

# Cholinergic Innervation of the Human Striatum, Globus Pallidus, Subthalamic Nucleus, Substantia Nigra, and Red Nucleus

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## ABSTRACT

The anatomical organization of cholinergic markers such as acetylcholinesterase, choline acetyltransferase, and nerve growth factor receptors was investigated in the basal ganglia of the human brain. The distribution of choline acetyltransferase-immunoreactive axons and varicosities and their relationship to regional perikarya showed that the caudate, putamen, nucleus accumbens, olfactory tubercle, globus pallidus, substantia nigra, red nucleus, and subthalamic nucleus of the human brain receive widespread cholinergic innervation. Components of the striatum (i.e., the putamen, caudate, olfactory tubercle, and nucleus accumbens) displayed the highest density of cholinergic varicosities. The next highest density of cholinergic innervation was detected in the red nucleus and subthalamic nucleus. The level of cholinergic innervation was of intermediate density in the globus pallidus and the ventral tegmental area and low in the pars compacta of the substantia nigra.

Immunoreactivity for nerve growth factor receptors (NGFr) was confined to the cholinergic neurons of the basal forebrain and their processes. Axonal immunoreactivity for NGFr was therefore used as a marker for cholinergic projections originating from the basal forebrain (Woolf et al., '89; *Neuroscience* 30:143-152). Although the vast majority of striatal cholinergic innervation was NGFr-negative and, therefore, intrinsic, the striatum also contained NGFr-positive axons, indicating the existence of an additional cholinergic input from the basal forebrain. This basal forebrain cholinergic innervation was more pronounced in the putamen than in the caudate. The distribution of NGFr-positive axons suggested that the basal forebrain may also project to the globus pallidus but probably not to the subthalamic nucleus, substantia nigra, or red nucleus. The great majority of cholinergic innervation to these latter three structures and to parts of the globus pallidus appeared to come from cholinergic neurons outside the basal forebrain, most of which are probably located in the upper brainstem.

These observations indicate that cholinergic neurotransmission originating from multiple sources is likely to play an important role in the diverse motor and behavioral affiliations that have been attributed to the human basal ganglia. © 1992 Wiley-Liss, Inc.

**Key words:** caudate, putamen, motor system, extrapyramidal

Markers of presynaptic cholinergic innervation have been detected within the striatum, globus pallidus, subthalamic nucleus, substantia nigra, and red nucleus of several mammalian species (Henderson and Greenfield, '87; Ikeda et al., '91; Martinez-Murillo et al., '89; Phelps et al., '85; Pickel and Chan, '90; Shute and Lewis, '67; Silver '74). In the human brain, these subcortical components of the

motor system have been associated with axonal acetylcholinesterase (AChE) activity and, in the case of the striatum, with choline acetyltransferase (ChAT)-like immunoreactivity (Graybiel, '90; Lehericy et al., '89; Mesulam and Geula, '88; Mizukawa et al., '86). The purpose of this report is to

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provide additional information on the cholinergic innervation of these structures with the help of whole-brain sections processed for the detection of cholinergic markers such as acetylcholinesterase, choline acetyltransferase, and nerve growth factor receptors (NGFr).

## MATERIALS AND METHODS

Five human brains [from subjects 47 (female), 55 (male), 55 (male), 71 (male), and 91 (male) years old] were obtained at autopsy from individuals with no history of prior chronic neurological disease or dementia. Cause of death was cerebellar hemorrhage in one (91 years old), pneumonia in one (47 years old), and myocardial infarction in the other three. The fixation and cutting procedures have been described elsewhere (Mesulam and Geula, '88, '91). Four of the brains (47, 55, 55, and 71 years old) were cut in whole-brain sections. In order to eliminate the potential contribution of changes due to aging, the principal conclusions on the distribution of ChAT and NGFr were derived from the two 55 year old specimens. Only those observations that were common to both specimens were taken into consideration. The other cases provided confirmatory data and additional cytoarchitectonic guidelines.

Representative series of sections were processed for Nissl substance with cresyl violet, for AChE histochemistry with a modified Koelle-Friedenwald procedure (Mesulam and Geula, '88), and for ChAT (German et al., '85) and NGFr (Marano et al., '87) immunocytochemistry with specific antibodies raised against human placenta and melanoma, respectively. The immunocytochemical reaction product was based on avidin-biotin-horseradish peroxidase (HRP) binding to sites of antigen-antibody complexes, diaminobenzidine (DAB) polymerization by  $H_2O_2$  at sites of HRP activity, and the subsequent intensification of the DAB polymers with silver, as described by Kitt et al. ('88). Controls for AChE histochemistry were obtained by adding the specific AChE inhibitor BW284C51 to the incubation solution. Controls for immunocytochemistry were obtained by carrying out the incubation with an irrelevant IgG instead of the antibody. Additional adsorption controls for ChAT immunocytochemistry were obtained by preincubating the antibody in the presence of purified human ChAT. For these blocking controls, 10  $\mu$ l of purified ChAT (1  $\mu$ g/ $\mu$ l) was added to 5  $\mu$ l of ChAT antibody (2  $\mu$ g/ $\mu$ l) and placed in 100  $\mu$ l of vehicle [phosphate-buffered saline (PBS) containing 10% normal goat serum and 0.5% Triton X-100].

This solution was left for 2 hours at room temperature and overnight at 4°C. The volume was then brought to 5 ml with vehicle, resulting in a final concentration of 2  $\mu$ g/ml of ChAT and a 1/1,000 dilution of ChAT antibody. This solution was used for immunostaining of control sections with ChAT-adsorbed antibody.

## RESULTS

### Acetylcholinesterase and choline acetyltransferase

The AChE histochemical procedure resulted in dense precipitates of reaction product in all the regions of interest (Fig. 1). No reaction product was obtained when the incubation medium contained the specific AChE inhibitor BW 284C51. The striatum, globus pallidus, subthalamic nucleus, substantia nigra, and red nucleus contained both AChE-rich cell bodies and also AChE-rich axonal and dendritic processes.

The intensified ChAT immunohistochemical reaction resulted in a black and granular precipitate. Consistently ChAT-rich perikaryal staining was found in striatal neurons, in the neurons of the Ch1-4 cell groups (i.e., the cholinergic cells centered on the medial septal complex, nuclei of the diagonal band, nucleus basalis of Meynert), the Ch5-6 cell groups (i.e., the cholinergic cells centered on the pedunculopontine and laterodorsal tegmental nuclei), the medial habenula (Ch7), the parabigeminal nucleus (Ch8), and the oculomotor-trochlear nuclei. The distribution of these neurons in the human brain has been described previously (Mesulam and Geula, '88; Mesulam et al., '89). These ChAT-rich neurons were characterized by an extremely dense perikaryal reaction product that extended into dendrites and axons. The reaction product was not obtained when the antibody had been preincubated (adsorbed) in the presence of pure ChAT or when it was substituted by an irrelevant IgG. Inconsistent, lighter staining of a diffuse or punctate nature was observed in some neurons of the cerebral cortex, thalamus, globus pallidus, and subthalamic nucleus. This staining tended to remain unaltered when the antibody had been preincubated in the presence of pure ChAT. We therefore provisionally interpreted the perikaryal staining outside of the striatum, Ch1-8 cell groups, and oculomotor-trochlear nuclei as being nonspecific. The substantia nigra and ventral tegmental area contained dark perikaryal staining,

#### Abbreviations

ac	anterior commissure	iml	internal medullary lamina
al	ansa lenticularis	In	insula
Am	amygdala	md	mediodorsal thalamic nucleus
av	anteroventral segment of globus pallidus	NA	nucleus accumbens
Cd	caudate nucleus	nST	nucleus of the stria terminalis
Ch4	cholinergic cell group centered around nucleus basalis	OT	olfactory tubercle
Ch4a	anterior sector of Ch4, pars compacta	p	posterior sector of Ch4, pars compacta
Ch4ai	anterointermediate sector of Ch4, pars compacta	Pt	putamen
cl	claustrum	r	reticular thalamic nucleus
cp	cerebral peduncle	RN	red nucleus
ec	external capsule	SN	substantia nigra
eml	external medullary lamina	SNC	pars compacta of substantia nigra
GPad	anterodorsal segment of globus pallidus	sp	septal area
GPav	anteroventral segment of globus pallidus	spb	striatopallidal fiber bundles
GPe	external segment of globus pallidus	ST	subthalamic nucleus
GPi	internal segment of globus pallidus	vpl	ventroposterolateral thalamic nucleus
Hy	hypothalamus	Vt	ventral tegmental area
ic	internal capsule	w	white matter fascicles

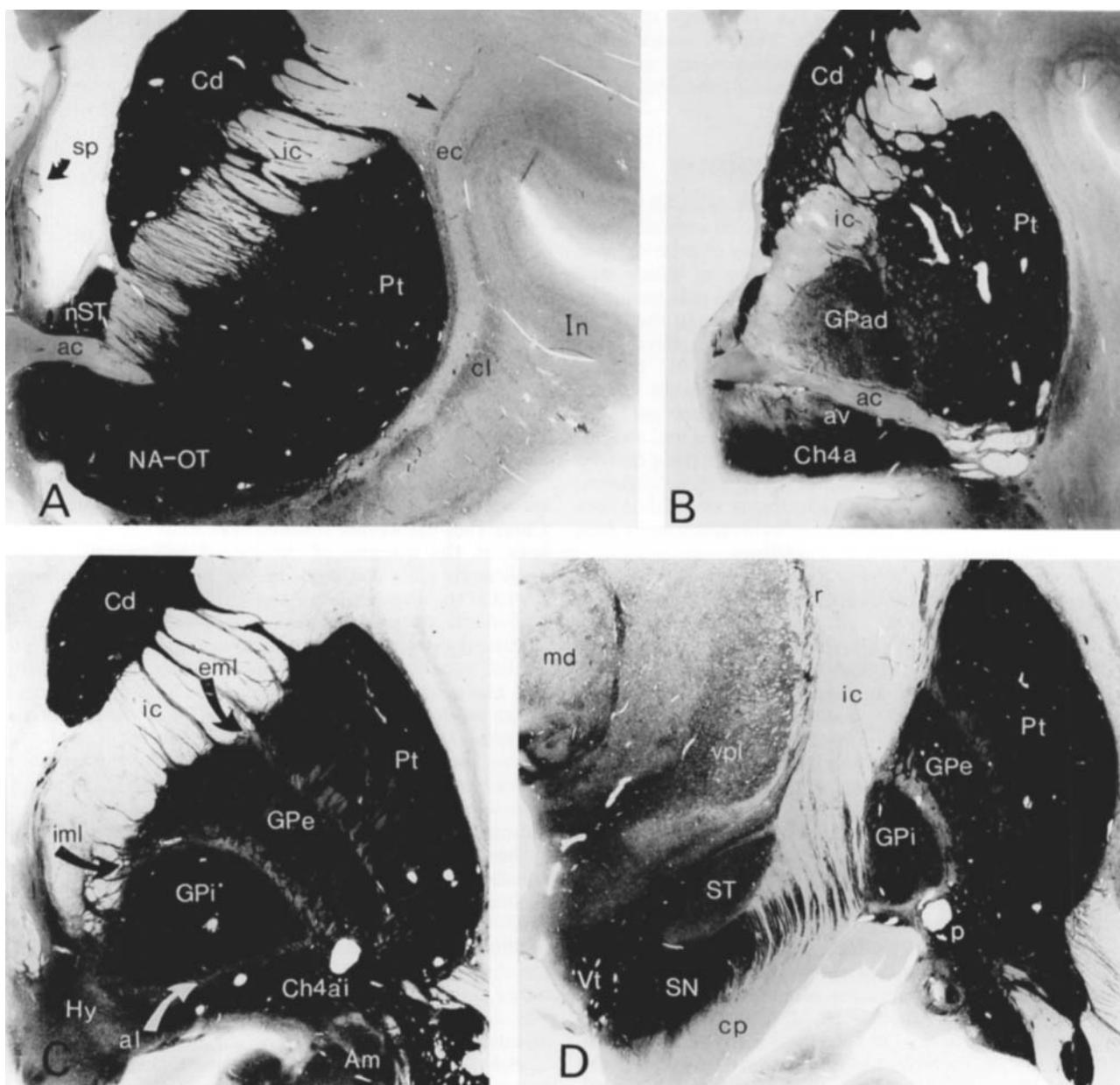


Fig. 1. Acetylcholinesterase (AChE) histochemistry of four coronal sections at progressively more posterior levels of the human cerebral hemisphere. A is most anterior, D most posterior. Medial is to the left, dorsal towards the top. Magnification in all four frames is  $\times 3$ . A: This level contains the three major divisions of the striatum—the putamen (Pt), the caudate (Cd), and the ventral striatum [nucleus accumbens (NA) and olfactory tubercle (OT)]. The high intensity of the AChE reaction product does not allow the visualization of the underlying patch-and-matrix pattern in striatal components. The unlabelled straight arrow points to an AChE-rich axonal fascicle which runs in the external capsule and which represents efferent axons of the Ch4 cell group.

B: The anterior commissure (ac) divides the anterior globus pallidus into anterodorsal (GPad) and anteroventral (av) segments. The anterior sector of the Ch4 cell group (Ch4a) provides a ventral boundary for the globus pallidus. C: The external medullary lamina (eml) separates the putamen (Pt) from the external globus pallidus (GPe) and the internal medullary lamina separates the GPe from the internal globus pallidus (GPi). The ventral boundary for the globus pallidus is provided by the ansa lenticularis (al) and the anterointermediate sector of Ch4 (Ch4ai). D: The substantia nigra (SN), and components of the ventral tegmental area (Vt) display intense AChE activity.

but this was confined to the melanin granules and was not altered by ChAT preincubation and therefore did not seem to reflect ChAT immunoreactivity.

In addition to perikaryal staining, widespread ChAT-positive axonal staining was observed in the cerebral cortex, thalamic nuclei, in all of the subcortical nuclei being studied

for this report, and in some white matter tracts such as the fornix, cingulum, and external capsule. Some of the ChAT-positive axons (especially those running in the hemispheric white matter) were straight and thick, whereas those in gray matter structures displayed multiple varicosities. We assumed that the straight fibers represented predomi-

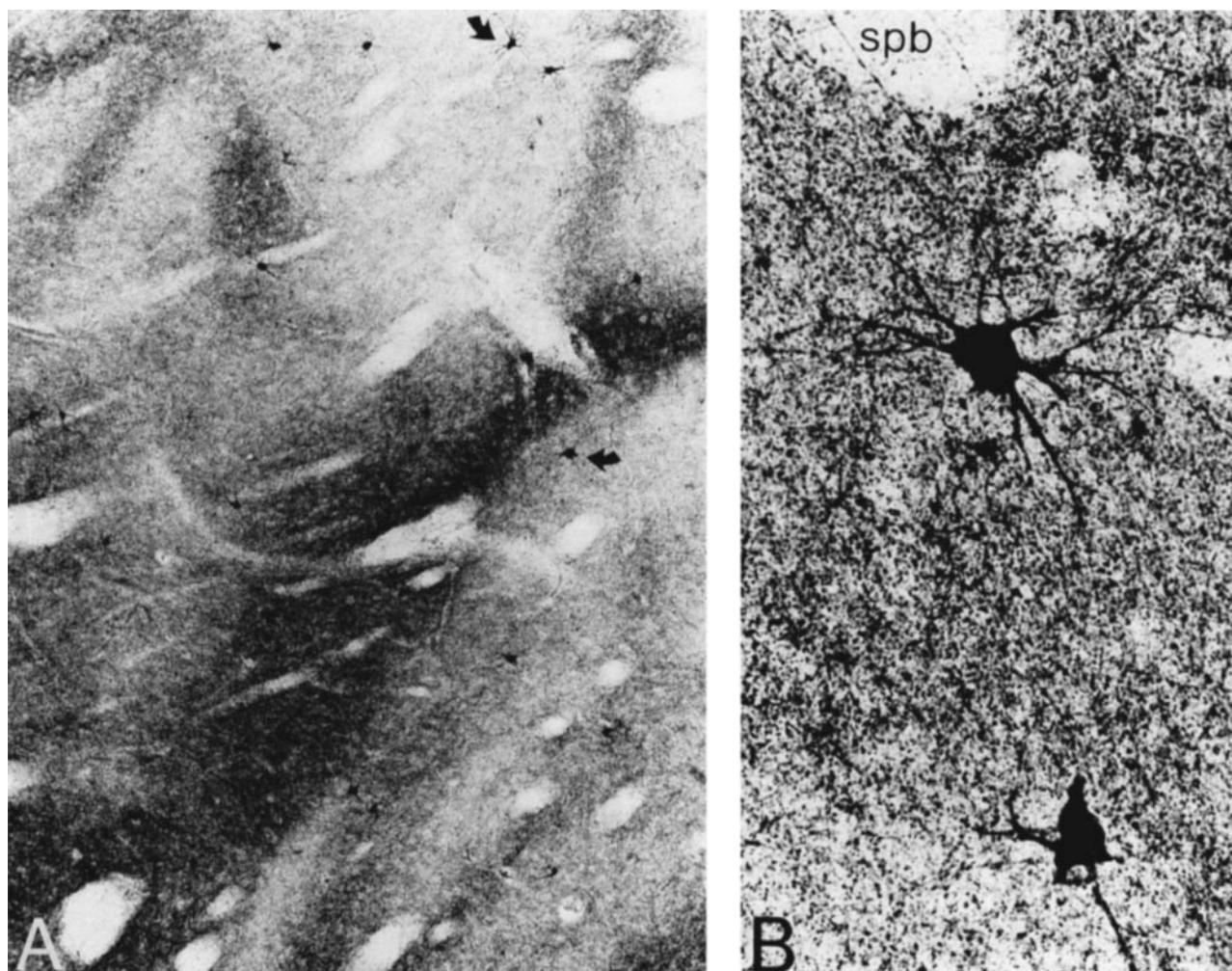


Fig. 2. Choline acetyltransferase (ChAT) immunocytochemistry in the putamen. **A:** Multipolar cholinergic neurons (curved arrows) are interspersed throughout the putamen. The ChAT-positive varicosities display intricate variations of density. The lightest areas correspond to the striatal patches (or striosomes) and the more intensely stained areas to the matrix. There is no obvious relationship between the density of ChAT-positive neurons and the density of neuropil staining. Dorsal towards the top, lateral towards the left. Magnification  $\times 64$ . **B:** Detail of putaminal

ChAT immunopositivity. Two ChAT-positive multipolar neurons are embedded in a dense bed of ChAT-positive preterminal profiles (or varicosities). The striatopallidal fiber bundles (spb) are immunonegative except for a few isolated axons. The bottom edge of the photomicrograph contains part of a striatal patch where the density of ChAT-positive varicosities is relatively low when compared to the matrix. Even in such patches, however, the density of immunopositive varicosities is quite high. Magnification  $\times 343$ .

nantly axons of passage and that the varicosities represented sites of presynaptic specializations involved in cholinergic transmission. Dense sheets of such ChAT-rich varicosities were interpreted to indicate the existence of axonal preterminal fields. In fact, electron microscopic investigations in the rat show that ChAT-positive punctate varicosities identified by light microscopy correspond to cholinergic preterminal axons and presynaptic boutons (Phelps et al., '85). On occasion, a ChAT-rich axon yielded a corkscrew profile or an extremely complex dense cluster containing multiple rosettes and preterminal arborizations.

The ChAT-rich axons, and varicosities were not seen in immunohistochemical preparations in which the ChAT antibody had been preincubated in the presence of pure ChAT or when it had been replaced by an irrelevant IgG. The distribution of ChAT-rich axons in the human cerebral cortex and thalamus has been described (Brandel et al., '90;

Mesulam et al., '92; Heckers et al., in press). This report will focus on the distribution of these cholinergic fibers in the striatum, globus pallidus, substantia nigra, subthalamic nucleus, and red nucleus.

**Striatum.** All four major components of the striatal complex, (i.e., the caudate, the putamen, the nucleus accumbens, and the olfactory tubercle) displayed a very high density of ChAT-like immunostaining (Fig. 2). Each component contained large numbers of ChAT-rich axonal varicosities that formed a continuous sheet of putative cholinergic terminals interrupted only by bundles of immunonegative striato-pallidal fibers. Regional variations in the density of these cholinergic terminals resulted in a mosaic of lightly stained patches (or striosomes) embedded within a more intensely staining matrix (Fig. 2A). The density of ChAT-rich terminals was also very high within the lighter staining patches but not as high as in the matrix. The

striatal mosaic of ChAT-rich terminals was extremely complex and there were further variations of density among the patches and within different regions of the matrix. The patch and matrix organization of ChAT varicosities was analogous to that previously described on the basis of AChE histochemistry (Graybiel, '90).

All sectors of the striatum contained ChAT-positive perikarya with a relatively uniform multipolar appearance (Fig. 2). The dendrites and, whenever they could be identified, the axons of these neurons were also ChAT-positive. We were unable to find consistent differences in the density of ChAT-positive striatal perikarya when the patches were compared to the matrix. The ChAT-positive striatal perikarya were embedded in a sea of dense ChAT-positive terminals. No major differences in the distribution of ChAT-positive terminals and perikarya were noted among the four components of the striatum.

**Globus pallidus.** Sections stained for AChE histochemistry helped to delineate the four major components of the human globus pallidus (Fig. 1). Anteriorly, the globus pallidus is divided by the anterior commissure into anterodorsal and anteroventral segments. More caudally, the globus pallidus is divided by the internal medullary lamina into external and internal segments. The globus pallidus was surrounded by the ChAT-positive neurons of the nucleus basalis (Ch4 pars compacta) and by the interstitial components of the Ch4 complex embedded within the medullary laminae of the globus pallidus, the internal capsule, the anterior commissure, the ansa peduncularis, and the ansa lenticularis (Fig. 3). Rarely, a ChAT-rich cell body with isodendritic features characteristic of the Ch4 cell group could be seen within the traditional boundaries of the globus pallidus. These neurons were considered as ectopic components of the Ch4 complex.

The anteroventral and anterodorsal components of the globus pallidus contained a dense matrix of ChAT-rich neurites (Fig. 4A,B). Some of these were nonvaricose and thick and appeared to constitute dendrites of interstitial Ch4 neurons whose perikarya were embedded within the anterior commissure or the medullary lamina of the globus pallidus. Other straight neurites seemed to represent axons of passage. However, there were also numerous varicose axons, some of which were very fine. Concentrations of these varicosities gave the appearance of terminal cholinergic fields of medium density within the anterior globus pallidus.

The ChAT-rich axonal staining in the more caudally situated external and internal sectors of the globus pallidus was somewhat less intense but also displayed a more differentiated pattern of distribution (Fig. 4C,D). In these more caudal sectors, the ChAT-rich varicose axons displayed a relatively heavier density along the boundaries of the external globus pallidus and along a crescent that lined the medial boundary of the internal globus pallidus (Fig. 3). The neuropil staining displayed ChAT-positive straight

fibers, corkscrew profiles, dense clusters, and also distinct collections of finely varicose axons (Figs. 4, 5). The varicosities were concentrated within the pallidal patches containing neuronal clusters. The intrapallidal white matter bundles (probably representing the striato-pallido-nigral bundles) were generally free of ChAT staining except for a few isolated axons (Fig. 4C,D).

The ChAT-rich varicose axons displayed an intimate relationship to pallidal neurons, especially within the external globus pallidus (Fig. 5). An individual ChAT-rich axon or a small fascicle of such axons could be seen to approach the soma and then wrap itself around the elongated dendrite (Fig. 5A-C). Varicosities of these axons seemed to make contact with both the cell body and dendrites. The globus pallidus also contained ChAT-rich dense clusters, sporadically distributed among pallidal neurons (Fig. 5D).

**Substantia nigra.** Thick ChAT-rich axons entered the substantia nigra mostly from its dorsal aspect and invested the pars compacta with medium and thin axons bearing multiple varicosities (Fig. 6A). Occasionally, a varicosity seemed to make contact with the cell body of a melanin-containing substantia nigra neuron (Fig. 7A). The density of ChAT-rich axons was relatively low in the middle and lateral parts of the pars compacta.

The density of ChAT-rich varicosities became much higher in the more medial part of the substantia nigra, in the region that corresponds to the ventral tegmental area of Tsai (the A10 dopaminergic cell group). The cells in the ventral tegmental area were somewhat smaller than those of the more lateral substantia nigra and were embedded in a moderately dense matrix of ChAT-rich varicosities (Fig. 7B).

**Subthalamic nucleus.** ChAT-rich thick and straight axons entered the subthalamic nucleus from its capsule, mostly dorsally, and then invested the subthalamic nucleus with a very dense matrix of ChAT-rich axons some of which were straight but the majority of which bore multiple varicosities (Figs. 6B, 7C). Complex dense clusters were also identified sporadically within the subthalamic nucleus. Under high power, very finely varicose axons seemed to encircle subthalamic neurons, giving the appearance of a honeycomb pattern.

**Red nucleus.** The red nucleus contained a matrix of ChAT-rich axons at a density similar to that of the subthalamic nucleus. The overall density in the red nucleus and subthalamic nucleus was higher than in all the other structures described above except for the striatum. Fascicles of ChAT-rich axons, many of which displayed varicosities, ran in a somewhat dorsoventral direction, displaying a much heavier density over the cell islands of the red nucleus than over the intercalated white matter bundles that probably represent the fibers of the superior cerebellar peduncle (Figs. 6C, 7D). As in the subthalamic nucleus, the red nucleus also contained complex dense clusters of ChAT-rich axons. No obvious difference in cholinergic innervation

Fig. 3. Choline acetyltransferase (ChAT) immunohistochemistry. The silver intensification of the ChAT reaction product has been carried to a point at which the density variations that differentiate the putaminal (Pt) patches from the matrix are no longer discernible. The striatopallidal fiber bundles (spb) are largely immunonegative. The external (GPe) and internal (GPi) sectors of the globus pallidus are surrounded by the ChAT-positive neurons of the nucleus basalis (Ch4) and by interstitial Ch4 neurons in the internal capsule (ic), external

medullary lamina (eml), and internal medullary lamina (iml). Unlabelled straight arrows point to the ChAT-positive cell bodies, dendrites, and axons in the internal capsule and medullary laminae. The neuropil of the globus pallidus contains many ChAT-positive axons but at an overall density that is much lower than that of the putamen. The ChAT-positive varicose axons display their greatest densities in the periphery of the GPe and along a medial crescent in GPi (open arrows). Magnification  $\times 16$ .

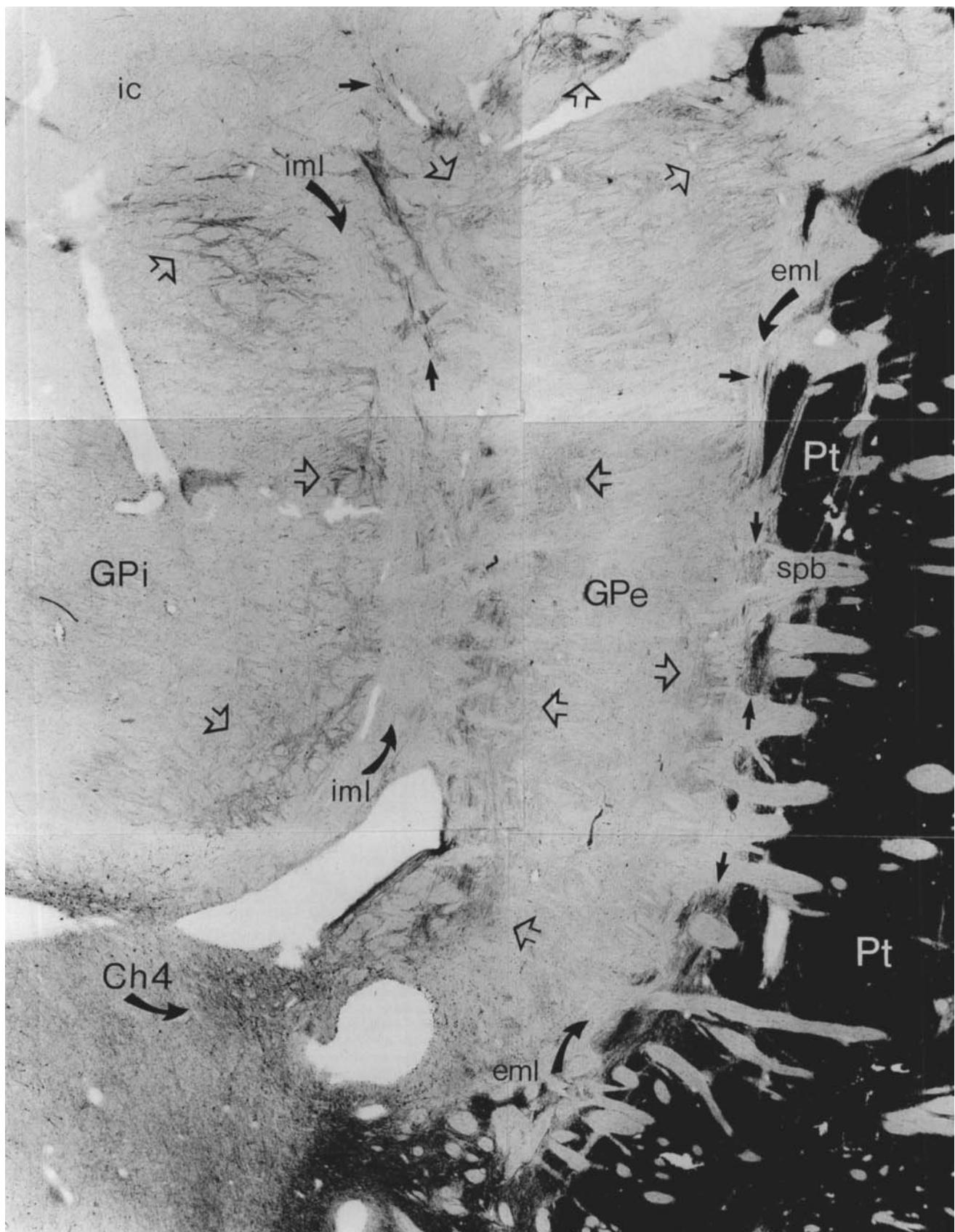


Figure 3

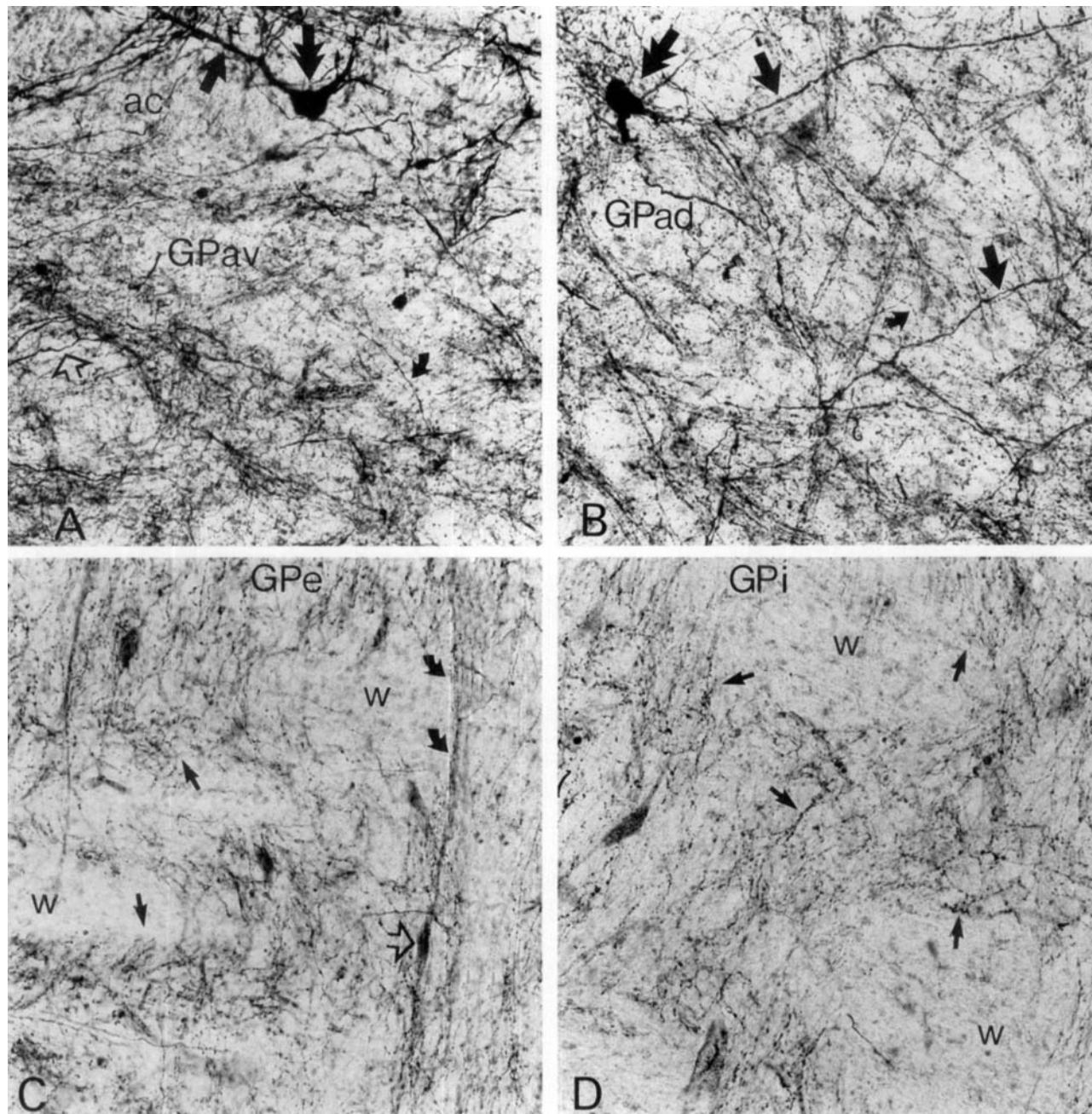


Fig. 4. Choline acetyltransferase (ChAT) immunohistochemistry in the globus pallidus. Dorsal is towards the top, lateral to the left. Magnification  $\times 262$ . **A:** Anteroventral segment of the globus pallidus (GPaV). A ChAT-positive neuron (double arrow) with immunopositive dendrites (single arrow) and axon is seen dorsally. This is an interstitial Ch4 neuron that is embedded partly in the anterior commissure (ac). The GPaV also contains ChAT-positive varicose axons (curved arrow), thick and relatively straight neurites (open arrow), and a moderate density of fine ChAT-positive punctate varicosities (terminals) in the background. **B:** The anterodorsal segment of the globus pallidus (GPad). A ChAT-positive interstitial Ch4 neuron (double arrow) that is partly embedded in the medullary lamina of the globus pallidus extends its dendrites (single arrow on the top) into the GPad. Other ChAT-

positive thick neurites also represent dendrites of Ch4 neurons (single arrow on the bottom). ChAT-positive varicose axons (curved arrow) and a background of fine ChAT-positive varicosities are also seen in the GPad. **C:** The external segment of the globus pallidus (GPe). Clusters of finely varicose ChAT-positive axons (straight arrows) are separated by noncholinergic white matter bundles (w) which contain the striatopallidal and pallidothalamic fibers. A small fascicle of ChAT-positive varicose axons (curved arrows) is closely apposed to the dendrite of a nonspecifically stained ChAT-negative pallidal neuron (open arrow). **D:** The internal segment of the globus pallidus (GPI) also contains patches of ChAT-positive varicose axons (straight arrows) separated by noncholinergic white matter (w) bundles.

could be discerned when the relatively small magnocellular division of the red nucleus was compared to the parvocellular division.

### Nerve growth factor receptors

As previously reported (Hefti et al., '86; Mesulam et al., '89; Mufson et al., '89), NGFr-like immunoreactivity was identified in the vast majority of the Ch1–4 neurons but not in the neurons of the striatum, the Ch5–8 cell groups, or the oculomotor-trochlear nuclei. In the striatum and in many other regions of the brain, dense and coarse clumps of nonspecific (or possibly glial) NGFr reaction product deposits were also detected. These were easily differentiated from axonal or neuronal staining.

The Ch1–4 neurons displayed not only ChAT and NGFr-positive perikarya but also ChAT- and NGFr-positive axons (Fig. 8A,B). Furthermore, the ChAT-positive white matter bundles that represent Ch1–4 efferents (e.g., parts of the fornix, cingulum, and external capsule) and targets of Ch1–4 efferents (e.g., cerebral cortex, hippocampus) also contained NGFr-positive axons. In the hippocampal formation, for example, the pattern displayed by ChAT-rich varicose axons was nearly identical to that displayed by NGFr-rich varicose axons (Fig. 8C,D). With some exceptions described elsewhere (Heckers et al., *in press*), the dense ChAT-positive thalamic projection fields of the Ch5–6 cell groups did not display NGFr-like immunoreactivity. The perikaryal and axonal NGFr staining was not obtained when the antibody was substituted by an irrelevant IgG.

In NGFr-immunostained sections, the putamen was flanked laterally by a thick bundle of nonvaricose NGFr-positive axons that ran in the external capsule (Fig. 9A). These axons had displayed intense staining in sections processed for AChE (Fig. 1A) histochemistry and ChAT immunocytochemistry and represent one of the lateral efferent pathways of the Ch1–4 complex. Rare NGFr-positive neurons were seen embedded within the external capsule. Medially, the putamen was flanked by numerous NGFr-positive axons and some NGFr-positive perikarya embedded within the external medullary lamina. The NGFr-positive perikarya of the external capsule and external medullary lamina represent interstitial elements of the Ch4 complex while the NGFr-positive axons of the external medullary lamina represent another efferent pathway for the cholinergic neurons of the Ch4 complex.

NGFr-positive neurites entered the putamen laterally from the external capsule, medially from the external medullary lamina, and also ventrally from the compact sector of Ch4 located in the nucleus basalis. Some of these neurites coursed within the fascicles containing the otherwise NGFr-negative striato-pallidal bundles. Some of the thick NGFr-positive, nonvaricose neurites in the putamen may have represented dendrites of the NGFr-positive Ch4 neurons, and others may have represented axons of passage. However, there were also many thin varicose axons that seemed to provide sites of presynaptic specialization (Fig. 10A). Some of these varicosities appeared to contact putaminal perikarya (Fig. 10B).

The varicose NGFr-positive axons were most numerous in the lateral and especially the lateroventral part of the putamen. In contrast to the putamen, the caudate nucleus contained only rare NGFr-positive axonal profiles (Fig. 9). The density of NGFr-positive axons was of intermediate magnitude in ventral striatal components such as the nucleus accumbens and olfactory tubercle.

The anteroventral and anterodorsal sectors of the globus pallidus contained dendrites of NGFr-positive Ch4 neurons and also NGFr-positive axons, only a few of which displayed varicosities. Few NGFr-positive straight and varicose axons were also seen in the external and internal sectors of the globus pallidus. The density of NGFr-positive varicosities in the external globus pallidus was lower than in the putamen and the anterior globus pallidus but higher than in the internal globus pallidus (Fig. 10C,D).

In the subthalamic nucleus, substantia nigra, and red nucleus NGFr-like axonal staining was distinctly rare. Occasionally, a solitary axon, almost always without varicosities, could be encountered running through one of these structures.

### DISCUSSION

The distribution of ChAT-positive varicose axons indicated that the human striatum, globus pallidus, subthalamic nucleus, red nucleus, and substantia nigra receive substantial cholinergic innervation. The density of this cholinergic innervation was very high in the striatum, high in the subthalamic nucleus and red nucleus, moderate in the globus pallidus and ventral tegmental area, and low in the pars compacta of the substantia nigra. This cholinergic innervation displayed a very orderly but also complex organization within each of these subcortical structures.

Immunoreactivity for NGFr was detected in the cholinergic cells and axons of the basal forebrain (Ch1–4) but not in those of the upper brainstem (Ch5–8) or striatum. Non-cholinergic sources of telencephalic NGFr may exist in the rodent (Pioro and Cuello, '90) but have not yet been identified in the human brain, except during the advanced senium (Mufson and Kordower, '92), at an age considerably beyond that of our specimens. It is therefore reasonable to assume that in the adult human brain ChAT-positive varicosities represent cholinergic input from all sources whereas NGFr-immunoreactive varicosities are likely to represent cholinergic innervation emanating predominantly, if not exclusively, from the Ch1–4 cell groups.

Experiments based on axonally transported tracers have shown that the vast majority of striatal cholinergic innervation is intrinsic and that it originates from ChAT-positive (but NGFr-negative) striatal neurons (Woolf and Butcher, '81). Our observation that the great majority of ChAT-positive striatal terminals were NGFr-negative suggests that a similar organization is likely to exist in the human brain. However, the presence of NGFr-positive varicose axons, albeit at a much lower density than that of ChAT-positive varicosities, showed that the human striatum also receives an extrinsic cholinergic innervation from the Ch1–4 cell groups, probably arising mostly from the nucleus basalis of Meynert (i.e., Ch4, pars compacta). This is consistent with observations in the monkey, in which HRP injections into the caudate have resulted in retrograde labeling within the nucleus basalis (Arikuni and Kubota, '84).

The overall cholinergic innervation of the four striatal components was of comparable intensity and each component showed an equally complex mosaic of ChAT-positive varicosities, organized in the form of light and dark patches. The NGFr-positive cholinergic projection from the basal forebrain, however, was not uniformly distributed and was considerably more intense in the putamen than in the caudate. Tracer experiments in the squirrel monkey raise the possibility that the striatum may receive yet a third cholinergic input from the Ch5–6 cell groups of the brain-

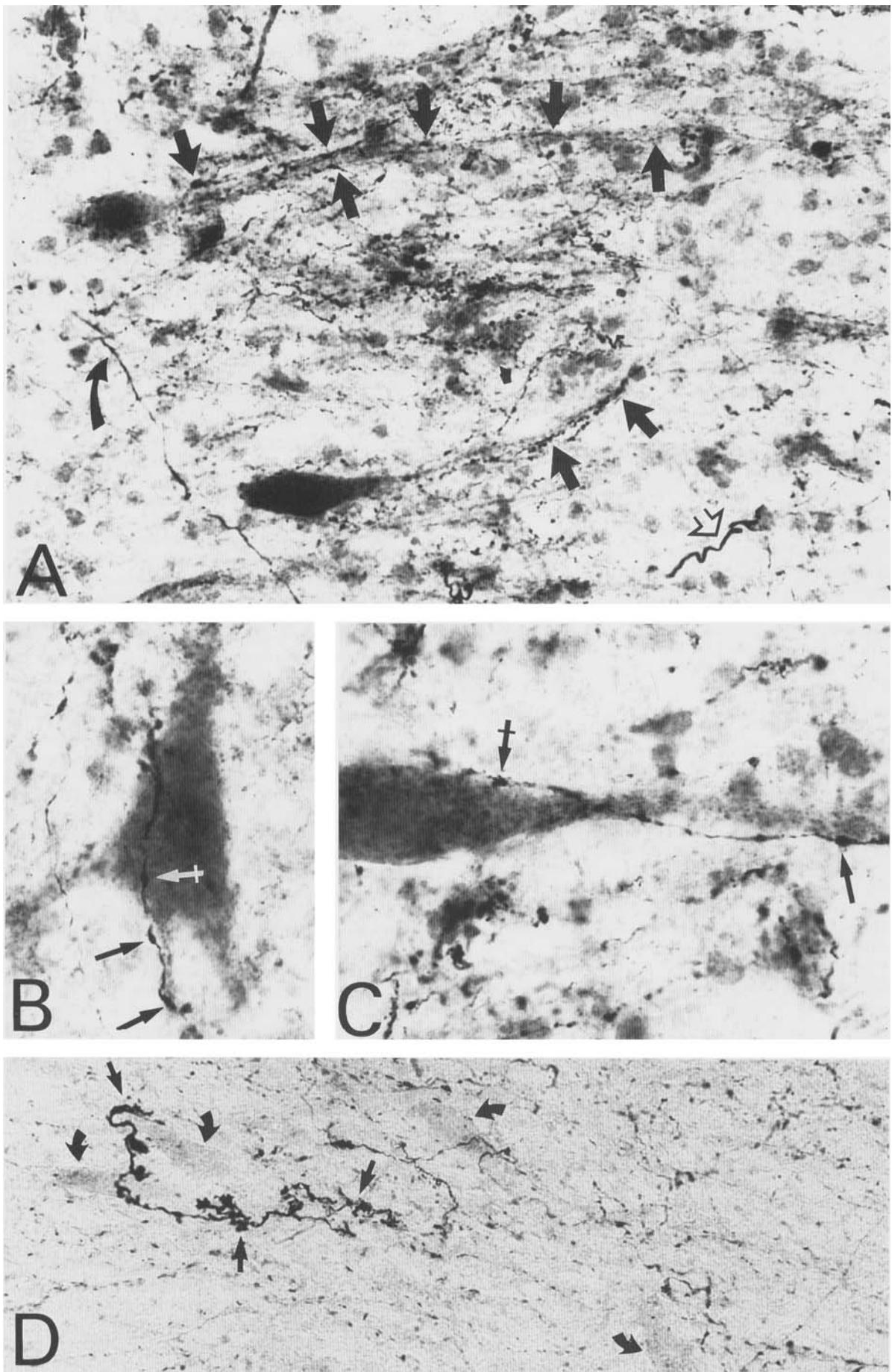


Figure 5

stem and that this projection may be more intense in the caudate than in the putamen (Smith and Parent, '86). Such a projection may also exist in the human brain, adding yet another dimension of complexity to the already immensely intricate neurochemical organization of the striatum.

Although the individual components of the striatum have a nearly identical cytoarchitectonic appearance in Nissl preparations, recent observations have shown that the primate caudate and putamen display substantial differences in the density of somatostatin- and calbindin-containing perikarya and also in the density of substance P and leucine-enkephalin (Martin et al., '91; Selden et al., '90). The differential distribution of cholinergic input from the basal forebrain provides an additional neurochemical feature that differentiates the putamen from the caudate.

The intimate spatial relationship of ChAT-positive varicosities with pallidal perikarya indicated that the globus pallidus receives a cholinergic input. The density of this input was modest in comparison to that of the striatum. The rarity of ChAT-positive axonal staining within the striato-pallidal bundles eliminated the striatum as a major source of this input. The presence of some NGFr-positive varicosities showed that part of this cholinergic input (especially in the anterior and external pallidal segments) is likely to arise from the Ch1–4 cell groups of the basal forebrain. The majority of the cholinergic innervation for the globus pallidus, especially for its internal sector (which corresponds to the entopeduncular nucleus of nonprimates), however, appears to arise from cholinergic cells outside of the forebrain. Most of this projection is likely to come from the Ch5–6 cell groups of the pedunculopontine and laterodorsal tegmental nuclei. The existence of such a projection from the pedunculopontine nucleus to the globus pallidus and entopeduncular nucleus has been demonstrated experimentally in several species of animals (Gonya-Magee and Anderson, '83; Jackson and Grossman, '83; Saper and Loewy, '82; Spann and Grofova, '89; Woolf and Butcher, '86).

The perikarya of the subthalamic nucleus and red nucleus were embedded within a dense matrix of ChAT-positive varicosities. The paucity of NGFr-like immunoreactivity in these two structures suggests that almost all of this cholinergic innervation arises from sources outside the basal forebrain. A substantial component of this input probably originates in the pedunculopontine (Ch5) and laterodorsal tegmental (Ch6) nuclei of the rostral brainstem. It is important to note, however, that there has been considerable controversy concerning the magnitude of the Ch5–6 projection to the subthalamic nucleus (Canteras et al., '90; Carpenter and Jayaraman, '90; Jackson and Grossman, '83; Lee et al., '88; Moon-Edley and Graybiel, '83; Nomura et al., '80; Rye et al., '87; Saper and Loewy, '82; Scarnati et al., '87; Spann and Grofova, '89; Sugimoto and Hattori, '84; Woolf and Butcher, '81). Even those studies that have raised the strongest objections, however, still report the existence of at least a few retrogradely labeled

ChAT-positive Ch5–6 neurons after tracer injections within the subthalamic nucleus (Rye et al., '87). Conceivably, the subthalamic nucleus and the red nucleus could receive cholinergic input from brainstem sources other than Ch5–6. For example, it has been suggested (but not yet confirmed) that the red nucleus may receive the bulk of its cholinergic input from ChAT-positive neurons of the cerebellum (Ikeda et al., '91).

Our observations also showed that the melanin containing (presumably dopaminergic) neurons of the human substantia nigra receive cholinergic innervation. The absence of axonal NGFr suggests that the vast majority of this input arises from sources outside of the Ch1–4 cell groups. Some experiments in rats suggest that the Ch5–6 cell groups provide the major source of nigral cholinergic input while others provide conflicting evidence (Beninato and Spencer, '87; Jackson and Crossman, '83; Lee et al., '88; Moon-Edley and Graybiel, '83; Rye et al., '87; Saper and Loewy, '82; Spann and Grofova, '89; Sugimoto and Hattori, '84; Tokuno et al., '88; Woolf and Butcher, '86). Within the substantia nigra, we found that the medially situated pigmented neurons of the ventral tegmental area received a more intense cholinergic innervation than the more laterally situated neurons of the pars compacta. Such neurochemical differences may underlie the different behavioral affiliations displayed by these two major groups of dopaminergic neurons (see Beckstead et al., '79, for review). For example, the pars compacta of the substantia nigra plays a major role in extrapyramidal motor control whereas the ventral tegmental area seems to be more closely affiliated with the coordination of motivation and related functions of the limbic system.

Electron microscopic investigations in the striatum of the rat show that the dominant mode of cholinergic neurotransmission occurs through symmetrical synapses upon the medium-sized and noncholinergic [presumably  $\gamma$ -aminobutyric acid (GABA)ergic] projection neurons (Phelps et al., '85; Pickel and Chan, '90). Assuming that symmetrical synapses are mostly inhibitory, such an input on inhibitory GABAergic projections neurons would have a net excitatory effect upon the targets of striatofugal GABAergic pathways such as the substantia nigra and the globus pallidus. Only 2–3% of cholinergic terminals in the striatum were found to make synaptic contact with the cholinergic interneurons (Pickel and Chan, '90). It is not known if the extrinsic cholinergic terminals that arise from the basal forebrain have a synaptic organization that sets them apart from the far more numerous intrinsic terminals. This question can be addressed with the help of ultrastructural observations based on NGFr immunocytochemistry. In the monkey, muscarinic receptor autoradiography shows that the striatum contains considerably more M1 than M2 receptors (Mash et al., '88). Physiological experiments in tissue slices show that muscarinic agonists have complex excitatory and

Fig. 5. Choline acetyltransferase (ChAT) immunohistochemistry in the globus pallidus. **A:** ChAT-positive varicose axons and their varicosities are apposed to the dendrites of pallidal neurons (straight solid arrows). The perikaryal and dendritic staining in the pallidal neurons reflects the neutral red counterstain. The pallidal neuropil also contains ChAT-positive straight axons (curved arrow) and corkscrew neurites (open arrow). Magnification  $\times 570$ . **B and C:** Varicosities of ChAT-

positive axons display potential sites of axosomatic (crossed straight arrows) and axodendritic (straight arrows) contacts with globus pallidus neurons that have been counterstained with neutral red. Magnification  $\times 1,000$ . **D:** ChAT-positive axons form complex dense clusters (straight arrows) displaying prominent varicosities and rosette formations among the immunonegative perikarya (curved arrows) of the globus pallidus. Magnification  $\times 262$ .

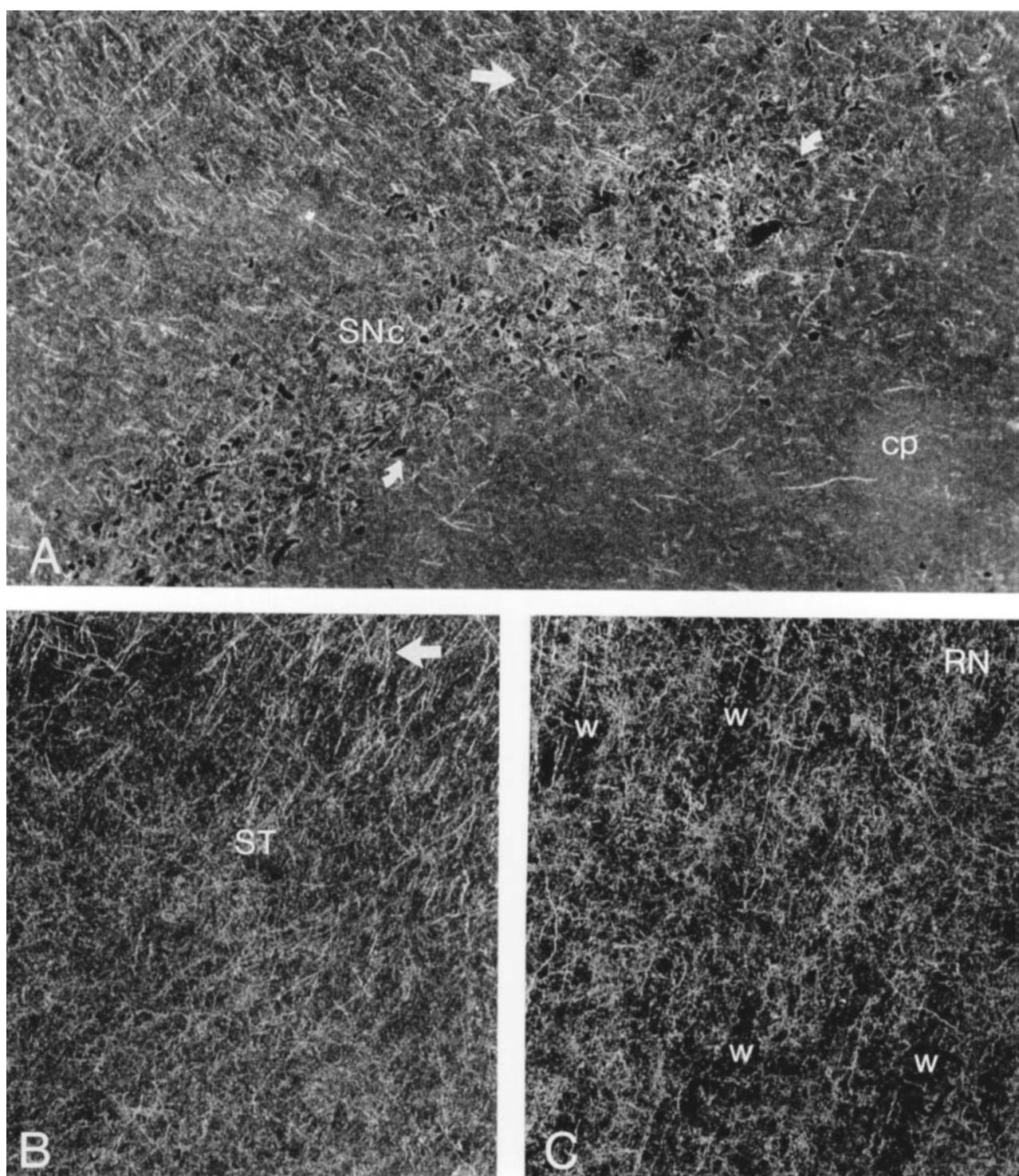
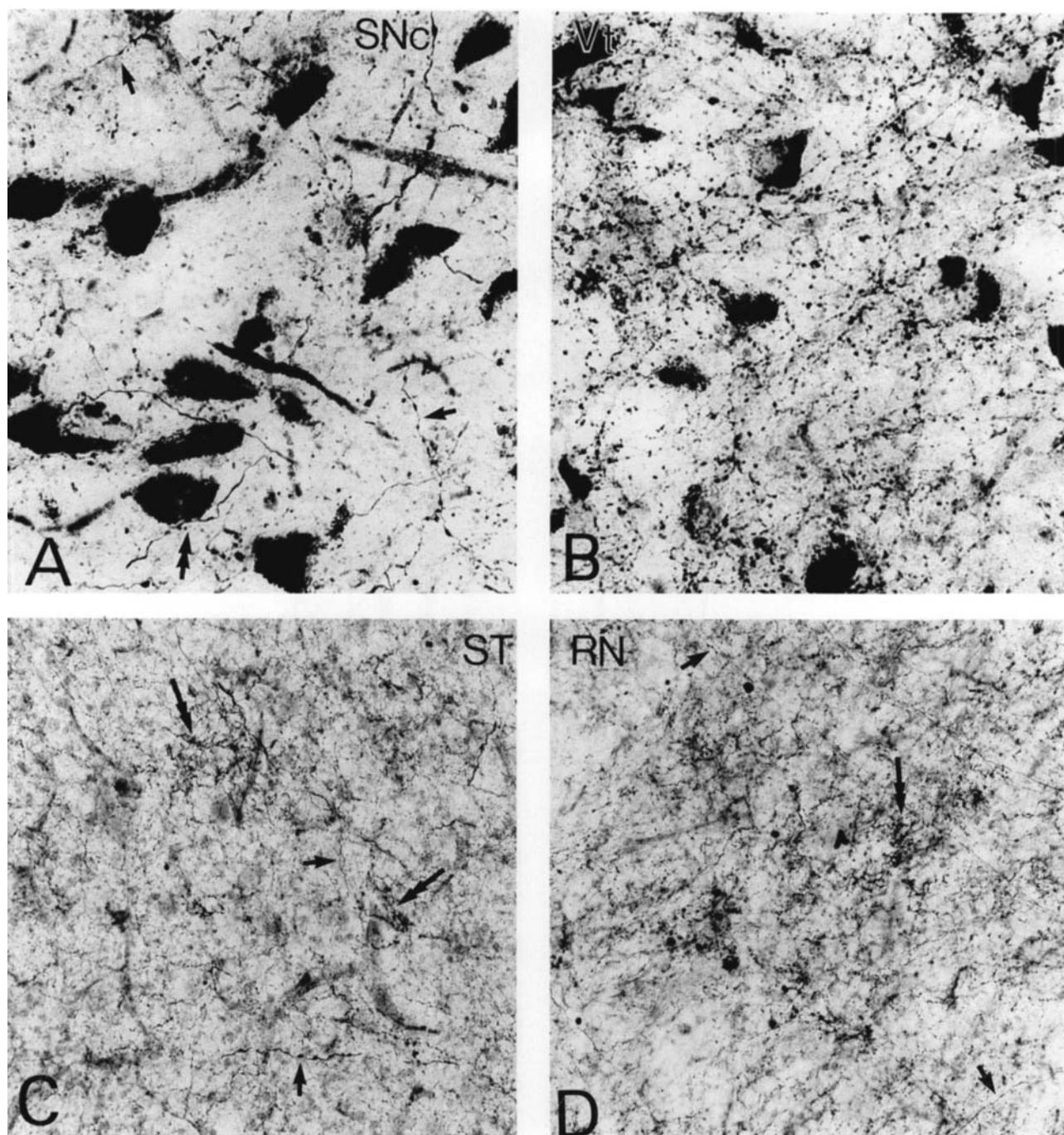


Fig. 6. Darkfield photomicrograph of choline acetyltransferase (ChAT) immunocytochemistry in the substantia nigra, subthalamic nucleus, and red nucleus. Dorsal is towards the top and medial towards the right. **A:** ChAT-positive thick axons (straight arrow) approach the dorsal boundary of the substantia nigra, pars compacta (SNC). The melanin-containing SNC perikarya (curved arrows) are surrounded by ChAT-positive varicose axons and their varicosities. Magnification  $\times 64$ . **B:** Thick ChAT-positive axons (straight arrow) are seen in the dorsome-

dial capsule of the subthalamic nucleus (ST). The nucleus itself displays a dense matrix of ChAT-positive varicose axons and punctate varicosities. Magnification  $\times 140$ . **C:** A dense matrix of ChAT-positive axons and their varicosities are seen over the cellular patches of the red nucleus (RN). The white matter bundles (w), probably representing cerebellar efferents display a much lower density of immunopositive fibers. Magnification  $\times 126$ .



**Fig. 7.** Choline acetyltransferase (ChAT) immunohistochemistry in the substantia nigra, subthalamic nucleus, and red nucleus. **A:** Pars compacta of the substantia nigra (SNC). ChAT-positive varicose axons (straight arrows) course among the pigmented neurons of the SNC. Some varicosities display potential contact sites (double arrow) with SNC cells. Magnification  $\times 480$ . **B:** Photomicrograph from the same tissue section as in A showing the more medially located pigmented neurons in the ventral tegmental area (Vt). The pigmented neurons are surrounded by ChAT-positive axons and varicosities. The density of the

cholinergic innervation is much higher than in the SNC. Magnification  $\times 480$ . **C:** The subthalamic nucleus displays a dense matrix of ChAT-positive varicose axons (straight arrows) and sporadic dense clusters (double arrows). The background contains finely varicose axons surrounding the silhouettes of subthalamic perikarya, giving the appearance of a honeycomb pattern. Magnification  $\times 280$ . **D:** The red nucleus (RN) displays a staining pattern very similar to that of the subthalamic nucleus. There are ChAT-positive varicose axons (single arrows) and dense clusters (double arrow). Magnification  $\times 280$ .

inhibitory effects on striatal neurons (Dodd and Misgeld, '86).

In contrast to the predominance of symmetrical contacts within the striatum, the predominant type of cholinergic

contact in the subthalamic nucleus and substantia nigra is of the asymmetric and presumably excitatory type (Sugimoto and Hattori, '84; Martinez-Murillo et al., '89). In the pars compacta of the substantia nigra and in the ventral

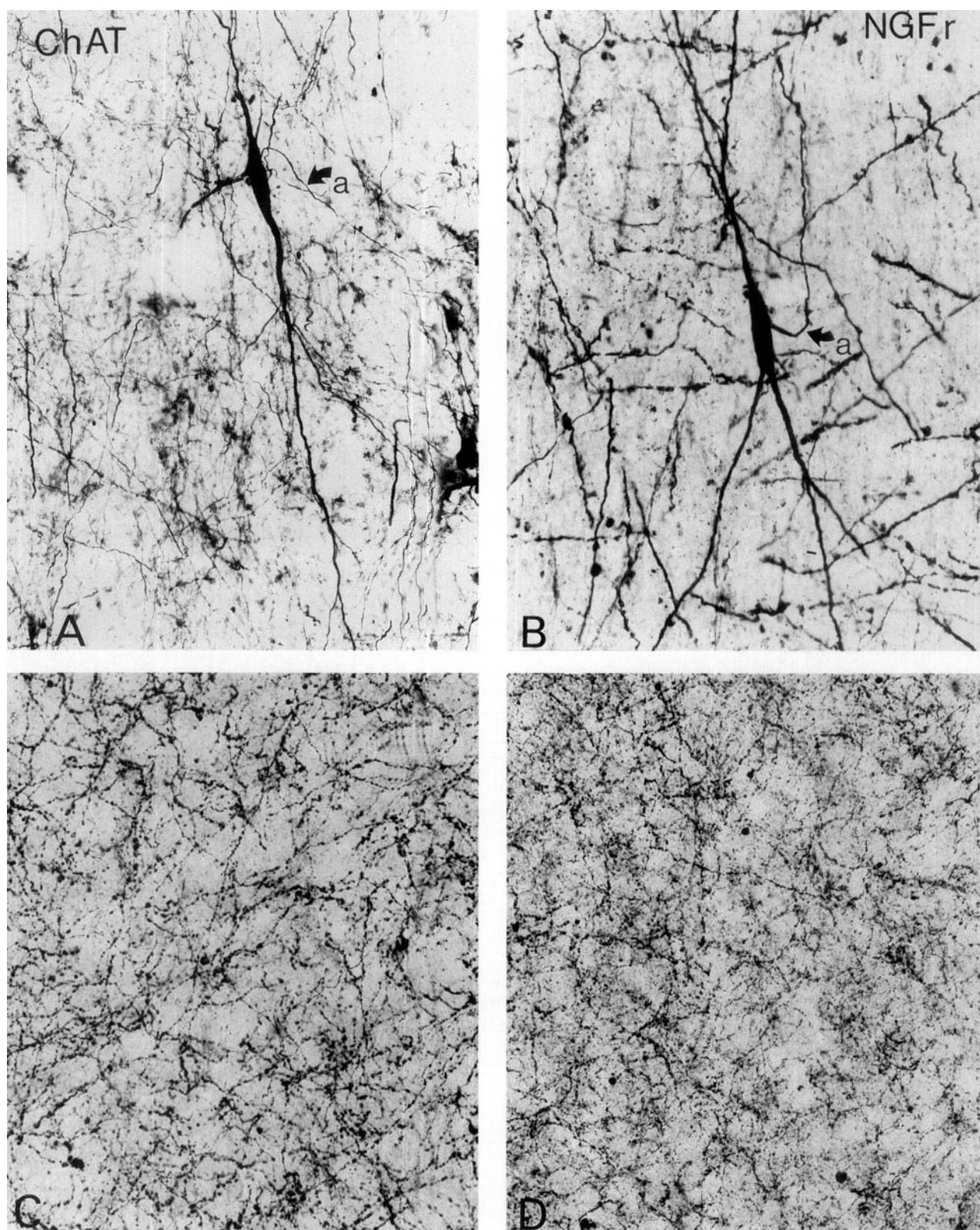


Fig. 8. Comparison of choline acetyltransferase (ChAT) and nerve growth factor receptor (NGFr) immunoreactivity. **A and B:** Ch4 perikarya from the anterior nucleus basalis. A shows that the Ch4 neuron has a ChAT-rich cell body, dendritic tree and axon (a). B shows that Ch4 neurons also have NGFr-rich cell bodies, dendrites, and axon (a). Nearly all Ch1–Ch4 neurons display these characteristics. Magnification  $\times 277$ . **C:** ChAT-positive projection field in the junction of the stratum pyramidale and stratum radiatum in the CA1 sector of the hippocampus. Magnification  $\times 333$ . **D:** Matching section of the same region stained for NGFr-like immunoreactivity shows a comparable distribution of NGFr-positive axons and varicosities. Magnification  $\times 333$ .

cation  $\times 277$ . **C:** ChAT-positive projection field in the junction of the stratum pyramidale and stratum radiatum in the CA1 sector of the hippocampus. Magnification  $\times 333$ . **D:** Matching section of the same region stained for NGFr-like immunoreactivity shows a comparable distribution of NGFr-positive axons and varicosities. Magnification  $\times 333$ .

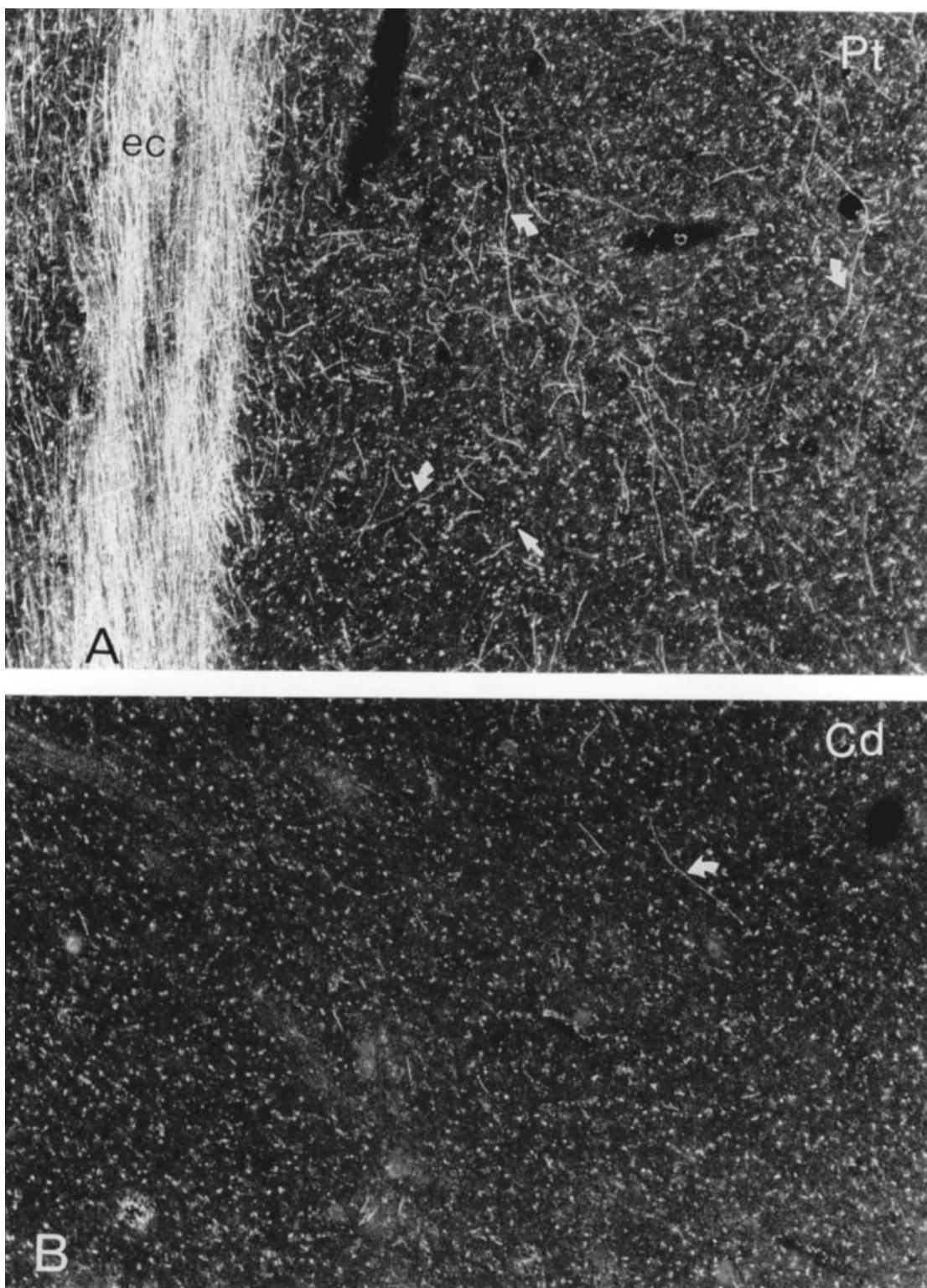


Fig. 9. Nerve growth factor receptor (NGFr) immunocytochemistry and darkfield photomicrography in the putamen (Pt) and caudate nucleus (Cd). Dorsal is towards the top and lateral to the left. Magnification  $\times 80$ . **A:** Thick bundles of NGFr-positive straight axons occupy part of the external capsule (ec). NGFr-positive axons can be seen to extend from the external capsule into the putamen. The

putamen contains numerous NGFr-positive straight and varicose axons (curved arrows). The coarse particulate profiles of reaction product in the neuropil (straight arrow) represent nonspecific deposits. **B:** The caudate nucleus contains a much lower density of NGFr-positive axons (curved arrow) than the putamen.

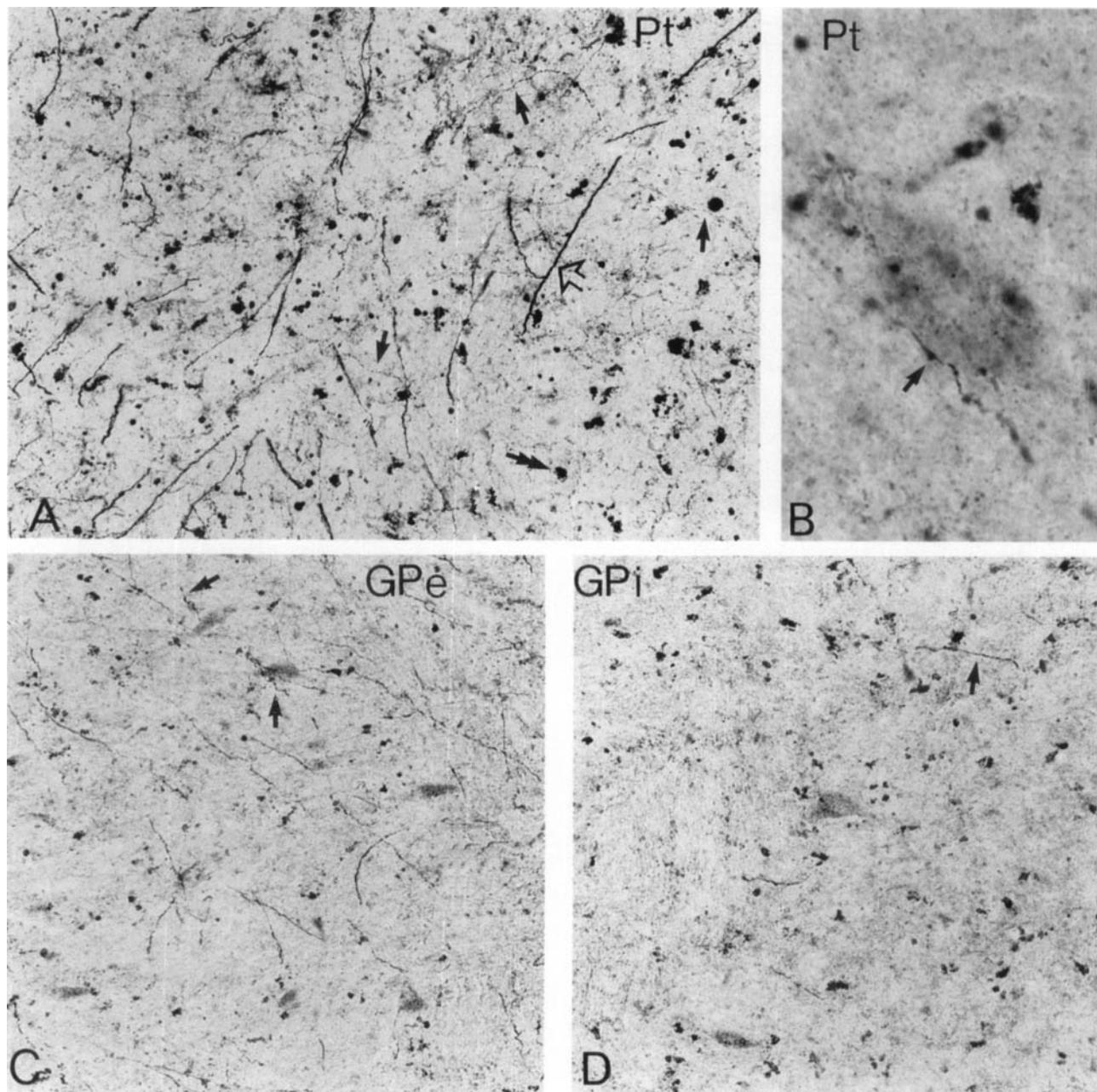


Fig. 10. Nerve growth factor receptor (NGFr) immunohistochemistry in the putamen and globus pallidus. **A:** The putamen (Pt) displays numerous NGFr-positive straight (open arrow) and varicose (straight arrows) axons. The background contains a moderately dense matrix of punctate immunopositive varicosities. Double arrow points to nonspecific depositions of reaction product. Magnification  $\times 240$ . **B:** An NGFr-positive varicosity (single arrow) displays a site of potential

synaptic contact with the silhouette of an immunonegative putaminal neuron. Magnification  $\times 1,250$ . **C:** The external segment of the globus pallidus (GPé) displays a relatively low density of NGFr-positive fibers. Some of these display varicosities (straight arrows). Magnification  $\times 300$ . **D:** The internal sector of the globus pallidus (GPI) displays a lower density of NGFr-positive fibers (straight arrow) than the external sector (GPé). Magnification  $\times 300$ .

tegmental area, cholinergic neurotransmission is mediated predominantly by M1-like receptors and tends to increase the rate of spontaneous action potentials of the dopaminergic neurons (Lacey et al., '90).

The red nucleus provides a pivotal relay for the pyramidal motor system, whereas the striatum, globus pallidus, subthalamic nucleus, and substantia nigra provide the principal constituents of the extrapyramidal motor system. A

very large body of clinical and experimental evidence has revealed the intricate interrelationship among these structures and their relevance to motor function (Alexander and DeLong, '85; DeLong et al., '85; Graybiel, '90). Exactly how the basal ganglia are related to movement remains somewhat mysterious. The suggestion has been made that they may be involved in the selection, readout, and suppression of motor plans (Marsden, '82; Penney and Young, '83).

In the human brain, the motor and premotor cortical areas and their corresponding thalamic nuclei are innervated by numerous cholinergic fibers (Heckers et al., in press; Mesulam et al., '92). The observations in this report show that the subcortical components of the pyramidal and extrapyramidal systems also receive a substantial and highly organized cholinergic input. It appears, therefore, that cholinergic neurotransmission is strategically positioned to influence all stages of motor integration. Clinical evidence shows that cholinergic agents tend to produce tremor whereas anticholinergic agents are quite effective for treating the rigidity and bradykinesia of parkinsonism (Penney and Young, '83). This influence upon motor activity has traditionally been attributed to the well-established cholinergic innervation of the striatum. Our observations show, however, that the globus pallidus, red nucleus, substantia nigra, and subthalamic nucleus may also participate in mediating the effects of cholinergic-active agents upon extrapyramidal function.

The behavioral affiliations of the basal ganglia are not confined to motor function. The cortical connectivity patterns suggest that the striatum may also provide a site of complex associative integration and that it could act as an efference synchronizer for distributed neurocognitive networks (Mesulam, '90; Van Hoesen et al., '81). There is, in fact, considerable support for dividing the striatum into three major spheres of influence: the *putamen*—related mostly to motor function; the *caudate*—related mostly to associative (cognitive) behaviors; and the ventral striatum (*nucleus accumbens* and *olfactory tubercle*)—related mostly to motivational behaviors characteristic of the limbic system (Alexander and DeLong, '85; Apicella et al., '91; Beckstead et al., '79; Künzle, '75; Mesulam, '85; Nishino et al., '81). In keeping with this classification, clinical experience shows that putaminal lesions are generally associated with disturbances of extrapyramidal motor function whereas lesions of the caudate may also lead to complex cognitive and neuropsychiatric disturbances (Caplan et al., '90; Fross et al., '87; Weilburg et al., '89). A parallel organization probably exists in the globus pallidus which may also have motor, associative and limbic components (Heimer and Wilson, '75). Damage to pallidal components, for example, can lead not only to extrapyramidal dysfunction but also to complex deficits of species-specific display behaviors and to severe disturbances of volition and activation (Laplane et al., '82; MacLean, '78). The observations in this report indicate that cholinergic neurotransmission may figure prominently also in the modulation of these cognitive and comportmental affiliations of the human basal ganglia.

According to information based on the distribution of axons with ChAT-like immunoreactivity, we can now definitively conclude that the cholinergic pathways of the human brain are in a position to exert a major influence upon neural activity not only at the cortical and thalamic levels but also at the level of basal ganglia circuitry. At each of these levels, cholinergic innervation displays a baffling diversity with respect to origin, projection density, synaptic specialization, receptor subtype, and physiological effect upon postsynaptic membranes. Additional details concerning these individual characteristics, their distribution, and the ways in which they are interrelated may help to elucidate further the intricate mechanisms through which cholinergic neurotransmission influences the function of nearly all major behavioral systems in the human brain.

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