongated perikarya (straight arrows) are more common in Ch5d than in Ch5c. The coronal level is equivalent to that of Fig. 1C. Dorsal is towards the top, medial towards the right. Magnification ×175.

There is no precise delineation between Ch5 and Ch6. The two groups are related to each other in the form of partially overlapping constellations rather than discrete nuclei with firm boundaries. There are two reasons for using the Ch5-Ch6 nomenclature to designate these cholinergic neurons: 1) the overlap with traditional cytoarchitectonic boundaries is not perfect so that neither Ch5 nor Ch6 is synonymous with any existing anatomical label; 2) there is considerable intermingling with neurons belonging to other pharmacosystems and the Ch5-Ch6 terminology is the only means for designating specifically the cholinergic neurons in the relevant areas. The Ch5 and Ch6 groups of the human brain display an overall pattern of distribution quite similar to that described in the brains of rodents, cats, dogs, and monkeys (Kimura et al., '81; Armstrong et al., '83; Mesulam et al., '83; '84; Satoh and Fibiger, '85; Isaacson and Tanaka, '86; DeLima and Singer, '87; Hallanger et al., '87; Jones and Beaudet, '87). As in the other mammalian species that have been investigated, the Ch5-Ch6 groups of the human brain constitute one of the most conspicuous and extensive cell groups of the entire rostral brainstem.

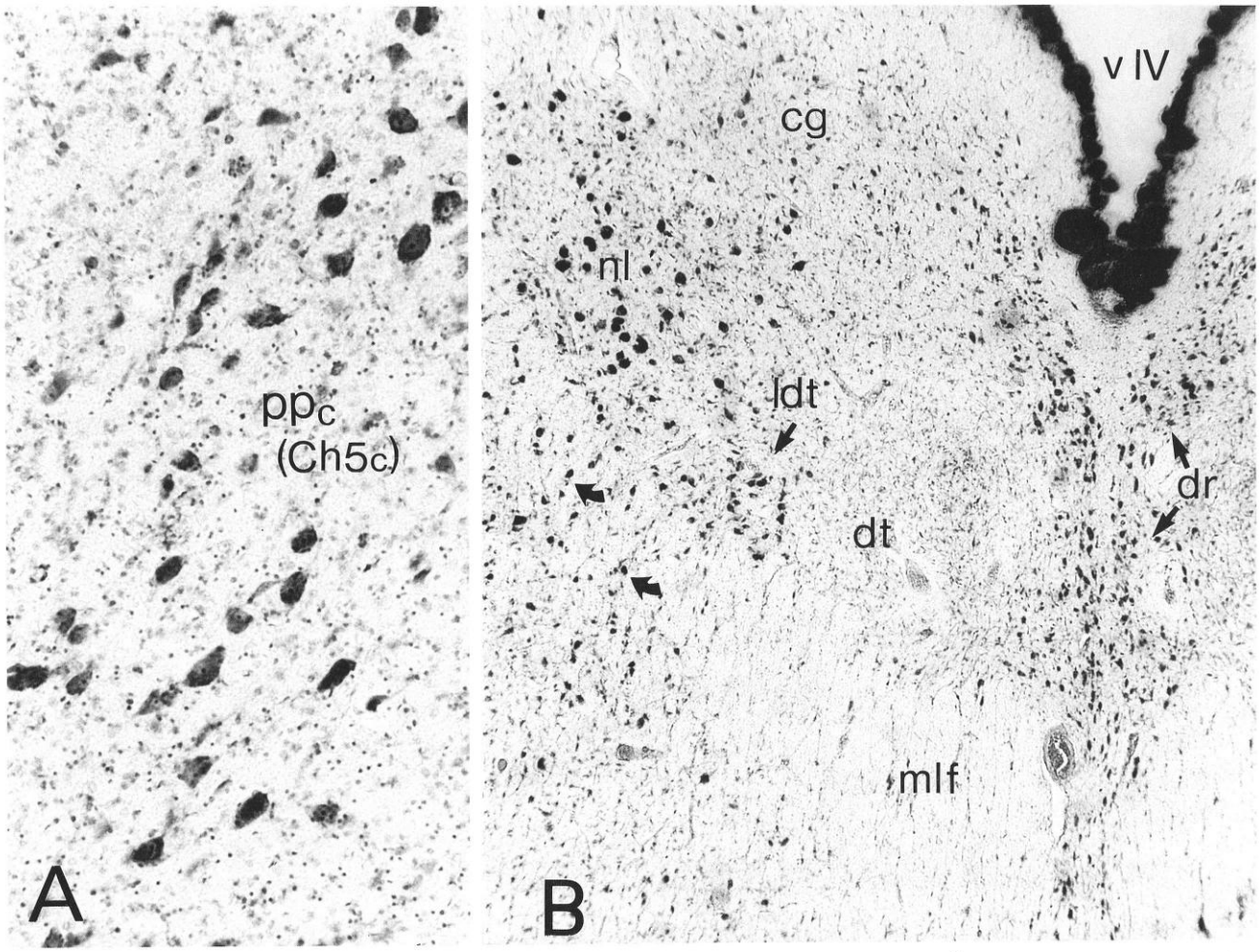

Fig. 7. Cresyl violet stain. **A**: Compact sector of the pedunculopontine nucleus corresponding to the level shown in Figure 1C. Medial is towards the right, dorsal towards the top. Magnification ×185. **B**: The periventricular region at a level corresponding to that shown in Figure 1C. The curved arrows point to neurons embedded within the medial longitudinal fasciculus (mlf) with cytological characteristics identical to neurons of the laterodorsal tegmental nucleus (ldt). Many of these neurons are ChAT-positive and part of the Ch6 group. Note how the nuclear boundaries become indistinct in the more dorsal reaches of the central gray (cg). Magnification ×50.

Connectivity and behavioral affiliations of Ch5 and Ch6

Moruzzi and Magoun ('49) had introduced the concept of an ascending reticular activating system that acted to desynchronize the cortical electroencephalogram via a relay in the thalamus. Subsequent work revealed that a most important component in this system consists of a cholinergic reticulothalamic pathway that promotes the activation of corticopetal relay neurons in the thalamus (Phillis et al., '67; McCance et al., '68; Shute and Lewis, '67; Dingledine and Kelly, '77; Hoover and Jacobowitz, '79). The advent of methods for the concurrent visualization of axonal transport and cholinergic perikaryal markers made it possible to trace the origin of this projection to the Ch5 and Ch6 cell groups (Hoover and Baisden, '80; Mesulam et al., '83; Sofroniew et al., '85; Isaacson and Tanaka, '86; Woolf and Butcher, '86; DeLima and Singer, '87; Hallanger et al., '87; Steriade et al., '88). This pathway supplies the vast majority of the thalamic cholinergic innervation, it represents the major output of the Ch5-Ch6 neurons, and it provides one of the most substantial afferents that the thalamus receives from the brainstem (Mesulam et al., '83; Hallanger et al., '87). Some thalamic nuclei, especially the reticular and mediodorsal, also receive cholinergic afferents from the basal forebrain, but these appear to be of lesser magnitude than the overall input from the brainstem to the thalamus (Mesulam et al., '83; Hallanger et al., '87; Steriade et al., '87).

The physiological relevance of this pathway to the reticular activating system was demonstrated by Kayama and colleagues ('86). They identified the Ch5-Ch6 neurons with NADPHd histochemistry and showed that electrical stimulation of these neurons caused a scopolamine-sensitive activation of lateral geniculate neurons and even an occasional enhancement of their response to photic stimulation. Thus, the Ch5-Ch6 neurons can influence the transthalamic (and ultimately corticopetal) processing of sensory information in ways that could modulate complex behavioral states such as arousal and attention.

The rostral brainstem contains state-dependent neurons whose firing rates reflect the level of electrocortical and behavioral arousal (Goodman et al., '68; Steriade, '81; Ray et

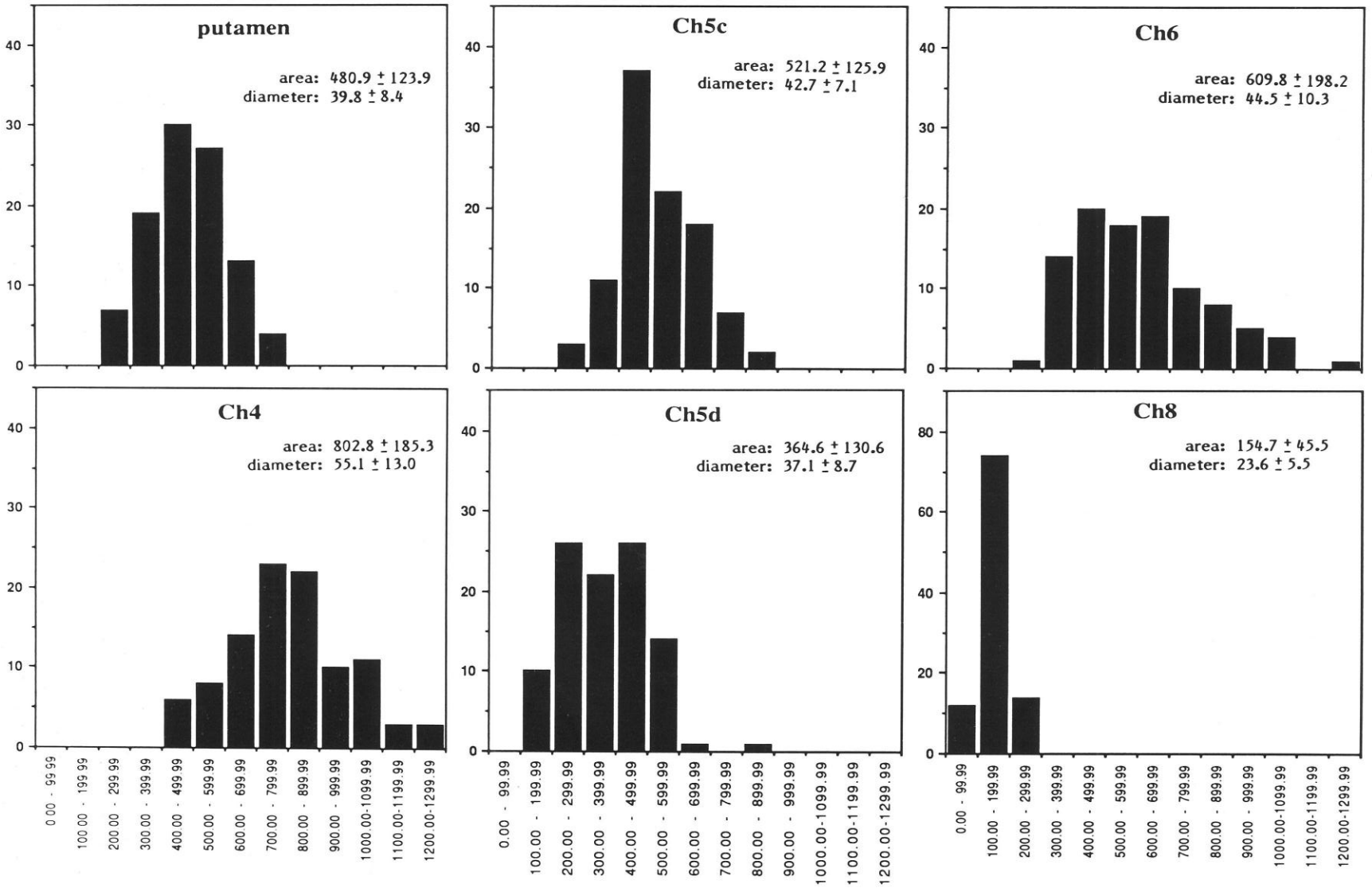

Fig. 8. Area and diameter measurements. At each site, 100 neurons were measured. All neurons were from the brain of a 76-year-old man. The y-axis indicates the number of neurons in each area range. The x-axis indicates the ranges of perikaryal area (in square microns). The scale for the x-axis is identical in all six frames. The numbers in the upper right corner of each frame indicate means and standard errors for area (in square microns) and maximum diameter (in microns).

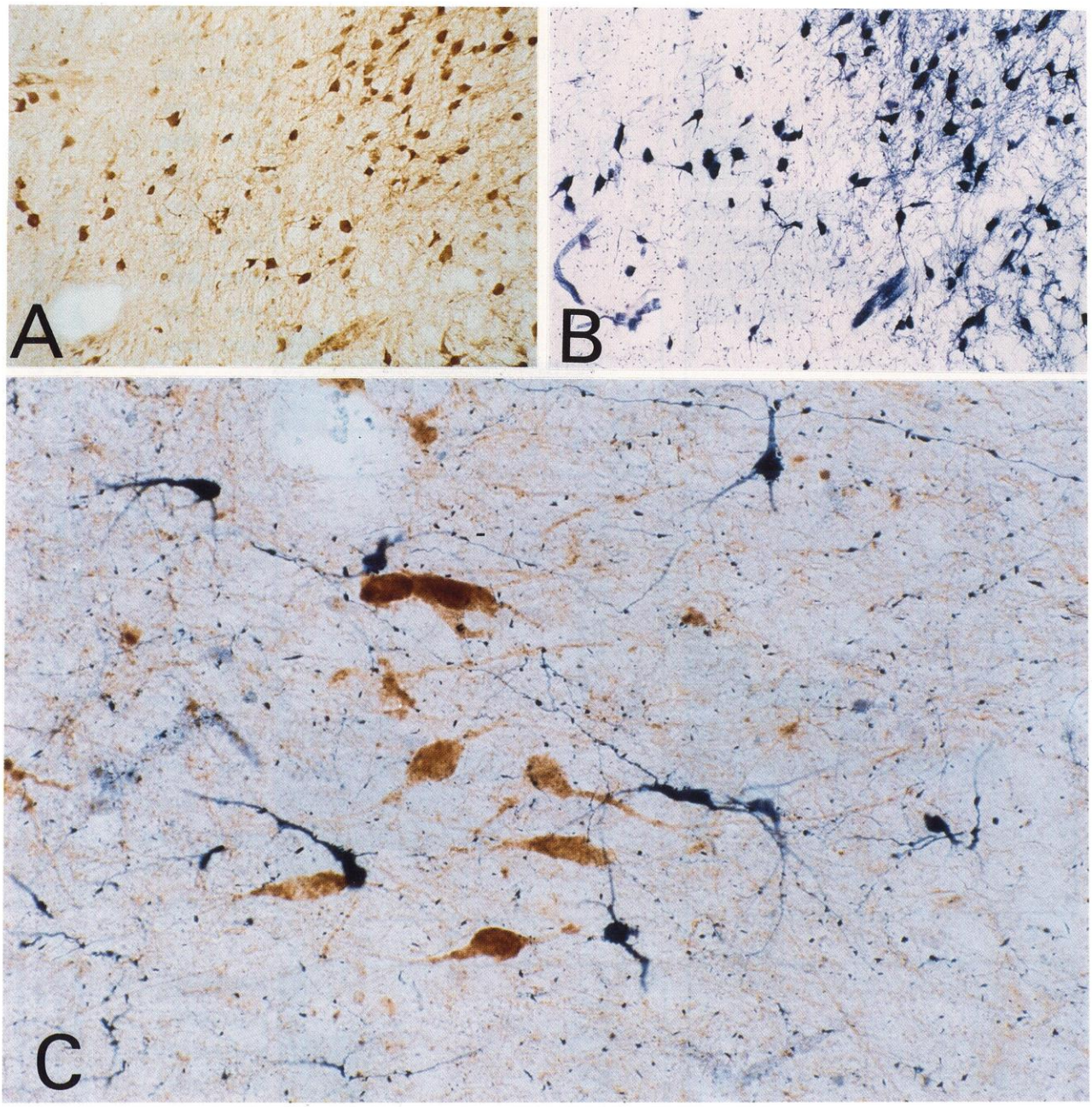

Fig. 9. **A**: ChAT immunohistochemistry of Ch5. Dorsal is towards the top, medial towards the right. Magnification ×75. **B**: NADPHd histochemistry of an adjacent section. Same orientation and magnification as in A. Note the identical distribution of ChAT and NADPHd. **C**: Concurrent ChAT and NADPHd staining in the nucleus basalis. Note the ChAT-positive (brown) Ch4 neurons and the smaller NADPHd-positive (blue) neurons. There is no overlap between the two populations. Magnification ×275.

al., '82). It will be interesting to see if these neurons belong to the Ch5-Ch6 complex. In keeping with their involvement in the reticular activation system, the Ch5 and Ch6 neurons have also been implicated in the modulation of rapid eye movement sleep (Mitani et al., in press).

In addition to thalamic projections, Ch5 and Ch6 may also provide a relatively sparse but direct monosynaptic cholinergic projection for parts of the cerebral cortex (Mesulam et al., '83; Mufson et al., '84; Satoh and Fibiger, '86). The Ch5-Ch6 neurons also project to the hypothalamus and to the basal forebrain regions containing the cortically projecting cholinergic neurons of the nucleus basalis (Woolf and Butcher, '86; Hallanger and Wainer, '88; Semba et al., '88; Vertes, '88). These projections could enable the Ch5-Ch6 neurons to influence the cholinergic innervation of the cortical mantle.

In addition to their role in the ascending reticular activating system, the Ch5 and Ch6 neurons may also participate in

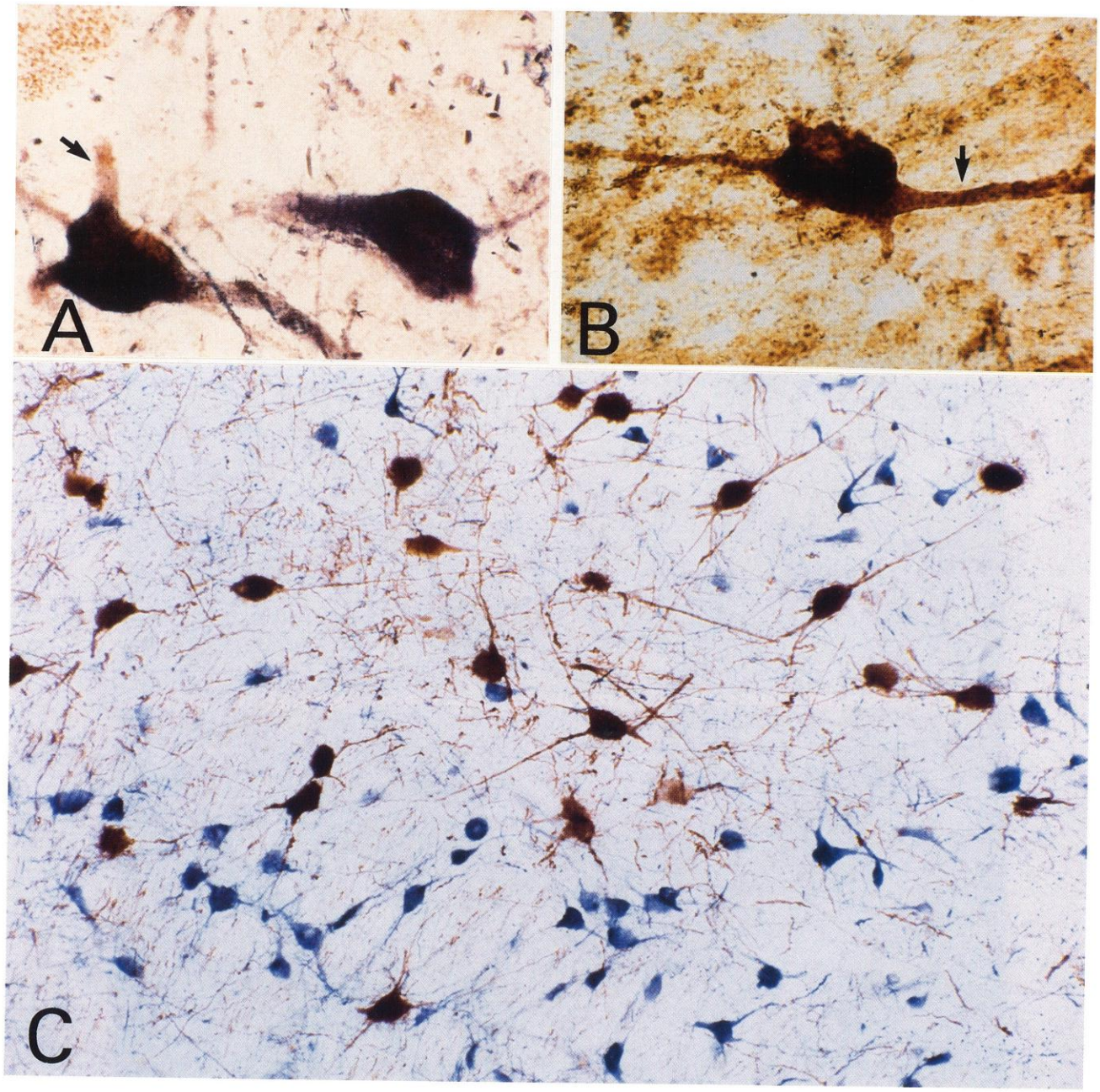

Fig. 10. **A**: Concurrent demonstration of ChAT (brown) and NADPHd (purple) in Ch6. The two Ch6 neurons in this photomicrograph are labeled with both reaction products. The ChAT reaction product is more easily seen in the axons and dendrites (arrow) whereas the NADPHd reaction product is more easily detected within the perikaryon. All Ch5 and Ch6 neurons display this type of double-labeling. Magnification ×818. **B**: Concurrent demonstration of AChE (black) and ChAT (brown). The photomicrograph shows a Ch6 neuron labeled with both reaction products. The ChAT reaction product is most clearly seen in the dendrites (arrow) and the AChE reaction product in the perikaryal region. Magnification ×818. **C**: Concurrent demonstration of TH (brown) and NADPHd (blue) in the laterodorsal tegmental-central gray region containing Ch6. The level is equivalent to that of Figure 1D. The NADPHd acts as a marker for the cholinergic Ch6 neurons. Note the intermingling between the two populations. There is no double staining. In the absence of immunohistochemical or histochemical stains, it would be difficult to decide by Nissl staining alone which neurons belong to Ch6 and which to the catecholaminergic cell groups. Magnification ×131.

extrapyramidal motor control. This possibility was raised by Nauta and Mehler ('66), who identified the pedunculopontine nucleus as the most caudal target of pallidofugal projections. This observation, subsequently confirmed with more modern tracing techniques (Kim et al., '76), suggested that the pedunculopontine nucleus could constitute an obligatory and therefore pivotal relay between the output of the basal ganglia and the motor nuclei of the brainstem. Numerous anatomical and physiological experiments reported projections (often reciprocal) between the peduncu-

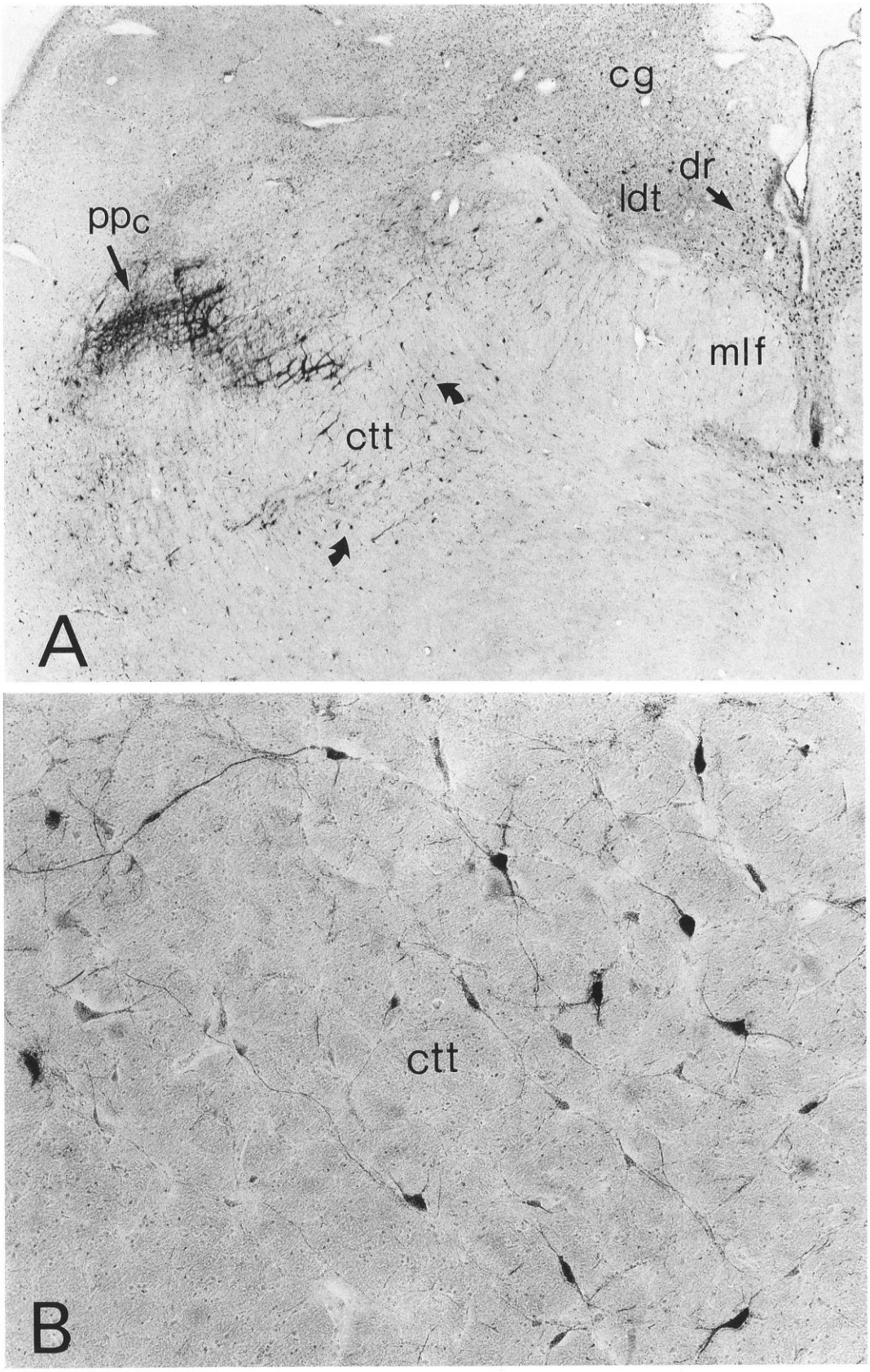

Figure 11

lopontine nucleus and several extrapyramidal structures such as the globus pallidus, entopeduncular nucleus, substantia nigra, subthalamic nucleus, neostriatum, and even the spinal cord (Nauta and Cole, '78; Beckstead and Frankfurter, '82; Saper and Loewy, '82; Gonya-Magee and Anderson, '83; Jackson and Crossman, '83; Moon-Edley and Graybiel, '83; Rye et al., '88; Tokuno et al., '88). Sugimoto and Hattori ('84) also showed that the projections from the pedunculopontine nucleus to the intralaminar nuclei of the thalamus made direct synaptic contact with the thalamic neurons that project to the striatum.

The few studies that have employed cholinergic markers in combination with retrogradely transported tracers (e.g. HRP) have yielded somewhat conflicting results. Some investigators (Woolf and Butcher, '86; Beninato and Spencer, '87; Clarke et al., '87) reported double HRP-ChAT labeling in the pedunculopontine nucleus of the rat after HRP injections in the substantia nigra. Others (Rye et al., '87) found that the retrograde labeling after similar injections was located predominantly in the ChAT-negative neurons of this region. In favor of this latter set of results, Scarnati and colleagues ('86) found that the activation of substantia nigra cells by pedunculopontine stimulation appeared to be mediated by glutamate rather than by acetylcholine. Rye and colleagues ('87) also showed that the descending projections from the substantia nigra and entopeduncular nucleus terminated not within the compact pedunculopontine nucleus but just medial to it. Since there is a diffuse sector of Ch5 that extends medially into the pontomesencephalic tegmentum, however, these descending extrapyramidal projections could conceivably still terminate upon cholinergic Ch5 neurons. One set of experiments (in which ChAT immunohistochemistry had been used to verify the site of electrical stimulation) reported that the Ch5 cell group overlapped with the mesencephalic locomotor region (Garcia-Rill et al., '87). Although the precise relationship of cholinergic brainstem neurons to motor pathways remains to be elucidated, the available evidence suggests that there is either a partial overlap or a very close linkage between the pedunculopontine nucleus (Ch5) and the regions of the upper brainstem traditionally associated with the extrapyramidal motor system.

The connectivity of Ch5 is not identical to that of Ch6. The Ch5 neurons appear to be more closely interconnected with extrapyramidal structures than the Ch6 neurons (Woolf and Butcher, '86). Furthermore, the Ch6 group sends relatively few projections to sensory relay nuclei of the thalamus while it figures prominently (occasionally as the principal source) in projections to its limbic nuclei (Woolf and Butcher, '86; Hallanger et al., '87). In keeping with the limbic affiliations of Ch6, projections have been described from the laterodorsal tegmental nucleus of the baboon to such limbic structures as the cingulate and subicular cortices but not to parietal neocortex (Mikol et al., '84). Satoh and Fibiger ('86) also showed that in comparison to the pedunculopontine nucleus (i.e. Ch5), the cholinergic neurons of the laterodorsal tegmental nucleus (i.e. Ch6) had the more extensive projections to the lateral septum and medial prefrontal cortex. It appears, therefore, that Ch6 is more closely associated with the limbic system, whereas Ch5 participates more extensively in the neural networks subserving sensory processing and extrapyramidal motor control. This distinction further justifies the delineation of Ch5 from Ch6 despite the absence of strict cytoarchitectonic demarcations.

Progress in unravelling the behavioral affiliations of these cholinergic cell groups has been slow, probably because of the transmitter-related heterogeneity of the region containing Ch5 and Ch6 and also the extension of these neurons across several nuclear boundaries and into fiber tracks. The pattern of projections summarized above would suggest that these neurons are important constituents of reticular, extrapyramidal, and limbic networks. However, the more specific details of these behavioral affiliations remain to be determined.

The regions containing the Ch5 and Ch6 neurons have been examined in a number of human neurodegenerative diseases. Alzheimer's disease, characterized by severe dementia, is associated with substantial neuronal loss in Ch4 but a much lesser (if any) loss in Ch5-Ch6 (Zweig et al., '87; Jellinger, '88). Even though some degenerative changes in the form of neurofibrillary tangles does occur in the Ch5-Ch6 region of patients with Alzheimer's disease this is not nearly so severe and extensive as the changes in the cholinergic neurons (Ch4) of the nucleus basalis (Mufson et al., '87; Zweig et al., '87; Jellinger, '88). This differential involvement of cholinergic neurons probably reflects differences in connectivity patterns. According to this explanation, the Ch4 neurons are affected because they project to the cortical surface, the primary site of the pathological process in Alzheimer's disease, while the Ch5-Ch6 neurons remain relatively spared because they do not have equally extensive cortical projections (Mesulam, '86).

Substantial cell loss in the pedunculopontine region has been reported in the brains of patients with Parkinson's disease and progressive supranuclear palsy (Hirsch et al., '87; Zweig et al., '87; Jellinger, '88). The major clinical features of both diseases include extrapyramidal deficits, frequently accompanied by changes in mental state. The loss of Ch5 neurons could contribute to the extrapyramidal and behavioral difficulties experienced by these patients. However, Parkinson's disease and progressive supranuclear palsy also display extensive pathological changes elsewhere in the brain, including the cholinergic neurons of the nucleus basalis (Rogers et al., '85). The exact contribution of the Ch5 cell loss to the clinical symptomatology of these two neurodegenerative diseases is therefore difficult to determine.

The parabigeminal nucleus

In addition to the Ch5-Ch6 cell groups and the cranial nerve nuclei, neurons of the parabigeminal nucleus also gave a modest but definite ChAT reaction. We described the presence of cholinergic neurons in the parabigeminal nucleus of rodents and showed that these neurons (designated Ch8) provided the major source of cholinergic projections for the superior colliculus (Mufson et al., '86). Some of the cholinergic innervation of thalamic nuclei (e.g. the lateral geniculate nucleus) also arises from the Ch8 cells (Fitzpatrick et al., '88).

The parabigeminal nucleus is closely affiliated with the superior colliculus through strong reciprocal projections

Fig. 11. **A**: AChE histochemistry. The level is slightly more caudal than that of Figure 1B. The compact pedunculopontine nucleus (ppc) is intensely AChE-rich. There are AChE-rich neurons (curved arrows) in the central tegmental tract (ctt) and in the area of the laterodorsal tegmental nucleus (ldt). Note that the neurons of the dorsal raphe (dr) are also intensely AChE-positive. Magnification ×17. **B**: AChE histochemistry of interstitial neurons in the central tegmental tract. Note cytological heterogeneity, isodendritic arborizations and lack of common orientation. Magnification ×143.

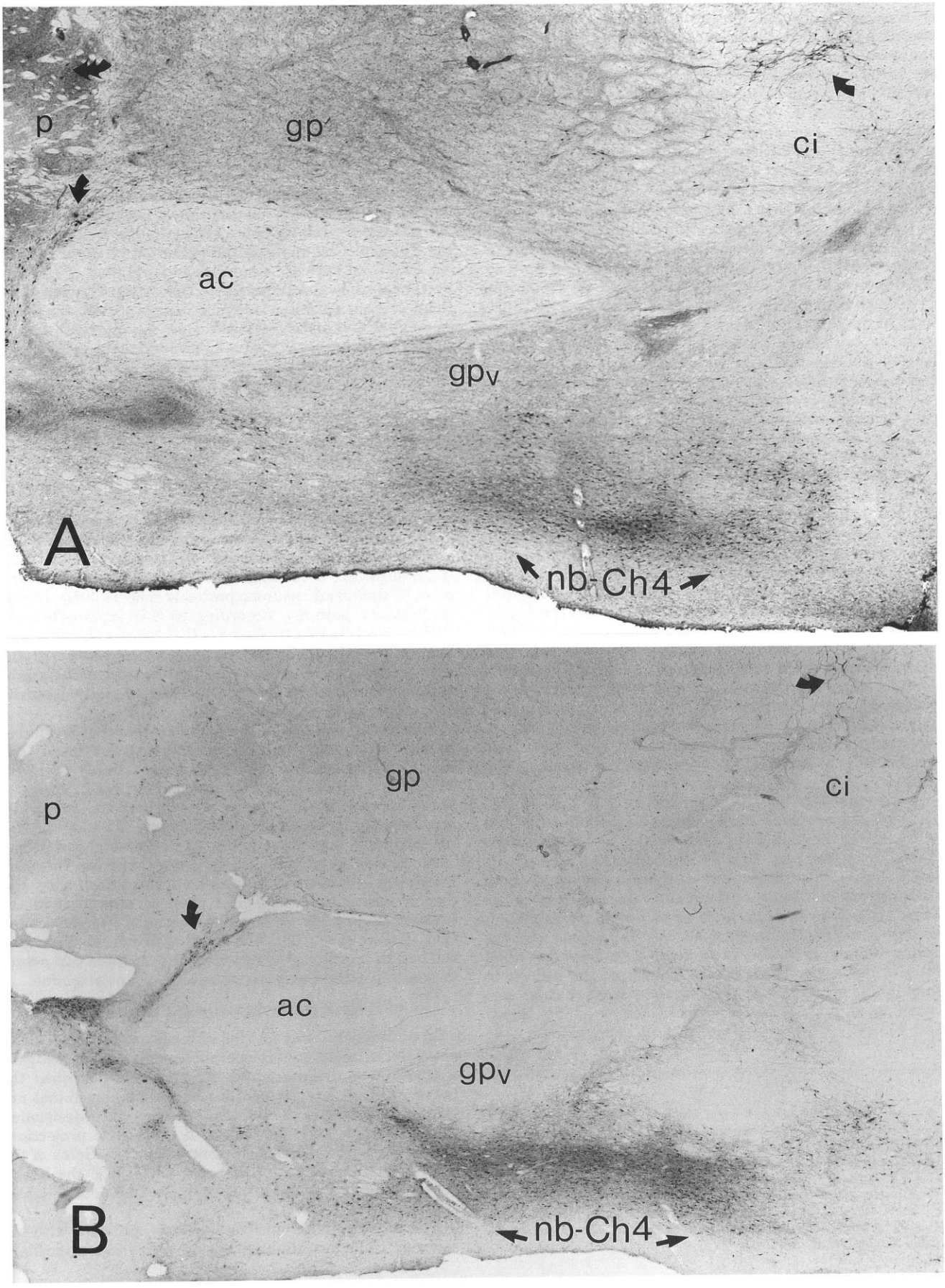

Figure 12

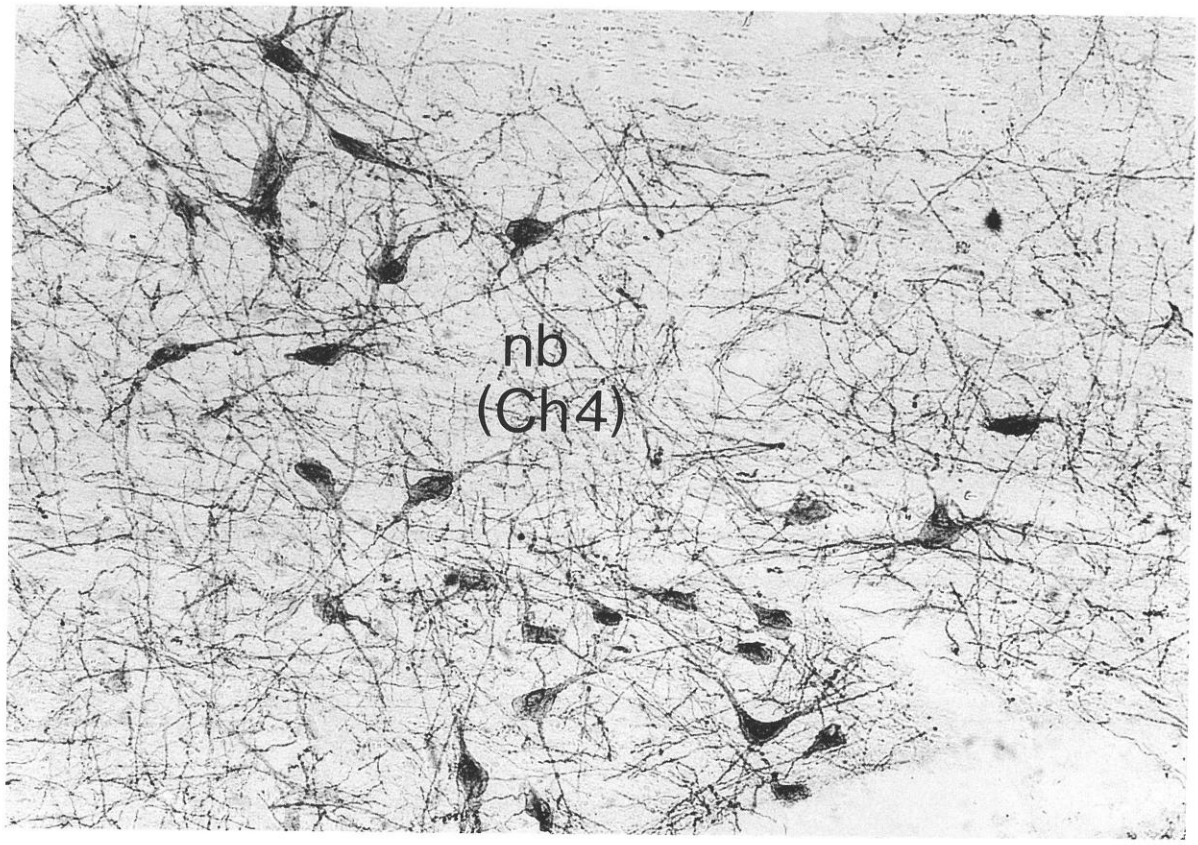

Fig. 13. NGFr immunoreactivity in the nucleus basalis (nb). Note the strongly positive reaction not only in the perikaryon but also in the dendrites and axons. Magnification ×134.

(Graybiel, '78; Sherk, '79a; '79b; Watanabe and Kawama, '79). In keeping with this connectivity pattern, parabigeminal neurons respond to visual stimuli in much the same way as neurons in the superficial retinorecipient layers of the superior colliculus (Sherk, '79a). How the cholinergic projection from the parabigeminal nucleus influences the processing of visual information within the superior colliculus remains to be determined.

Comparison of brainstem to forebrain cholinergic projection neurons

The basal forebrain contains four overlapping groups of cholinergic projection neurons focused around the medial septal nucleus (the Ch1 cell group), the vertical nucleus of the diagonal band (Ch2), the horizontal limb nucleus of the diagonal band (Ch3), and the nucleus basalis of Meynert (Ch4). These four groups provide the major cholinergic input for neocortical and limbic forebrain structures (see Mesulam, '88 for review). The Ch4 cell group, largely coextensive with the magnocellular nucleus basalis of Meynert, is the largest of the forebrain cholinergic constellations (Mesulam and Geula, '88). The suggestion had been advanced that the nucleus basalis might constitute a rostral extension of the brainstem reticular formation, especially of its cholinergic isodendritic core. We therefore made a series of morphological and cytochemical comparisons and observed that while Ch4 and Ch5-6 do share some common features, they also differ in important aspects.

Ramon Moliner and Nauta ('66) proposed a subdivision of brainstem nuclei into two major groups: 1) "closed" nuclei with relatively homogeneous perikaryal morphology and specialized, hodophobic dendrites that remain confined to nuclear boundaries; 2) "open" nuclei with nonspecialized (isodendritic) and overlapping dendritic branches, considerable cytological heterogeneity, and a hodophilic tendency for spread into nearby fiber bundles. This second set of features was considered characteristic of the reticular formation. Our observations with ChAT immunohistochemistry (previously in the basal forebrain (Mesulam and Geula, '88) and now in the brainstem) provide further confirmation for this concept. The oculomotor and especially trochlear nuclei, for example, displayed the characteristics of "closed"

Fig. 12. **A:** ChAT immunohistochemistry. ChAT-positive neurons are concentrated in the nucleus basalis (Ch4). Interstitial Ch4 neurons (single curved arrows) are seen in the internal capsule (ci) and around the anterior commissure (ac). Note the ChAT-positive neurons (double curved arrow) in the putamen (p). Medial is to the right, dorsal to the top. **B:** NGFr immunohistochemistry of a section from the same brain and at a level very close to the one in A. Note the concentration of immunoreactive neurons in the nucleus basalis (nb-Ch4). There are also interstitial immunoreactive neurons (single arrows) in the internal capsule (ci) and surrounding the anterior commissure (ac). The putamen (p) does not contain NGFr immunoreactive neurons. These two photomicrographs indicate that cholinergic neurons of Ch4 but not those of the striatum are NGFr-positive. Magnification ×17.

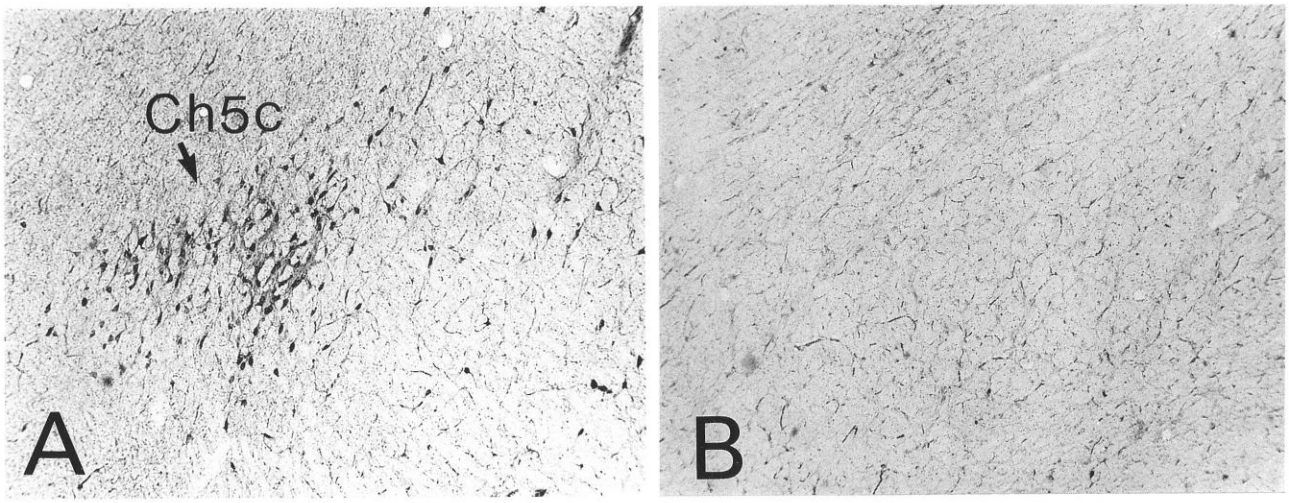

Fig. 14. **A**: ChAT immunohistochemistry demonstrates the intensely immunoreactive perikarya of Ch5c. **B**: Immediately matching section processed for NGFr immunohistochemistry shows an absence of appreciable NGFr staining in the Ch5 neurons. Magnification for A and B ×33.

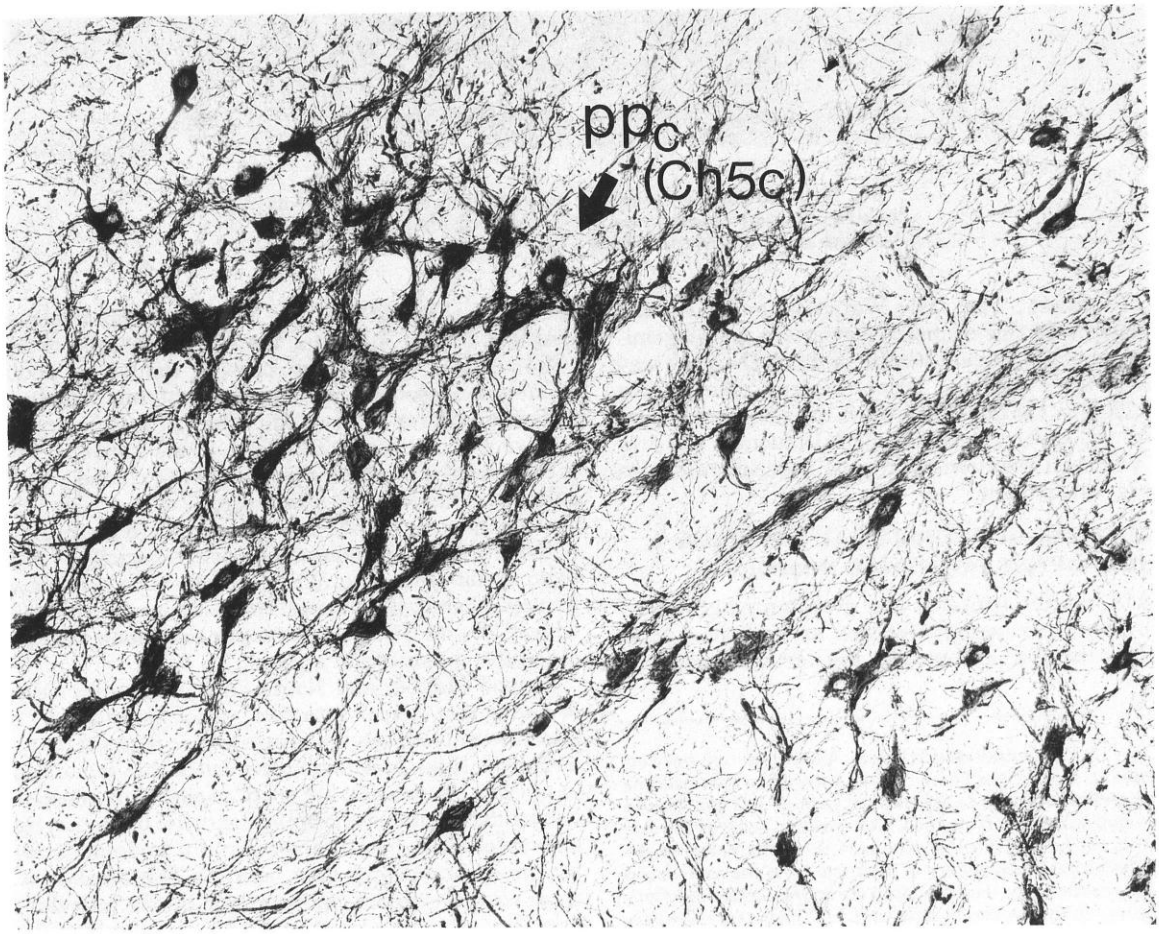

Fig. 15. Immunohistochemistry with an antibody (SMI-32) to non-phosphorylated neurofilament protein. Intense staining of a fibrillar nature is seen in Ch5. Magnification ×163.

TABLE 1. Cytochemical Profiles of Some Cholinergic Neuron Types

	ChAT	AChE	SMI-32	NADPHd	NGFr
Ch4 (nucleus basalis)	+	+	+	–	+
Ch5 (pedunculopontine nucleus)	+	+	+	+	–
Ch6 (laterodorsal tegmental nucleus)	+	+	+	+	–
Ch8 (parabigeminal nucleus)	+	+	–	–	–
Striatum (putamen and caudate)	+	+	–	–	–
Oculomotor nucleus	+	+	+	–	–

nuclei. In contrast, the Ch4, Ch5 and Ch6 groups fit the description of "open" nuclei since their constituent neurons displayed considerable cytological heteromorphism, overlapping and nonspecialized dendritic branching patterns, and a propensity for extending into adjacent fiber bundles in the form of interstitial elements.

Another feature common not only to Ch4, Ch5, and Ch6 but to all cholinergic perikarya (including those of the striatum, cranial nerve nuclei, and parabigeminal nucleus) is a high content of AChE. However, a high perikaryal AChE content is not a specific feature of cholinergic neurons and was also observed in the neurons of the substantia nigra, ventral tegmental area, dorsal raphe, and nucleus locus coeruleus. A third property that Ch4 shares with Ch5 and Ch6 is the high content of non-phosphorylated neurofilament protein, a characteristic not present in the cholinergic cells of the striatum and parabigeminal nuclei. However, this cytochemical feature is not specific for cholinergic nuclei and may be a characteristic common to all large neurons with long projections, including the cholinergic cranial nerve motoneurons (Table 1). Neurons of Ch4 and Ch5-Ch6 display a particularly intense immunoreactivity for the pyruvate dehydrogenase complex, and this constitutes an additional feature shared by both sets of cholinergic neurons (Milner et al., '87).

There were also cytochemical differences that sharply differentiated Ch4 from Ch5-Ch6. For example, Ch5-Ch6 neurons displayed high contents of the mitochondrial enzyme NADPH diaphorase, whereas the cholinergic Ch4 neurons of the nucleus basalis gave a negative reaction. The presence of many NADPHd-rich neurons in the human nucleus basalis had been reported by Ellis et al. ('87), who suggested, on the basis of AChE histochemistry, that these were distinct from the cholinergic perikarya. The concurrent staining with ChAT in our experiments unequivocally confirms this suggestion by demonstrating that NADPHd-rich and ChAT-positive neurons constitute two completely independent populations. The cholinergic neurons of the striatum, cranial nerve nuclei, and parabigeminal nucleus were also lacking in NADPHd. Thus, the NADPHd-rich staining pattern sets Ch5-Ch6 apart from all other cholinergic neurons that we examined. The overlap between NADPHd-rich and ChAT-positive neurons in Ch5-Ch6 and the lack of such overlap in the striatum had been reported in the rat (Vincent et al., '83a; '83b).

A second major difference between the forebrain and brainstem cholinergic neurons occurred in the expression of NGF receptor protein (NGFr). The presence of NGFr in the human Ch4 had been reported by Hefti and colleagues ('87). Our observations confirm this and suggest that most, if not all, ChAT-positive perikaryan in Ch4 are NGFr-positive. In contrast, none of the other cholinergic cells that we examined (i.e. Ch5, Ch6, striatal, cranial nerve, and parabigeminal neurons) expressed NGFr-like immunoreactivity. This further strengthens the notion that cholinergic projection neurons of the forebrain (but not striatal cholinergic interneurons or cholinergic projection neurons of the rostral brainstem) are under the trophic influence of NGF. It is thought that NGF is produced at cortical target regions and that it is retrogradely transported through a specific binding to NGF receptors expressed by the forebrain cholinergic neurons (Hefti et al., '87).

Many of the forebrain and brainstem cholinergic projection neurons also contain neuropeptides. Subsets of Ch5-Ch6 neurons have been shown to contain substance P, atriopeptin, corticotropin releasing factor, and bombesin/gastrin releasing peptide (Standaert et al., '86; Vincent et al., '86). The Ch4 neurons, on the other hand, have been shown to contain galanin and calbindin-d 28 (Celio and Norman '85; Walker et al., '87). The pattern of peptide expression in the forebrain cholinergic neurons has not yet been compared systematically to that of the brainstem cholinergic neurons. Conceivably, peptide content will constitute another set of properties that distinguish Ch4 neurons from those of Ch5-Ch6.

The observations in this report show that the cholinergic neurons of the human rostral brainstem follow a general plan of organization similar to that described in other primates, carnivores, and rodents. On the basis of animal experiments, it may be assumed that these neurons provide the cholinergic innervation of the human thalamus and that they participate in neural networks related to the reticular activating, limbic, and extrapyramidal systems. The comparisons between Ch4 and Ch5-Ch6 show that neuronal sets that employ the same primary transmitter and that have identical morphological features, can display marked variations in overall cytochemical signature. These individual cytochemical patterns can potentially influence the physiological properties of cholinergic neurons as well as their selective vulnerabilities to disease states and other metabolic perturbations.

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