

Distribution of Choline Acetyltransferase Immunoreactivity in the Pigeon Brain

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ABSTRACT

We have investigated the distribution of cholinergic perikarya and fibers in the brain of the pigeon (*Columba livia*). With this aim, pigeon brain sections were processed immunohistochemically by using an antiserum specific for chicken choline acetyltransferase. Our results show cholinergic neurons in the pigeon basal telencephalon, the hypothalamus, the habenula, the pretectum, the midbrain tectum, the dorsal isthmus, the isthmic tegmentum, and the cranial nerve motor nuclei. Cholinergic fibers were prominent in the dorsal telencephalon, the striatum, the thalamus, the tectum, and the interpeduncular nucleus. Comparison of our results with previous studies in birds suggests some major cholinergic pathways in the avian brain and clarifies the possible origin of the cholinergic innervation of some parts of the avian brain. In addition, comparison of our results in birds with those in other vertebrate species shows that the organization of the cholinergic systems in many regions of the avian brain (such as the basal forebrain, the epithalamus, the isthmus, and the hindbrain) is much like that in reptiles and mammals. In contrast, however, birds appear largely to lack intrinsic cholinergic neurons in the dorsal ("neocortex-like") parts of the telencephalon. © 1994 Wiley-Liss, Inc.

Key words: acetylcholine, basal ganglia, isthmus, motor nuclei, comparative neuroanatomy

During the last decade, the cholinergic systems of the brain have been described in a number of vertebrate groups on the basis of immunohistochemistry for choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine (*rats*: Houser et al., 1983; Tago et al., 1989; *cats*: Kimura et al., 1981; Vincent and Reiner, 1987; *guinea pigs*: Maley et al., 1988; *primates*: Mesulam et al., 1984; Satoh and Fibiger, 1985; *chickens*: Sorenson et al., 1989; *crocodiles*: Brauth et al., 1985; *turtles*: Mufson et al., 1984; Powers and Reiner, 1993; *lizards*: Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; *frogs*: Ciani et al., 1988; *teleosts*: Brantley and Bass, 1988; Ekström, 1987). These studies have shown many similarities in the cholinergic systems of the brains of vertebrates. For example, diverse vertebrate species have now been shown typically to possess cholinergic neurons in the basal telencephalon, the hypothalamus, the habenula, the isthmic region, the reticular formation, and the cranial motor nuclei.

On the basis of their similarity in location, connectivity, and acetylcholine content, the cholinergic cell groups of the habenula, isthmic region, and cranial motor nuclei of the various vertebrate species appear homologous, and their presence appears to represent a primitive condition for at least jawed vertebrates (Medina et al., 1993). Less is known, however, about the connections, topographic organization, and interspecies differences in the cholinergic cell bodies of

the basal forebrain and the hypothalamus. In mammals, there are two main groups of cholinergic cell bodies in the basal telencephalon: 1) intrinsic cholinergic neurons of the striatum, the nucleus accumbens, and the olfactory tubercle; and 2) cholinergic projection neurons of the pallidal areas and the substantia innominata. Both groups of cholinergic neurons play important roles in the brain by modulating the activity of basal ganglia projection neurons or by direct projections to the cortex and the pontomesencephalic reticular formation. Comparable cholinergic cell groups are present in the basal telencephalon of reptiles, amphibians, and fishes, although their connections are still unknown. Data on the cholinergic systems of the brain, and particularly the basal telencephalon, of birds, however, are lacking. For these reasons, we have investigated the distribution of cholinergic perikarya and fibers in the brain of pigeon (*Columba livia*). Our results in the pigeon were compared with those in other vertebrates in order to gain further insight into the comparative neuroanatomy and evolution of the cholinergic systems of vertebrates.

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Abbreviations

ac	anterior commissure	LPO	lobus parolfactorius
Ad	archistriatum dorsale	LS	lateral septum
AL	ansa lenticularis	M	mammillary region
Amb	nucleus ambiguus	MDT	mediodorsal tegmental nucleus
APH	area parahippocampalis	MH	medial habenula
ApR	perirhinalic area	MLd	nucleus mesencephali lateralis, pars dorsalis (inferior colliculus)
Av	archistriatum ventrale	mot	tractus olfactorius medialis
Bas	nucleus basalis of birds; in Figure 27, Bas indicates the nucleus basalis of Meynert of mammals	MS	medial septum
BCS	brachium colliculi superioris	N	neostriatum
Cb	cerebellum	NI	neostriatum intermedium
CbI	nucleus cerebellaris internus	n III-XII	III-XII cranial nerves
CbL	nucleus cerebellaris lateralis	nBOR	nucleus of the basal optic root
CbM	nucleus cerebellaris intermedium	NdB	nucleus of the fasciculus diagonalis Brocae
CDL	dorsolateral corticoid area	Noa	nucleus olfactorius anterior
CE	nucleus cuneatus externus	nST	nucleus of the stria terminalis
CoS	nucleus commissurae septi	OB	olfactory bulb
CPi	cortex piriformis	OC	optic chiasm
Ctx	cortex	OI	nucleus olivaris inferior
CT	commissura tectalis	onl	olfactory nerve layer
CTz	corpus trapezoideum (Papez)	oph	periventricular hypothalamic organ
Cu	nucleus cuneatus	Ov	nucleus ovoidalis
D	nucleus of Darkschewitsch	PA	paleostriatum augmentatum
DLAmc	nucleus dorsolateralis anterior thalami, pars magnocellularis	PAG	periaqueductal gray
DLL	nucleus dorsolateralis anterior thalami, pars lateralis	PaM	nucleus paramedianus
DLM	nucleus dorsolateralis anterior thalami, pars medialis	PB	parabigeminal nucleus
DMA	nucleus dorsomedialis anterior thalami	pc or PC	posterior commissure
DMP	nucleus dorsomedialis posterior thalami	PCpc	nucleus parvocellularis of the posterior commissure
DS	decussatio supraoptica	PCV	processus lateralis cerebello-vestibularis
DVR	dorsal ventricular ridge	Pe	periventricular hypothalamic nucleus
E	ectostriatum	PE	external preoptic nucleus
epl	external plexiform layer	PGL	nucleus paragigantocellularis lateralis
EW	nucleus of Edinger-Westphal	PH	posterior hypothalamic nucleus
FPL	fasciculus prosencephalicus lateralis	PMH	nucleus medialis hypothalami posterioris
FR	reticular formation	PO	preoptic region
FRL	formatio reticularis lateralis mesencephali	POA	nucleus preopticus anterior
fr	fasciculus retroflexus	POM	nucleus preopticus medialis
GC	nuclei gracilis et cuneatus	PP	paleostriatum primitivum
gcl	granule cell layer	PPC	nucleus principalis precommisuralis
GLv	nucleus geniculatus lateralis, pars ventralis	PPN	pedunculopontine tegmental nucleus
gl	glomerular layer	PrV	nucleus sensorius principalis nervi trigemini
GT	nucleus griseum tectalis	Pt	prectectum
GP	globus pallidus	PT	nucleus pretectalis
HA	hyperstriatum accessorium	PTM	nucleus pretectalis medialis
HD	hyperstriatum dorsale	PVM	nucleus periventricularis magnocellularis
HIS	hyperstriatum intercalatum superior	RFa	retrofacial motor nucleus of the nervus glossopharyngeus
HM	nucleus habenularis medialis	Rgc	nucleus reticularis gigantocellularis
Hp	Hippocampus	RL	nucleus reticularis lateralis
HV	hyperstriatum ventrale	Rot	nucleus rotundus
Ico	nucleus intercollicularis	RP	nucleus reticularis pontis caudalis
ID	disseminated isthmic nucleus	Rpc	nucleus reticularis parvocellularis
IHA	intercalated nucleus of the HA	RSv	nucleus reticularis superior, pars ventralis
III-XII	III-XII cranial nerve nuclei	Ru	nucleus ruber
IIId	nucleus nervi oculomotorii, pars dorsalis	SCE	stratum cellulare externum
IIlv	nucleus nervi oculomotorii, pars ventralis	SCI	stratum cellulare internum
Imc	nucleus isthmi, pars magnocellularis	SCN	suprachiasmatic nucleus
Inf	infundibulum	SG	substantia gelatinosa Rolandi (trigemini)
INP	nucleus intrapenduncularis	SL	lateral septal nucleus
IO	nucleus isthmo-opticus	SLu	nucleus semilunaris
IP	nucleus interpenduncularis	SN	substantia nigra
Ipc	nucleus isthmi, pars parvocellularis	SM	supramammillary nucleus
IPS	nucleus interstitio-pretecto-subpretectalis	SMe	stria medullaris
IV	nucleus nervi trochlearis	SP	nucleus subpretectalis
IXm	nucleus motorius nervi glossopharyngei	SPC	nucleus superficialis parvocellularis (nucleus tractus septomesencephalicus)
IX-Xm	nucleus motorius nervi glossopharyngei and nucleus motorius nervi vagi	SpL	nucleus spiriformis lateralis
Jc	nucleus juxtacommissuralis	SpM	nucleus spiriformis medialis
La	nucleus laminaris	SRt	nucleus subrotundus
LA	nucleus lateralis anterior thalami	SS	nucleus superficialis synencephali
LC	nucleus linearis caudalis	SSp	nucleus supraspinalis
LDT	laterodorsal tegmental nucleus	Str	striatum
LH	lateral hypothalamic area	td	dorsal thalamus
LHy	nucleus lateralis hypothalami	TeO	optic tectum
LLi	intermediate nucleus of the lemniscus lateralis	TO	tuberculum olfactarium
LM	nucleus lentiformis	TPO	area temporo-parieto-occipitalis
LoC	locus coeruleus	TrO	optic tract
lot	tractus olfactory lateralis	TTD	tractus descendens nervi trigemini
		TU	nucleus tuberis

Abbreviations (continued)

tv	ventral thalamus	VIII m	nucleus motorius nervi octavi
TV	nucleus tegmenti ventralis (Gudden)	VI m	nucleus motorius nervi abducens
VDL	nucleus vestibularis dorsolateralis (Sanders)	VLT	nucleus ventrolateralis thalami
Ve	vestibular nuclei	Vmd	nucleus motorius dorsalis nervi trigemini
VeD	nucleus vestibularis descendens	Vmv	nucleus motorius ventralis nervi trigemini
VeL	nucleus vestibularis lateralis	VP	ventral paleostriatum or ventral pallidum
VeM	nucleus vestibularis medialis	VS	nucleus vestibularis superior
VH	ventral horn	VTA	ventral tegmental area
VII d	nucleus motorius dorsalis nervi facialis	XII m	nucleus motorius nervi hypoglossi
VII v	nucleus motorius ventralis nervi facialis	Xm	nucleus motorius nervi vagi

MATERIALS AND METHODS

Sixteen adult pigeons of either sex, weighing 420–520 g, were used in the present study. Birds were deeply anesthetized with 0.4 ml 35% chloral hydrate (i.p.) and perfused with 20 ml of a sodium phosphate-buffered solution (0.01 M, pH 7.4) containing 0.75% NaCl and 1,000 units/ml heparin, followed by 400 ml of a sodium phosphate-buffered solution (0.1 M, pH 7.4) containing 4% paraformaldehyde, 1.8% lysine, and 0.2% sodium periodate. Two birds were colchicine treated prior to perfusion using methods that we have described previously (Anderson and Reiner, 1990).

After perfusion, brains were removed and immersed overnight in a sodium phosphate buffer (PB) solution (0.1 M, pH 7.4) containing 20% sucrose, 10% glycerol, and 0.02% sodium azide. The brains were then sectioned on a freezing microtome at 40 μ m in the frontal or sagittal plane, and sections were serially collected in PB containing 0.01% sodium azide. Sections were subsequently processed for ChAT immunohistochemistry by the peroxidase antiperoxidase (PAP) method.

After pretreatment with 0.5% H₂O₂ for 30 minutes and rinsing in PB, sections were incubated under constant, gentle agitation in a rabbit anti-chicken ChAT antiserum (generously provided by Dr. Miles Epstein, C.D. Johnson, and June Dahl, University of Wisconsin, Madison, WI) (Johnson and Epstein, 1986), diluted 1:1,000 in 0.1 M PB (pH 7.4) containing 0.3% Triton X-100 and 0.01% sodium azide for 48–72 hours at 4°C. Sections were next rinsed in 0.1 M PB (pH 7.4) and incubated in a secondary antiserum (donkey anti-rabbit, 1:50, Jackson) overnight at 4°C, and then rinsed and incubated in rabbit PAP (1:200, Sternberger) for 1 hour at room temperature. Secondary antiserum and PAP complex were diluted with the same diluent as the primary antiserum. Sections were then rinsed in PB and the immunolabeling visualized by immersing the sections in 0.1 M PB (pH 7.4) containing 0.05% diaminobenzidine (DAB), 0.04% nickel ammonium sulfate, and 0.01% H₂O₂ for 20 minutes. Finally, sections were rinsed, mounted, dehydrated, and coverslipped.

Several control procedures were carried out to examine labeling specificity. First, some sections were processed without the primary antiserum. Second, other sections were processed with the anti-chicken ChAT blocked with a mixture of chicken proteins similar but not identical to ChAT (chicken proteins kindly provided from M. Epstein, University of Wisconsin). Finally, some sections were processed using a primary antiserum raised in goat and directed against human placental ChAT (obtained from Chemicon International, CA). The monospecificity of this

antiserum for ChAT has been demonstrated previously (Shiromani et al., 1987). The nomenclature of Karten and Hodos (1967) was followed for most cell groups of the pigeon central nervous system (CNS).

RESULTS

Control procedures and general results

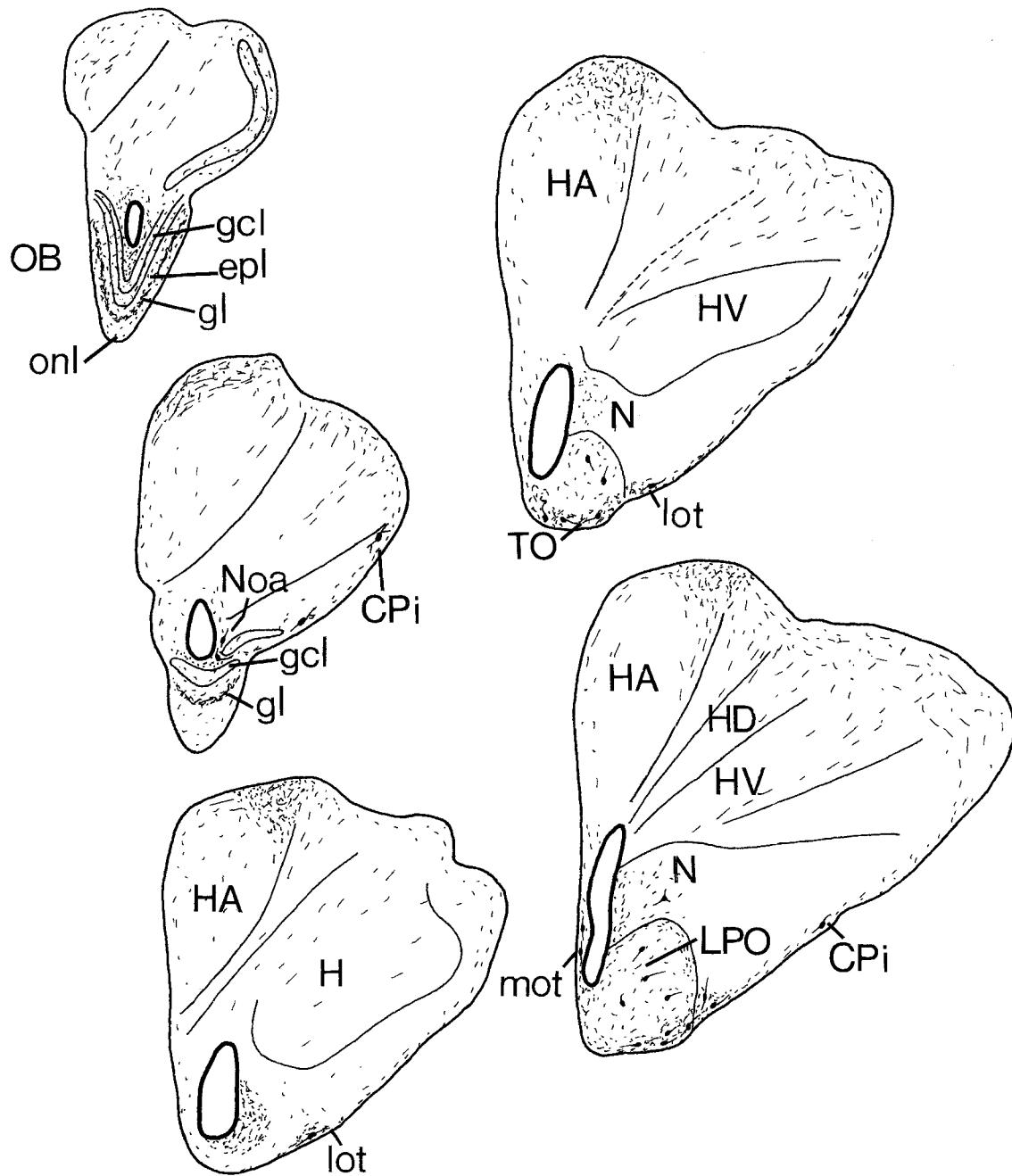
Sections processed without the primary antiserum yielded no labeling, and blocking the anti-chicken ChAT with non-ChAT chicken proteins did not alter the labeling pattern observed with this antiserum. Finally, the anti-human ChAT yielded labeling largely identical to that observed with anti-chicken ChAT. The lateral septal nucleus, the commissural nucleus of the septum, and the ventral thalamic reticular nucleus, however, showed perikaryal labeling with the anti-chicken ChAT, but a decrease or absence of perikaryal labeling with the anti-human ChAT (as discussed further below). The anti-chicken ChAT itself yielded labeling of neurons and fibers throughout the brain and spinal cord of pigeon. The pattern of perikaryal labeling was the same in colchicine-treated and noncolchicine-treated animals. The distribution of ChAT immunoreactivity (ChATi) with this antiserum is depicted in drawings of a rostral to caudal series of transverse sections through the brain of the pigeon *Columba livia* (Figs. 1–9). In the following text, we first describe the distribution of ChATi cell bodies, and then that of ChATi fibers. These descriptions are largely based on the labeling with the anti-chicken ChAT.

Distribution of ChAT-immunoreactive cell bodies

Telencephalon

Dorsal telencephalon (Wulst and DVR). The most rostrally located ChATi cell bodies were observed in the nucleus olfactory anterior (Noa). Further caudally, a few ChATi cell bodies were seen in the cortex piriformis (CPi) of dorsolateral telencephalon, as identified by Reiner and Karten (1985) (Figs. 1, 2, 4, 12B). An extremely small number of immunoreactive neurons were also observed at rostromedial levels of the neostriatum and, more dorsally, in the lamina hyperstriatica (Figs. 1, 12A).

Basal telencephalon. The largest field of ChATi cell bodies of the telencephalon was present in the basal forebrain, including both striatal and pallidal structures. Neurons throughout the basal telencephalon were intensely immunoreactive and possessed extensive dendritic labeling. Dispersed, multipolar or bipolar ChATi neurons



Figs. 1-9. Drawings of a series of transverse sections through the brain of the pigeon *Columba livia*, from rostral (Fig. 1) to caudal (Fig. 9) levels, showing the distribution of choline acetyltransferase immunoreactive (ChATi) cells and fibers.

were observed in the medial part of the dorsal striatum [i.e., lobus parolfactorius (LPO)] at rostral and intermediate levels of the basal ganglia (Figs. 1-3, 10A,B, 11A, 12A). ChATi neurons were more abundant in the lateral LPO than in the medial LPO. The diameter of these ChATi perikarya was 10-12 μm (short diameter) by 18-20 μm (long diameter). In addition, ChATi perikarya were observed in such ventral striatal structures as the olfactory tubercle (TO) and the bed nucleus of the stria terminalis (nST) (Figs. 1-4, 10A, 11A), as well as in the medial and

lateral olfactory tracts (mot, lot) at the brain surface (Figs. 1-5, 10, 11). Large scattered ChATi neurons were observed in the paleostriatum primitivum (PP; the dorsal pallidum in birds; Karten and Dubbeldam, 1973). Large, more clustered ChATi neurons were observed in the intrapeduncular nucleus (INP; considered by some as part of the avian basal ganglia; Karten and Dubbeldam, 1973). ChATi neurons were also observed in the ventral paleostriatum [or ventral pallidum (VP)] of the basal ganglia, within and around the fasciculus prosencephalicus lateralis [or lateral forebrain

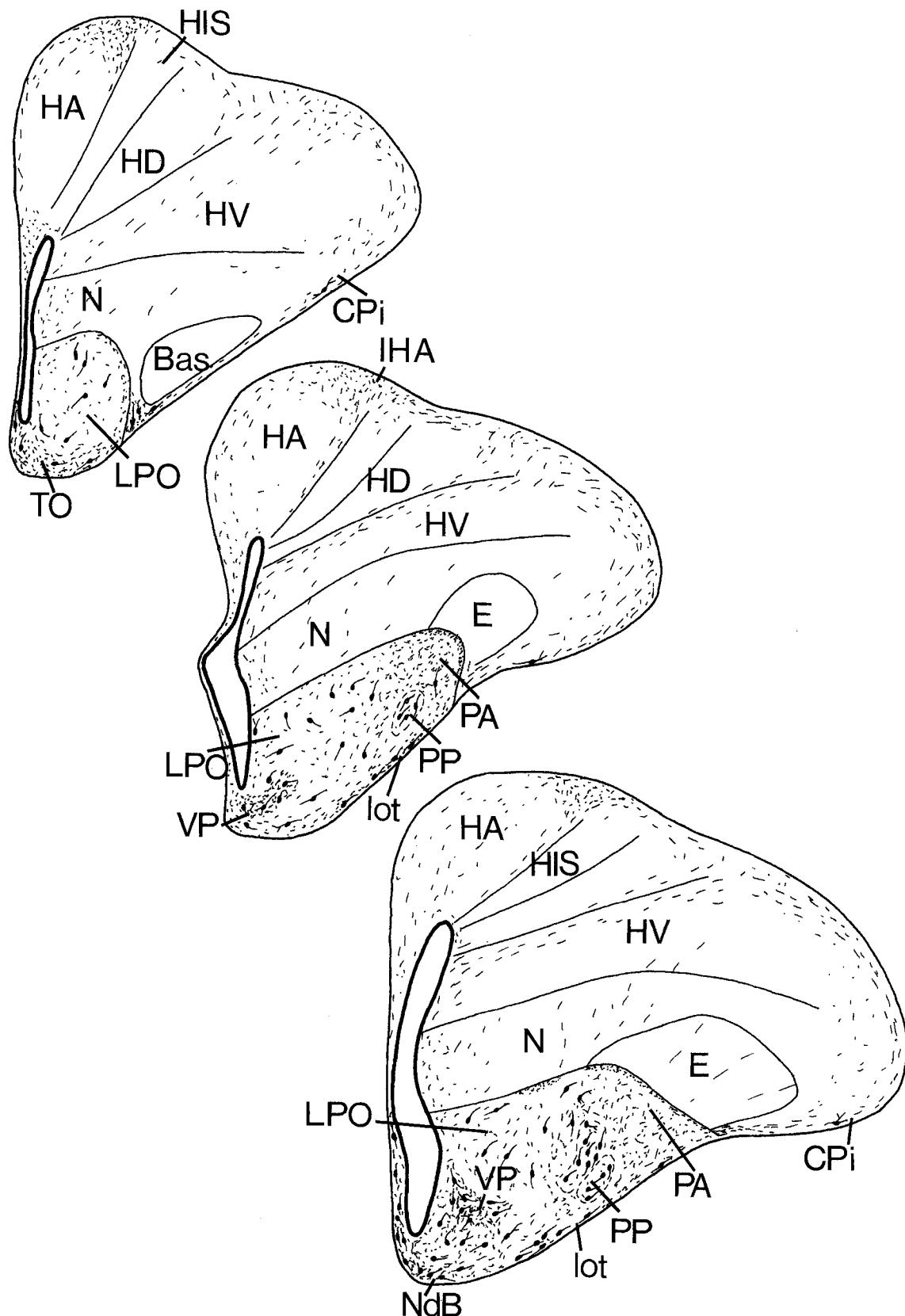


Figure 2

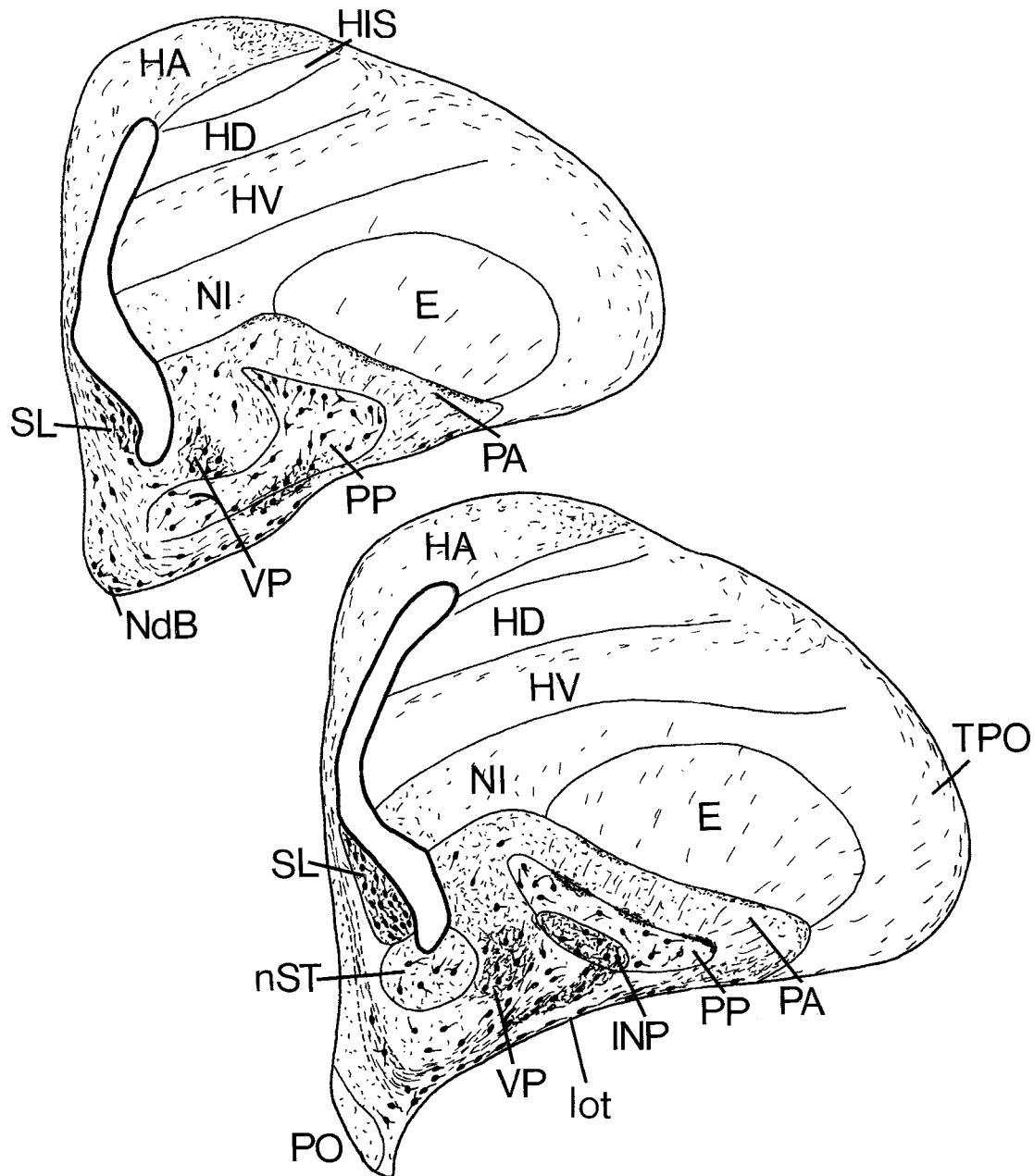


Figure 3

bundle (FPL)], and in the horizontal and vertical limbs of the nucleus of the diagonal band of Broca (NdB) (Figs. 2–5, 10B,C, 11, 12C). The ChATi neurons scattered across PP, INP, VP, FPL, and NdB appeared to constitute a large, somewhat continuous field of immunoreactive neurons.

Small ChATi neurons were also present surrounding the anterior commissure, in the lateral septal nucleus (SL), and in the nucleus commissurae septi (CoS) (Figs. 3–5, 11A). A few immunoreactive cell bodies were seen in the medial septal nucleus as well. Besides their smaller size, the cholinergic cell bodies of the septal region were distinct from the other basal telencephalic ChATi neurons in that they were only lightly labeled for the anti-chicken ChAT. Further, only a few neurons of the SL and CoS showed

labeling with the anti-human ChAT, and this labeling was extremely light.

Diencephalon

Hypothalamus. Several groups of ChATi neurons were present in the hypothalamus. In the rostral hypothalamus, lightly labeled ChATi cell bodies were observed in the nucleus preopticus medialis (POM) (Fig. 4). More caudally, at the level of the supraoptic decussation (DS), the nucleus medialis hypothalami posterioris (PMH) contained bipolar ChATi cell bodies, with numerous dendrites arranged parallel to the ventricle (Figs. 4, 5, 13B, 15B). Further caudally, ChATi cell bodies were also present in the infundibular region, a few of which were seen in the nucleus tuberis (TU) (Figs. 6, 15A). Within the lateral hypothala-

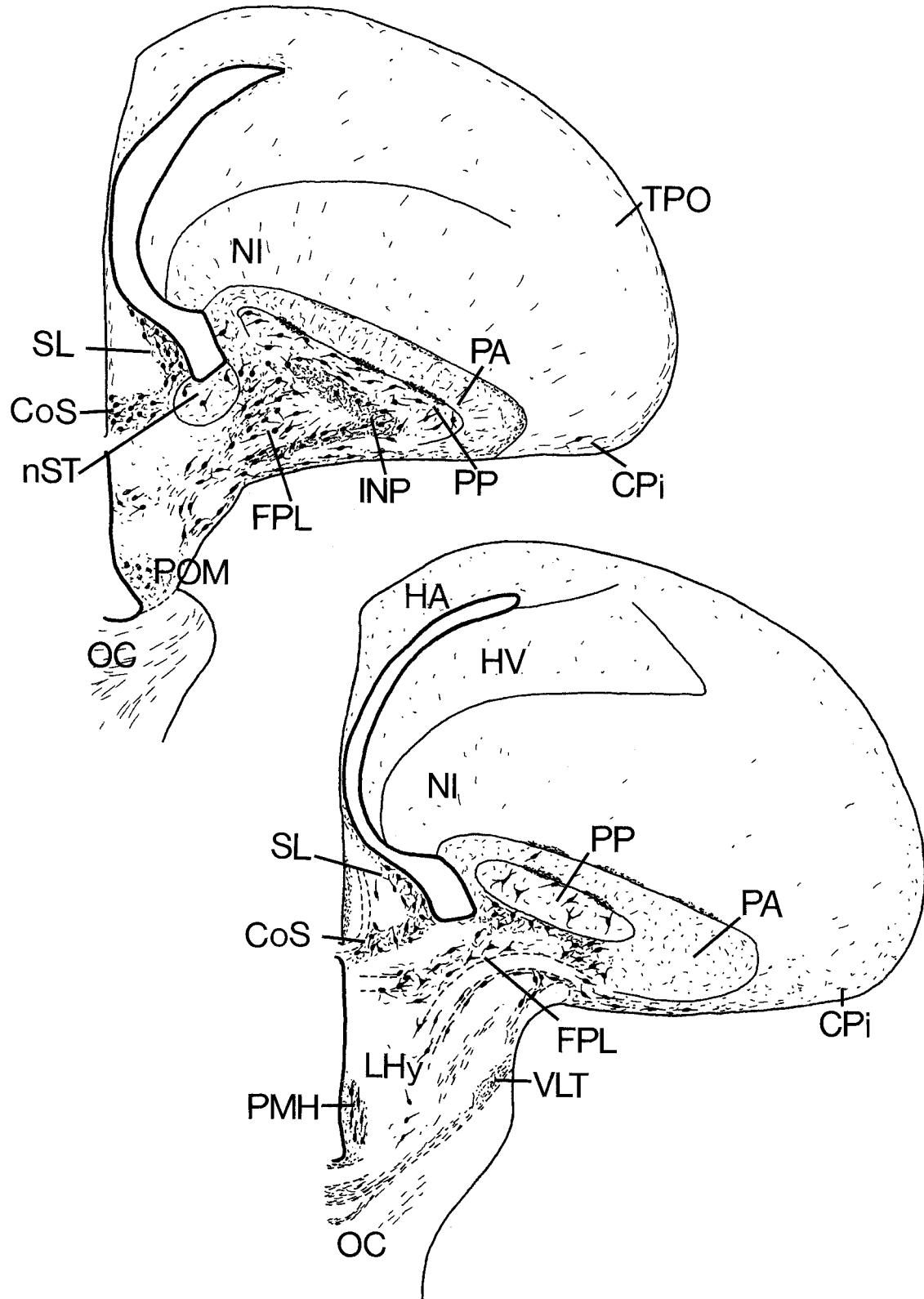


Figure 4

mus, dispersed ChATi neurons were present in the nucleus lateralis hypothalami (LHy) throughout its rostrocaudal extent (Figs. 4–6, 13B–D, 15B–D). Immunoreactive neu-

rons were observed in the stratum cellulare externum (SCE) and in the dorsal part of the stratum cellulare internum (SCI) (Figs. 5, 6, 13C).

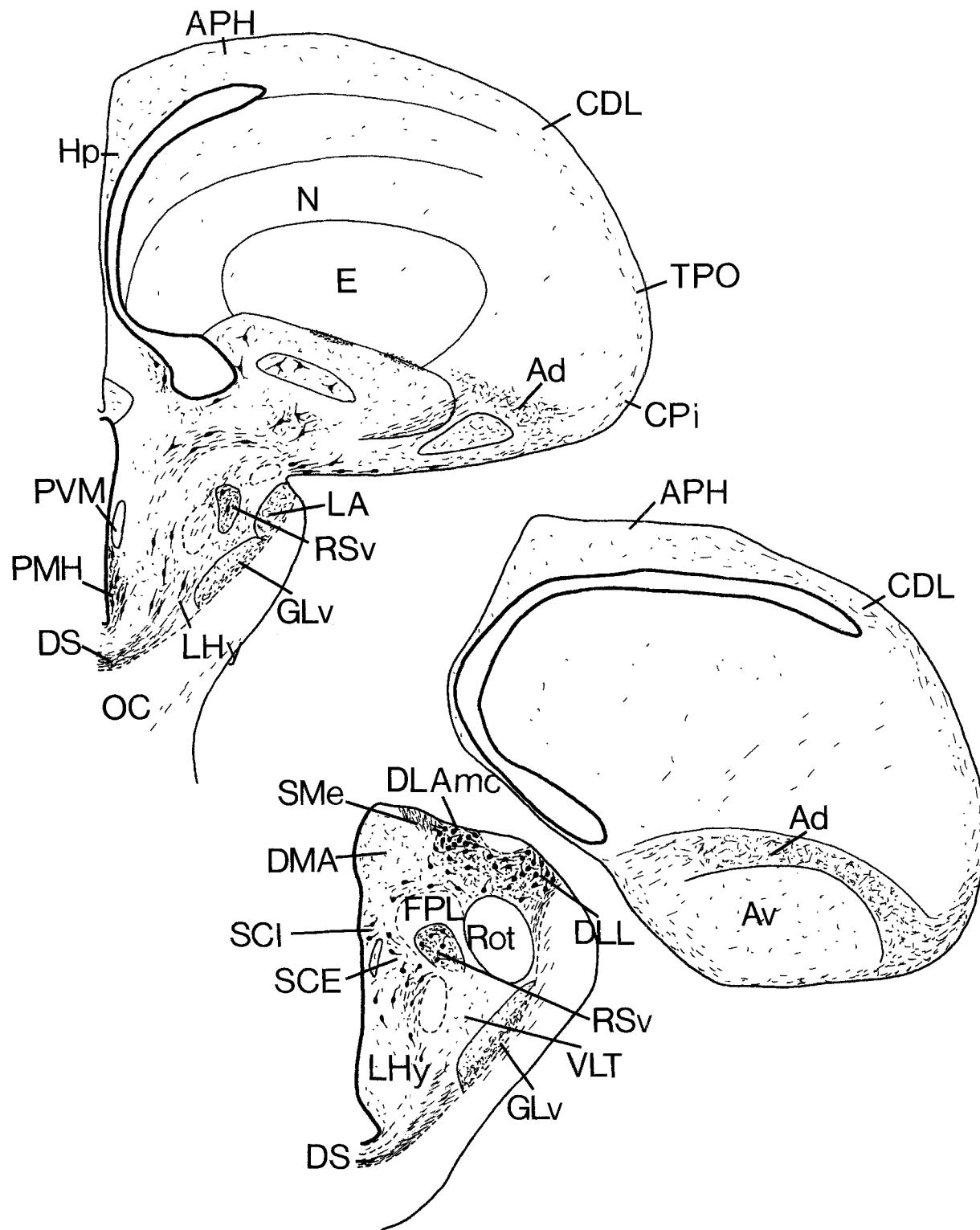


Figure 5

Thalamus and epithalamus. Extremely lightly labeled ChATi cell bodies were present in the nucleus reticularis superior pars ventralis (RSv) of the ventral thalamus (Figs. 5, 14A,B). The neurons of RSv did not label with the anti-human ChAT. In the dorsal thalamus, lightly labeled

neurons were observed in the three subdivisions of the nucleus dorsolateralis anterior thalami: pars magnocellularis (DLAmc), pars lateralis (DLL), and pars medialis (DLM) (Figs. 5, 6, 14A,C). These three cell groups make up the avian dorsal lateral geniculate nucleus and have been

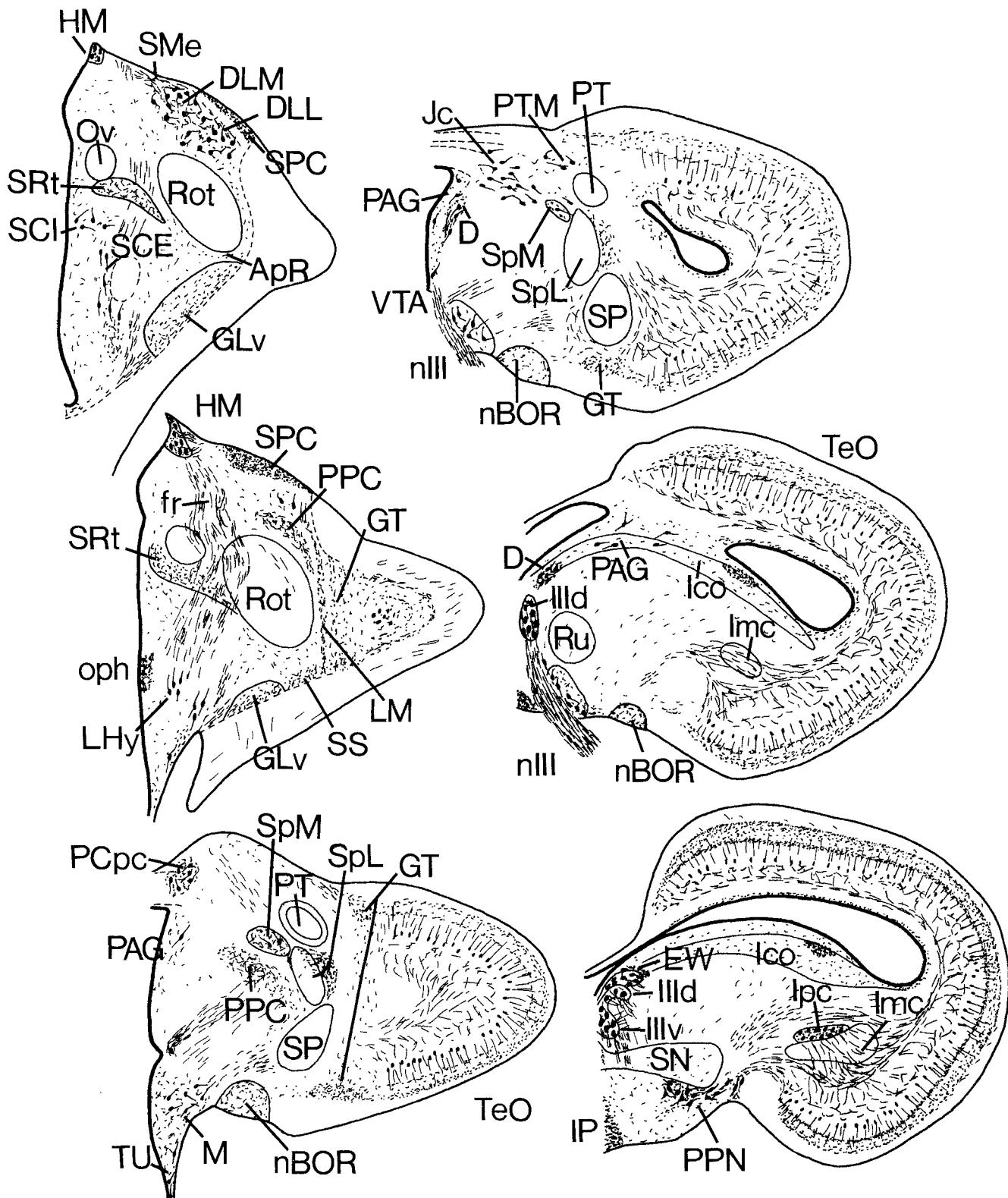


Figure 6

collectively referred to as the principal optic nucleus of the thalamus (OPT) (Karten et al., 1973). Just dorsal to the OPT, immunoreactive neurons were also observed in the

nucleus superficialis parvocellularis (SPC), surrounding the tractus septomesencephalicus (TSM) (Figs. 6, 16A). Neurons in SPC did not label for anti-human CHAT. In the

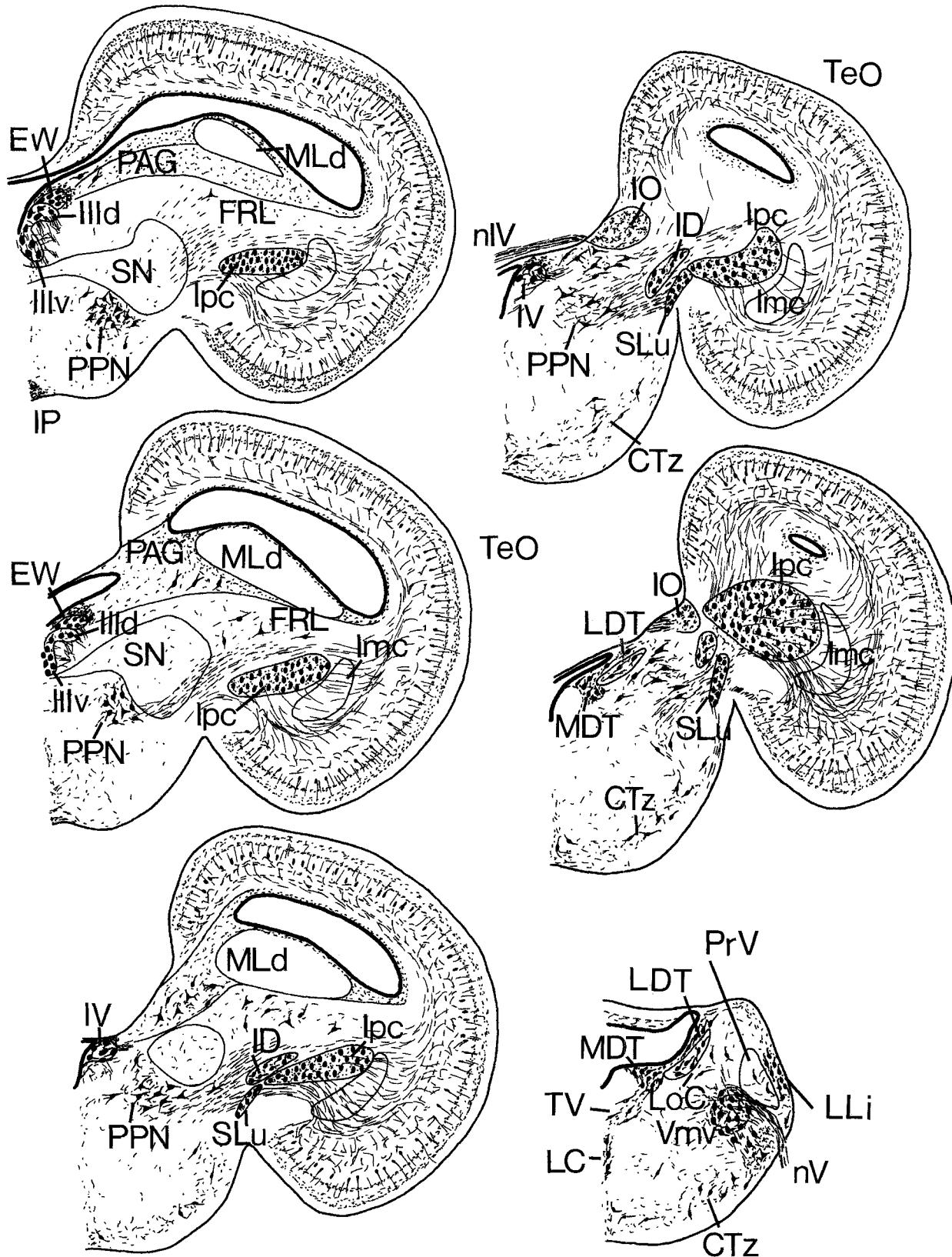


Figure 7

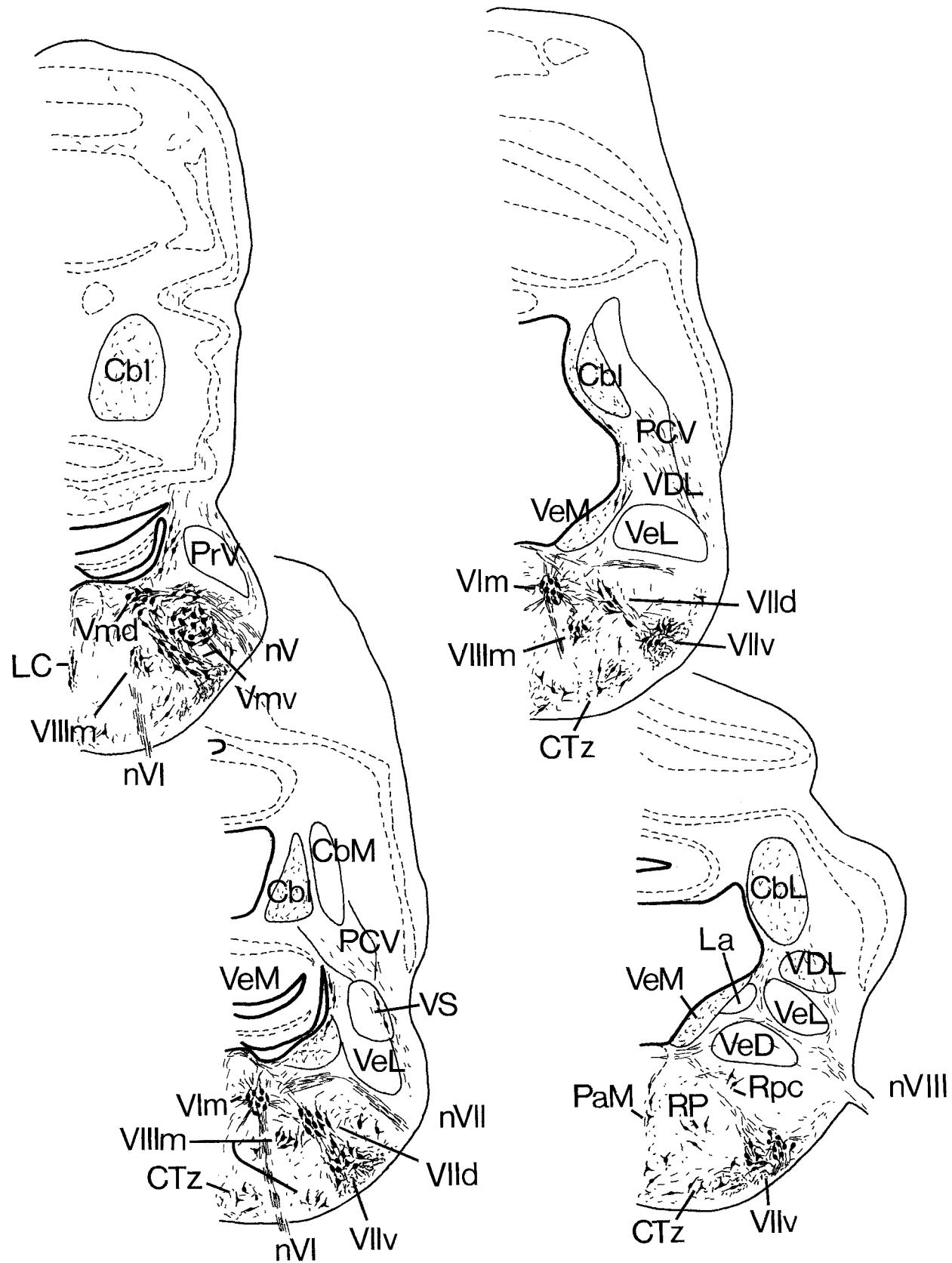


Figure 8

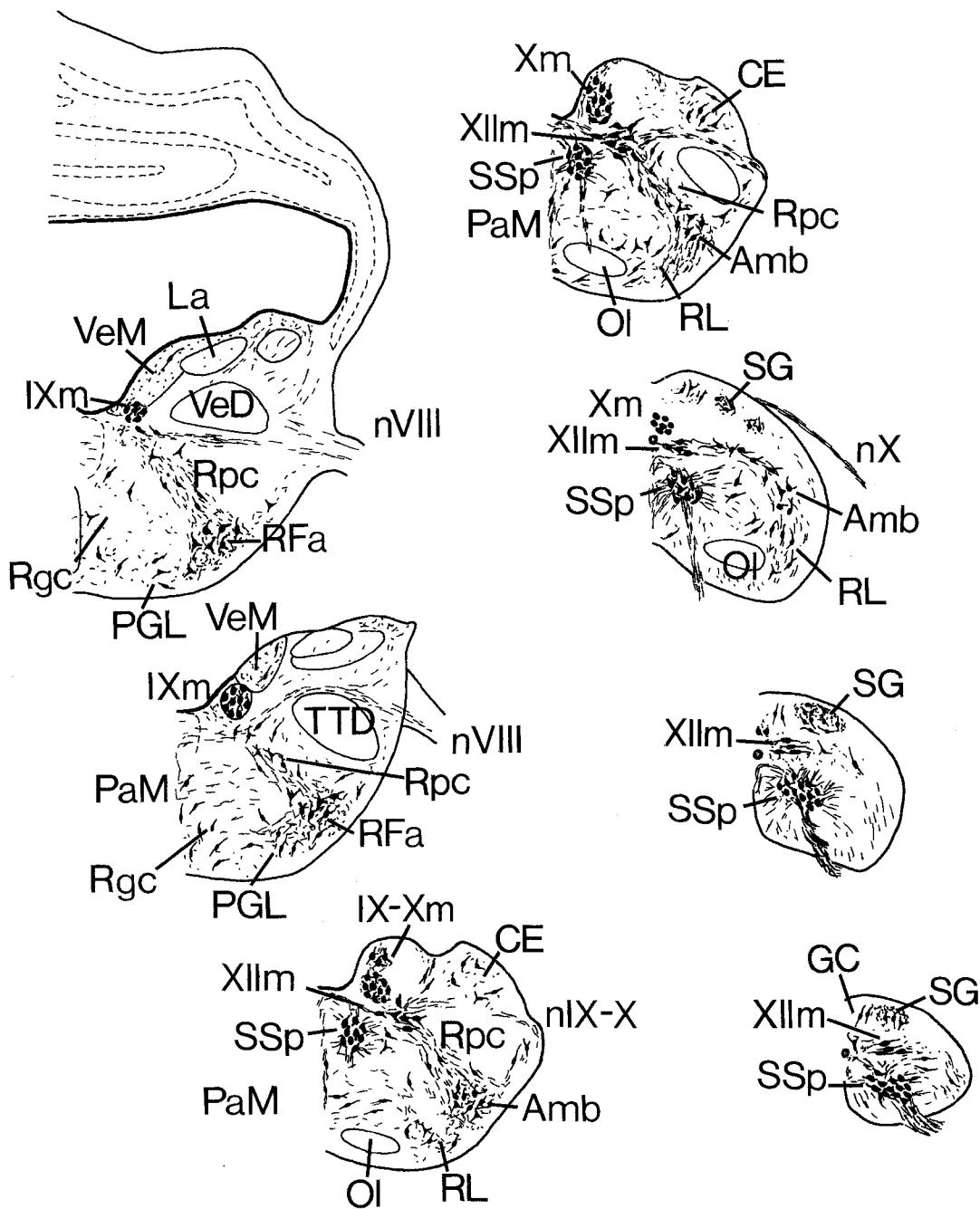


Figure 9

epithalamus, densely clustered ChATi cell bodies were present in the nucleus habenularis medialis (HM) (Figs. 6, 13C). Axons of these cells were seen to leave the HM and form the fasciculus retroflexus.

Pretectum. Several groups of ChATi neurons were present in the pretectum. A few immunoreactive cell bodies were present in the nucleus spiriformis medialis (SpM), while two distinct small groups of ChATi neurons were located medial to SpM among the fibers of the posterior commissure (Figs. 6, 17). One of these consisted of small, round cell bodies located laterodorsal to the posterior

commissure at rostral commissural levels, and we refer to it as the nucleus parvocellularis of the posterior commissure (PCpc) (Fig. 17A,C). The second of these commissural ChATi cell groups was located lateroventral to the commissure at its caudal levels, and it is called by us the nucleus juxtapcommisuralis (Jc) (Fig. 17B,D). Immunoreactive neurons were also seen in the nucleus pretectalis medialis (PTM) (Figs. 6, 16B). The cholinergic neurons of the SpM, Jc, and PTM were lightly immunoreactive for both anti-chicken ChAT and anti-human ChAT, while those of the PCpc were strongly immunoreactive.

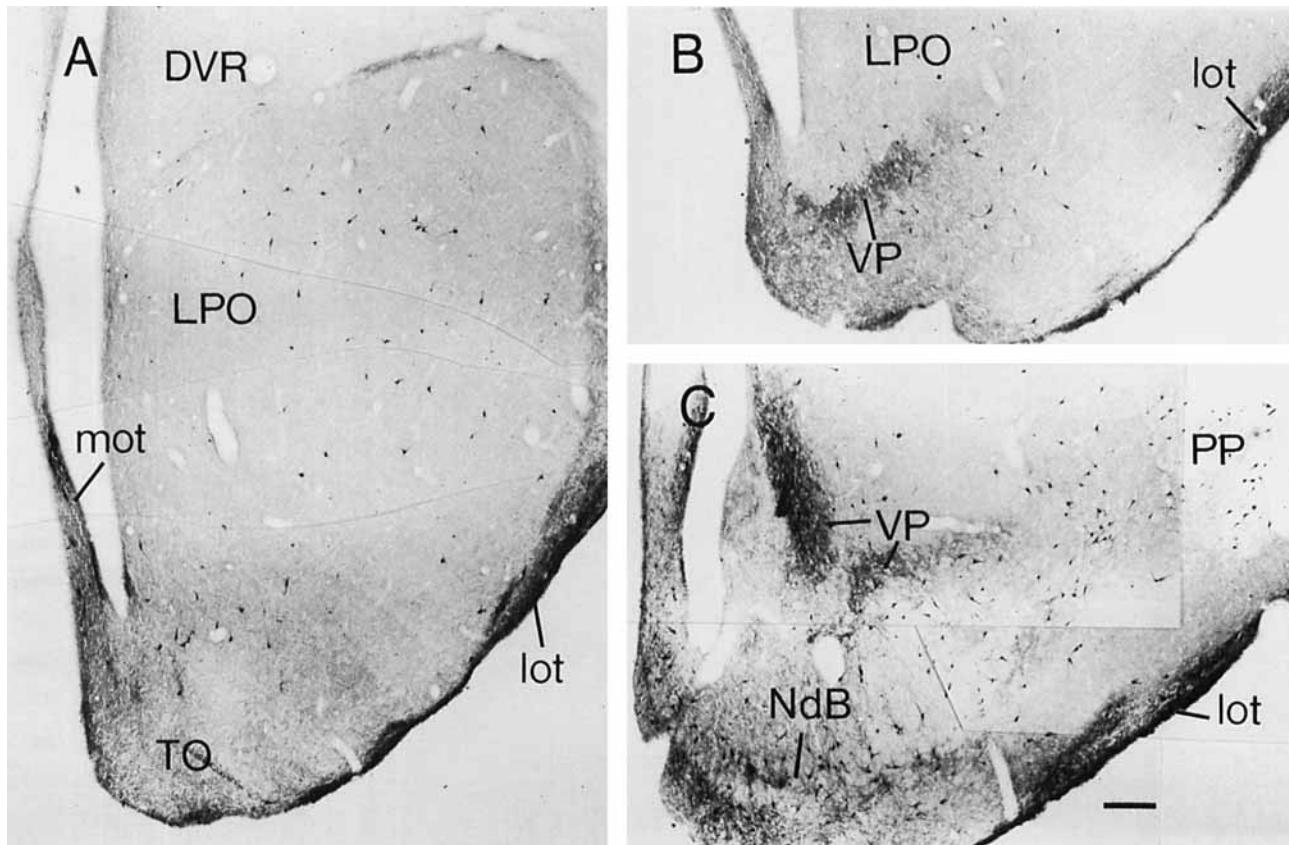


Fig. 10. Photomicrographs of transverse sections through the pigeon basal telencephalon, showing ChATi cells and fibers at several rostro-intermediate levels of the basal ganglia. Scale bar = 200 μm .

Mesencephalon

Optic lobe and tectum. Numerous bipolar ChATi cell bodies were observed in the stratum griseum et fibrosum superficiale of the optic tectum, mainly located in tectal layer 10 (Figs. 6, 7, 18). Each tectal ChATi neuron showed a long, radially disposed, apical process that ramified in tectal layers 3–7. A few immunoreactive neurons of the same type were also located in tectal layers 9 and 11.

Central gray and tegmentum. Scattered ChATi neurons were present in the periaqueductal gray (PAG), the nucleus of Darkschewitsch (D), and the lateral reticular formation (FRL) (Figs. 6, 7, 17B, 20C). More ventrally, ChATi neurons were also seen in the ventral tegmental area (VTA) (Figs. 6, 20A,B). More caudally, the motoneurons of the oculomotor complex, including the somatic motoneurons of the dorsal oculomotor nucleus (III^d) and ventral oculomotor nucleus (III^v) and the somewhat smaller visceral preganglionic neurons of the Edinger-Westphal nucleus (EW), showed a very strong ChAT immunoreactivity (Figs. 6, 7, 19A, 20A). The motoneurons of the somatic oculomotor complex possessed labeled dendrites that extended laterally into the PAG and the lateral part of the fasciculus longitudinalis medialis. The labeled dendrites of the EW motoneurons were largely confined to the nucleus, although many in the medial part of the nucleus extended dorsomedially toward the ventricle. The ChATi axons of the oculomotor complex motoneurons were prominently labeled as they coursed ventrally to exit the brain as the oculomotor nerve.

Isthmus. All or nearly all perikarya in the nucleus isthmi pars parvocellularis (Ipc) were ChATi (Figs. 6, 7, 19B, 21). Axons of these neurons were seen to course into the stratum album centrale of the tectum, and ramify in the overlying tectal layers. The semilunar isthmic nucleus (SLu) contained smaller cell bodies that showed a very intense ChAT immunoreactivity (Figs. 7, 19B, 21). All neurons of SLu appeared to be ChATi. Medial to the Ipc and SLu, a nucleus identified in the atlas of Karten and Hodos (1967) as nucleus lemnisci lateralis pars dorsalis (LLd) also contained ChATi neurons (Figs. 7, 19B). Tracing experiments have shown that this nucleus is not related to the auditory system of pigeons and therefore cannot be considered one of the nuclei of the lateral lemniscus (Arends and Zeigler, 1986; Wild, 1987). The location of this nucleus dorsomedial to cholinergic isthmic nuclei that project to the tectum (Ipc and SLu), and its demonstrated projections to the optic tectum (Brecha, 1978), indicate that this region may represent an additional cholinergic isthmotectal nucleus. Therefore, we believe this region should be considered an isthmic nucleus comparable to Ipc and SLu. Because this cell group is less well defined than SLu and Ipc, we have called it the disseminated isthmic nucleus (ID). ChATi cell bodies were observed surrounding the isthmo-optic nucleus (Fig. 21). The small size and location of these neurons is consonant with the possibility that they are ectopic IO neurons (Hayes and Webster, 1981). The isthmo-optic nucleus itself was observed to contain many extremely lightly labeled ChATi neurons in some pigeons, particularly

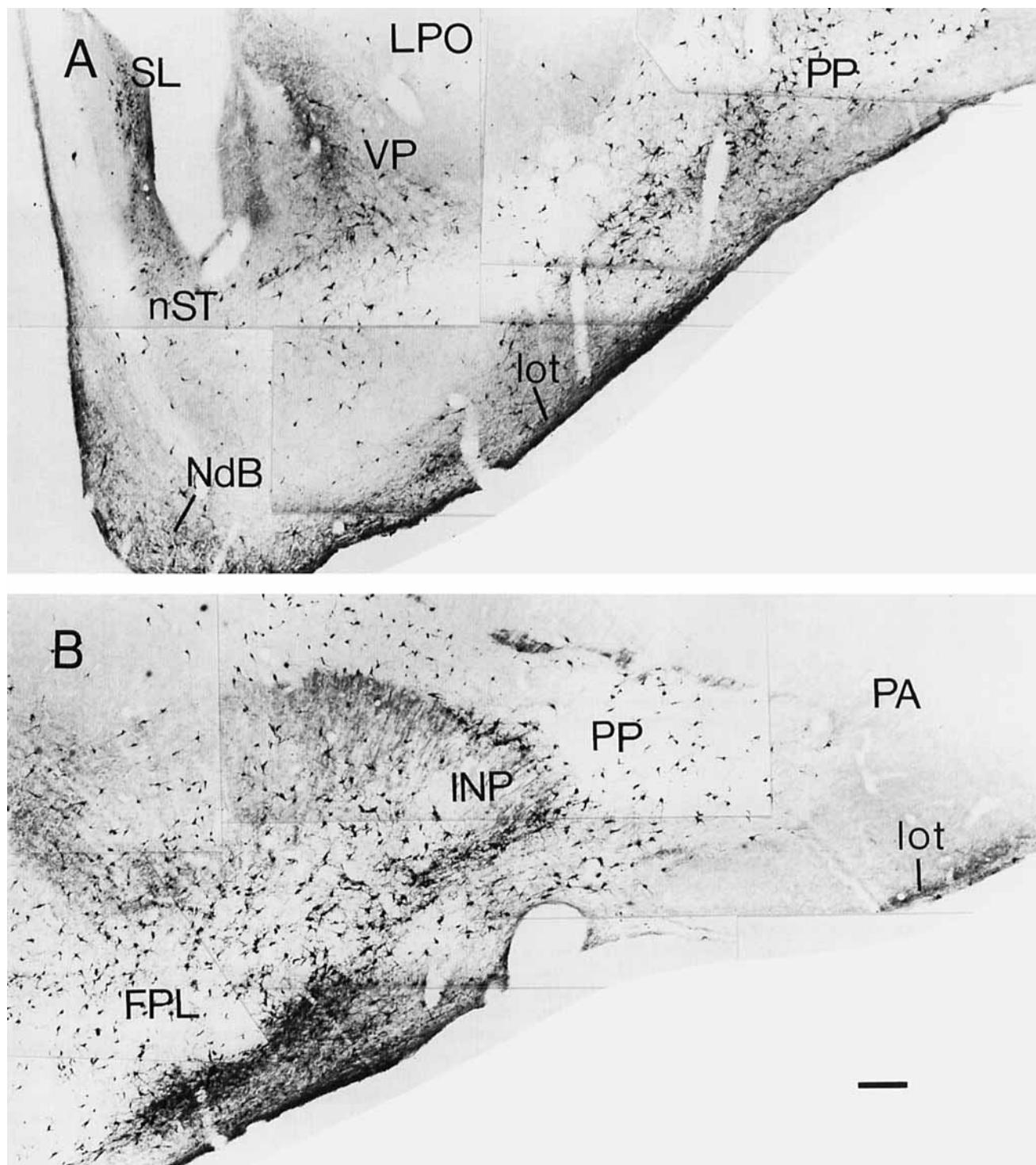


Fig. 11. Photomicrographs of transverse sections through the pigeon telencephalon, showing ChATi cells and fibers at caudal levels of the basal forebrain. Scale bar = 200 μ m.

those brains that were less well fixed (Fig. 21). Isthmo-optic neurons did not label with the human anti-ChAT.

A prominent group of large, multipolar ChATi neurons was present in the isthmic tegmentum (Figs. 6, 7, 19A, 21). These ChATi neurons spanned several cell groups, includ-

ing the nucleus profundus mesencephali pars ventralis rostrally, the oral pontine reticular formation (RPO) at intermediate tegmental levels, and the region below the midbrain central gray caudally. This cell group of ChATi neurons appeared to represent a single entity that highly

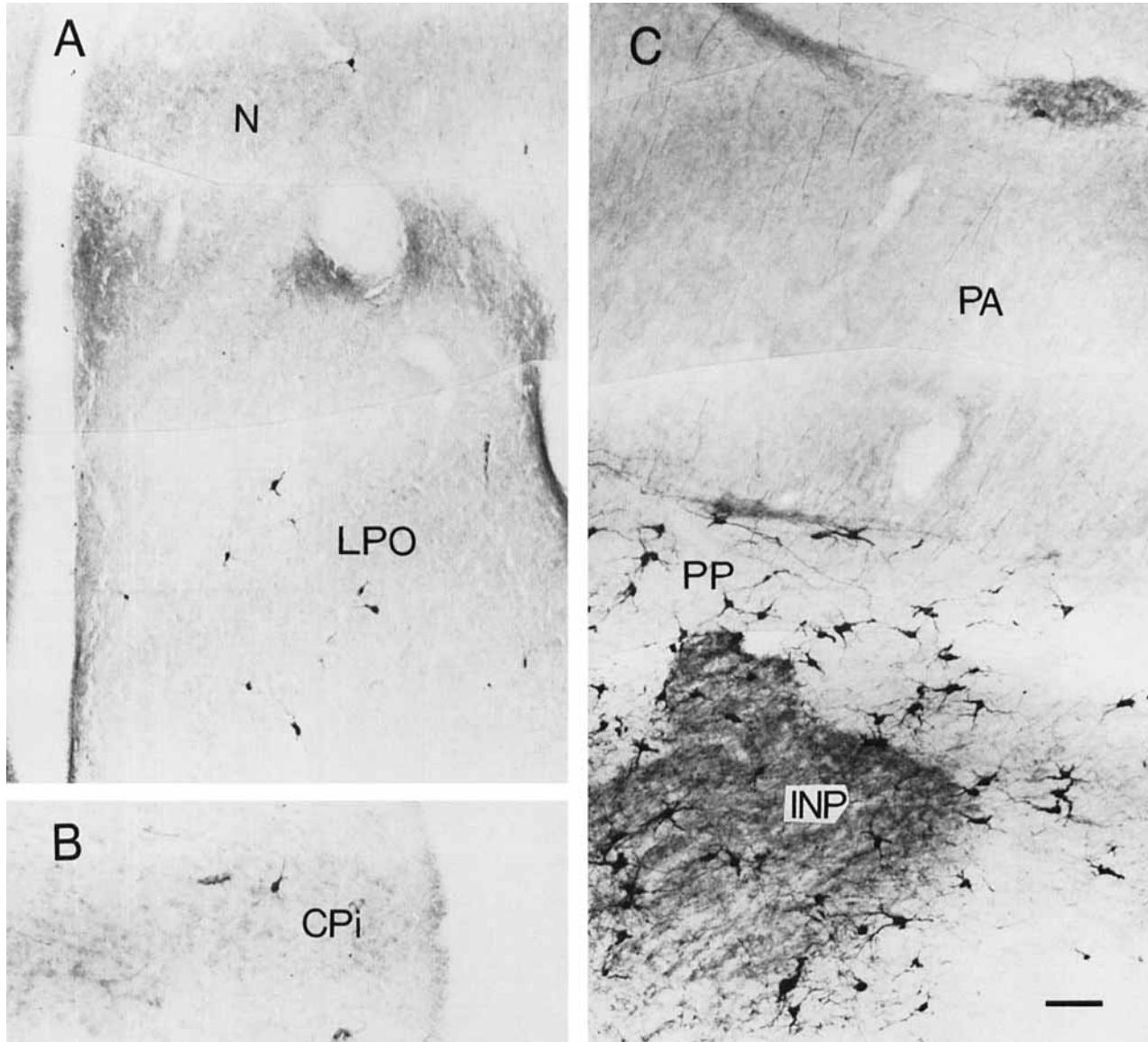


Fig. 12. High-magnification photographs of ChATi cells and fibers in the basal ganglia and DVR. **A:** ChATi neurons in the rostral striatum (LPO) and in the neostriatum. **B:** ChATi neurons in the cortex piriformis (CPi). **C:** Numerous large ChATi neurons in the pallidal regions of the basal ganglia (PP, INP). Note the high numbers of ChATi

fibers and varicosities in the INP. ChATi axons of pallidal neurons course into dorsalmost telencephalic regions, traversing the striatum (PA). Note the absence of ChATi neurons in the lateral striatum (PA). Scale bar = 100 μ m.

resembles the mammalian pedunculopontine tegmental nucleus (von Bartheld and Bothwell, 1992). Therefore, we called this group of ChATi neurons in birds the pedunculopontine tegmental nucleus (PPN) (Figs. 6, 7, 19A, 21). Note that the PPN of birds differs from the cell group previously called "nucleus tegmenti pedunculopontinus" in early work by Karten and coworkers (Karten and Hodos, 1967; Karten and Dubbeldam, 1973). The cell group previously termed the nucleus tegmenti pedunculopontinus is now referred to as the substantia nigra, and it contains dopaminergic neurons (Anderson and Reiner, 1990). In the transverse plane of the Karten and Hodos atlas (Karten and Hodos, 1967), the ChATi neurons of PPN are located ventral to the dopaminergic neurons of the substantia nigra. In the

sagittal plane, however, it is easier to appreciate the topographic relationship between these two cell groups—both are observed to consist of a column of neurons oriented rostroventral to dorsocaudal, with the cholinergic PPN cell band located in the isthmic tegmentum parallel and just caudal to the band of dopaminergic neurons of the substantia nigra located in the mesencephalic tegmentum.

Dorsocaudal to the PPN, ChATi neurons were observed at two sites in the central gray around the fourth ventricle. The more lateral of these two groups is located in the central gray beneath the lateral horn of the fourth ventricle, mainly dorsal to the noradrenergic neurons of the locus coeruleus (LoC), but overlapping the LoC to a small extent (Figs. 7, 22). We term this cholinergic cell group the

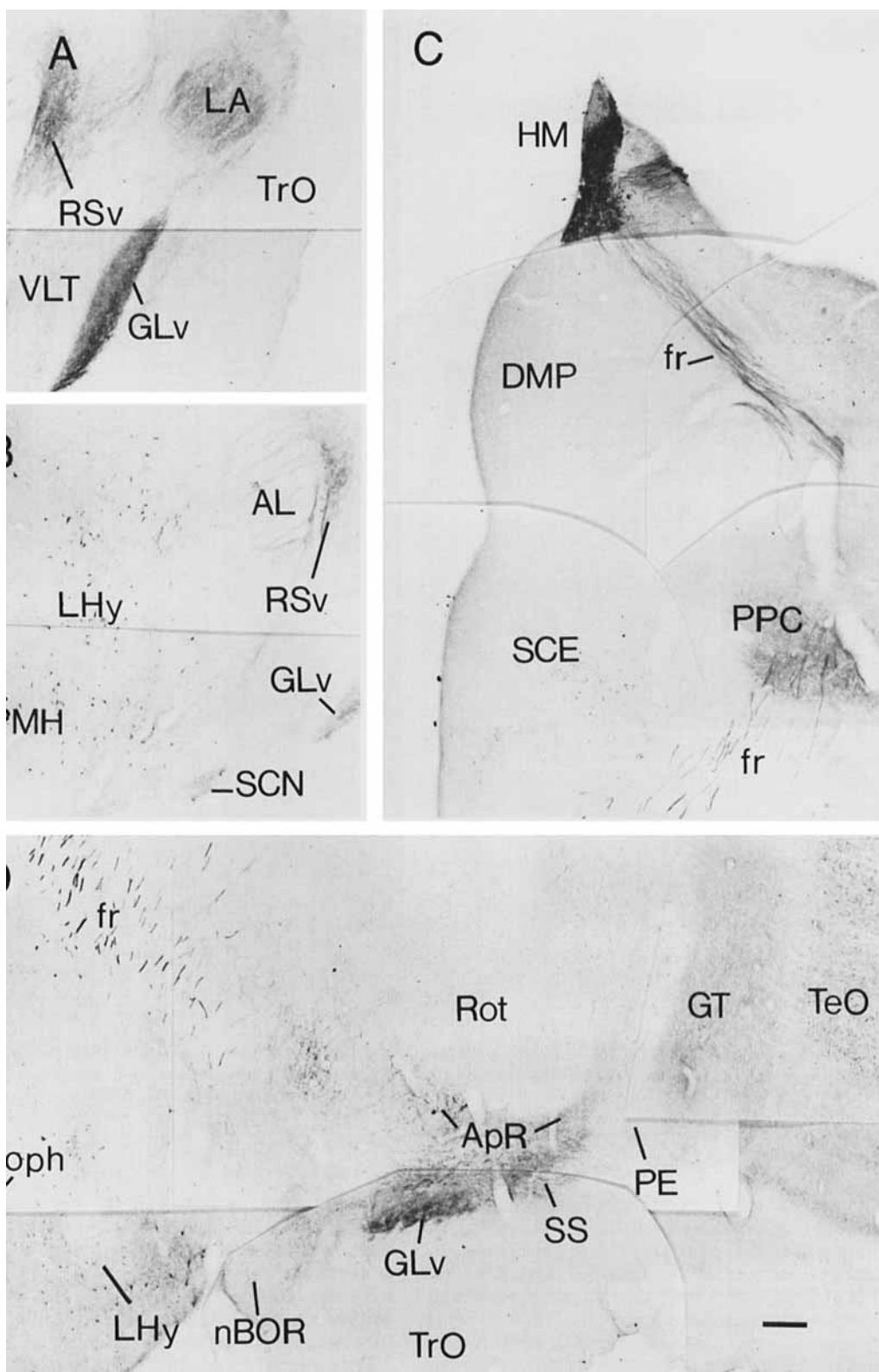


Fig. 13. Photomicrographs of transverse sections through the pigeon diencephalon and mesencephalon. **A:** Dense ChATi innervation in the optic neuropiles of the ventral thalamus (GLv, LA), as well as in the RSV. **B:** ChATi neurons in the periventricular (PMH) and lateral (LHy) hypothalamus. Note the moderate ChATi innervation in the suprachiasmatic nucleus (SCN). **C:** ChATi cell bodies in the medial habenula (HM), whose axons collect in the fasciculus retroflexus (fr). **D:** ChATi neurons and fibers at ventrocaudal hypothalamic levels. Note the extremely dense innervation in the oph. This photomicrograph also shows a dense ChATi innervation in the optic neuropiles of the thalamus (GLv), pretectum (SS), and mesencephalon (GT and optic tectum). Scale bar = 200 μ m.

Another bundle of ChATi axons arrives to the habenula, the stria medullaris (lateral to HM). This photomicrograph also shows ChATi neurons in the SCE of the hypothalamus and a dense ChATi innervation in the PPC of the pretectum. **D:** ChATi neurons and fibers at ventrocaudal hypothalamic levels. Note the extremely dense innervation in the oph. This photomicrograph also shows a dense ChATi innervation in the optic neuropiles of the thalamus (GLv), pretectum (SS), and mesencephalon (GT and optic tectum). Scale bar = 200 μ m.

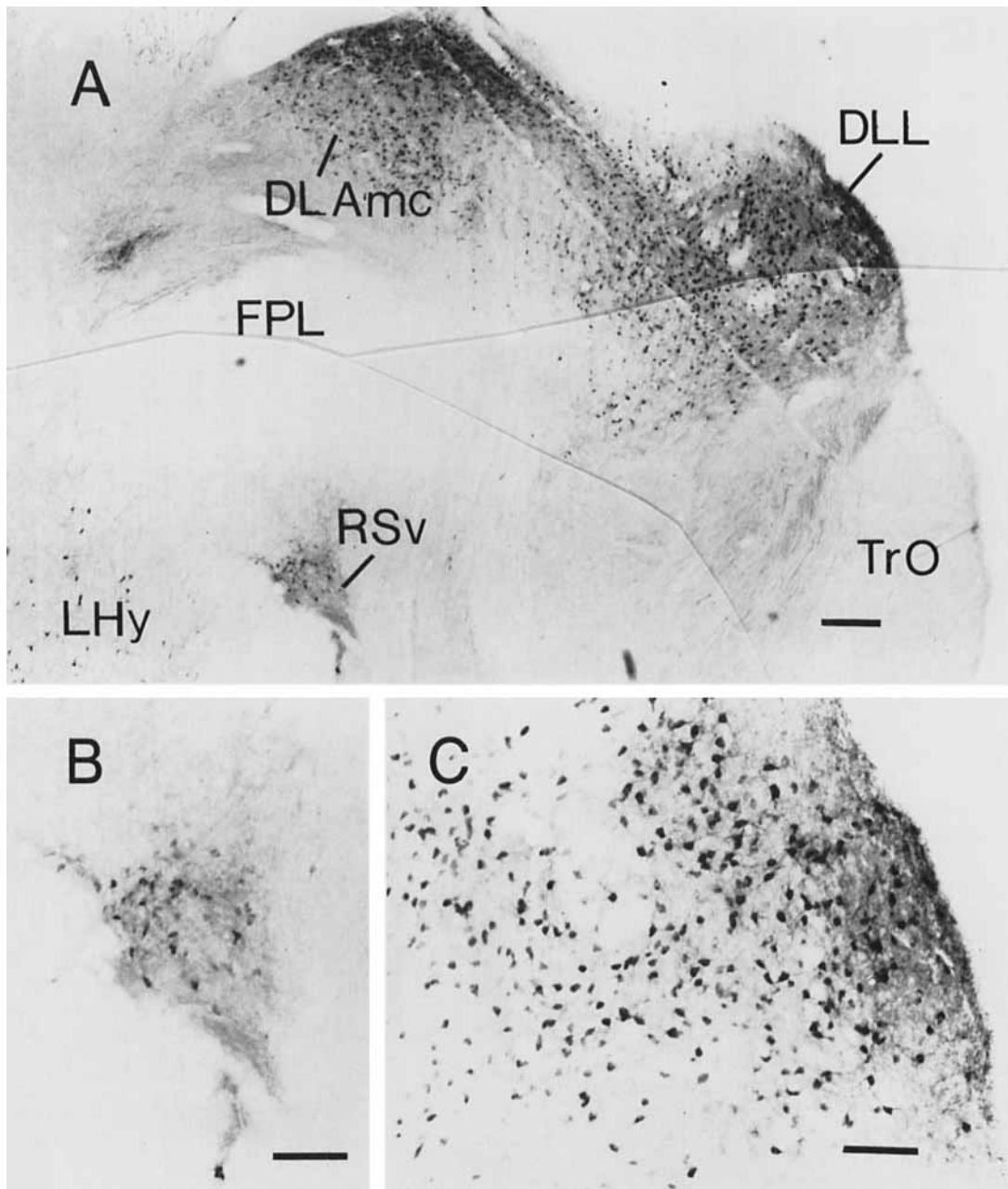


Fig. 14. Photomicrographs of ChATi cell bodies and fibers in the pigeon thalamus. **A:** ChATi cell bodies in the dorsal (DLL, DLAmc) and ventral (RSv) thalamus. **B,C:** Higher magnification photographs of RSv and DLL, respectively. Scale bars = 200 μm in A, 100 μm in B and C.

laterodorsal tegmental nucleus (LDT). The more medially located of the dorsal tegmental cholinergic cell groups is located in the central gray medial to the LoC, lateral to the FLM, and caudal to the trochlear nucleus. This cell group has been called the nucleus segmenti dorsalis (TD) (Karten and Hodos, 1967), but we will refer to it as the mediodorsal tegmental nucleus (MDT) to distinguish it from the LDT. Sparse ChATi neurons were also seen in the nucleus segmenti ventralis (TV) below the MDT. Finally, in the isthmic region rostral to MDT, the motoneurons of the trochlear nucleus were intensely ChATi. Their labeled axons decussated dorsal to the fourth ventricle, exiting the

brain through the contralateral trochlear nerve (Figs. 7, 21).

Rhombencephalon. At rostral rhombencephalic levels, a few small ChATi neurons were observed in the nucleus vestibularis medialis (VeM) and the nucleus vestibularis superior (VS), and adjacent to the processus lateralis cerebellovestibularis (PCV) (Figs. 8, 23).

In the ventral part of the rostral rhombencephalon, the trigeminal motor complex contained ChATi neurons. Although Wild and Zeigler (1980) identified several distinct subnuclei in the trigeminal motor complex of pigeons (Figs. 7, 8, 22, 23, 26), for simplicity we illustrate this cell group as

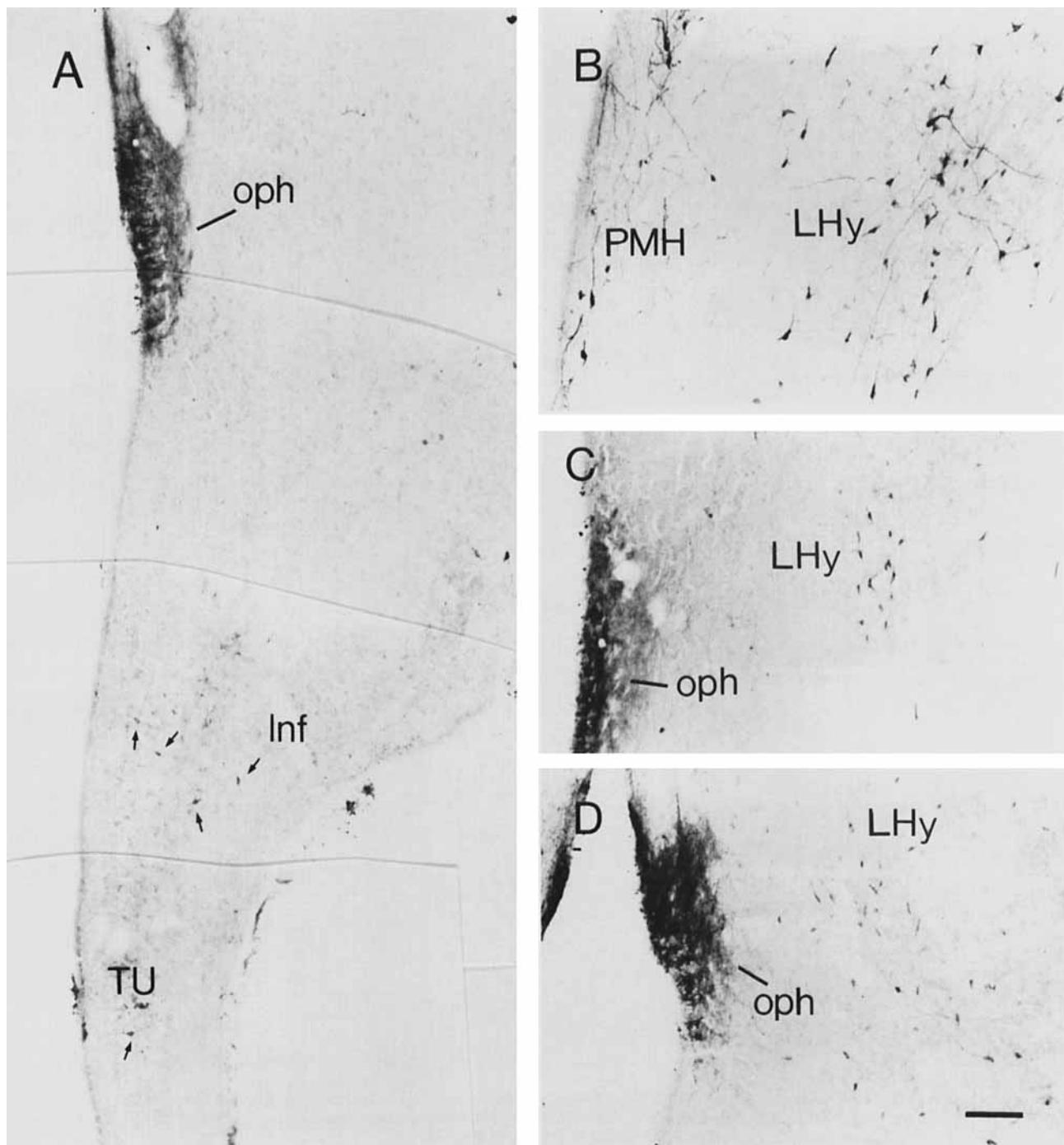


Fig. 15. Photomicrographs of ChATi cell bodies and fibers in the pigeon hypothalamus. **A,C,D:** ChATi neurons and fibers at a caudoven-tral hypothalamic level. Note the small ChATi neurons (arrows) in the

infundibular region (TU, Inf) and the dense ChATi innervation in the oph. **B:** ChATi neurons in the periventricular (PMH) and lateral (LHy) areas at an intermediate hypothalamic level. Scale bar = 100 μ m.

consisting of only dorsal or ventral nuclei (Vmd, Vmv) (Fig. 23). The motoneurons of the Vmv possess long labeled ventrolaterally directed dendrites that ramify near the pial surface. The labeled axons of the trigeminal motoneurons exit the brain laterally through the trigeminal nerve. Dorsolateral to the trigeminal motor complex, a compact group of small ChATi neurons was observed in what appeared to be the caudal part of intermediate nucleus of

the lateral lemniscus (LLi; Arends and Zeigler, 1986) (Figs. 7, 22). Caudal to the trigeminal complex, the facial motor complex also contained ChATi motoneurons in its dorsal (VIIId) and ventral nuclei (VIIIV) (Figs. 8, 24A, 26). The motoneurons of the VIIIV had very darkly stained dendrites that extended to and ramified at the pial surface. The labeled axons of the facial motoneurons were observed to course dorsomedially to the region lateral to the abducens

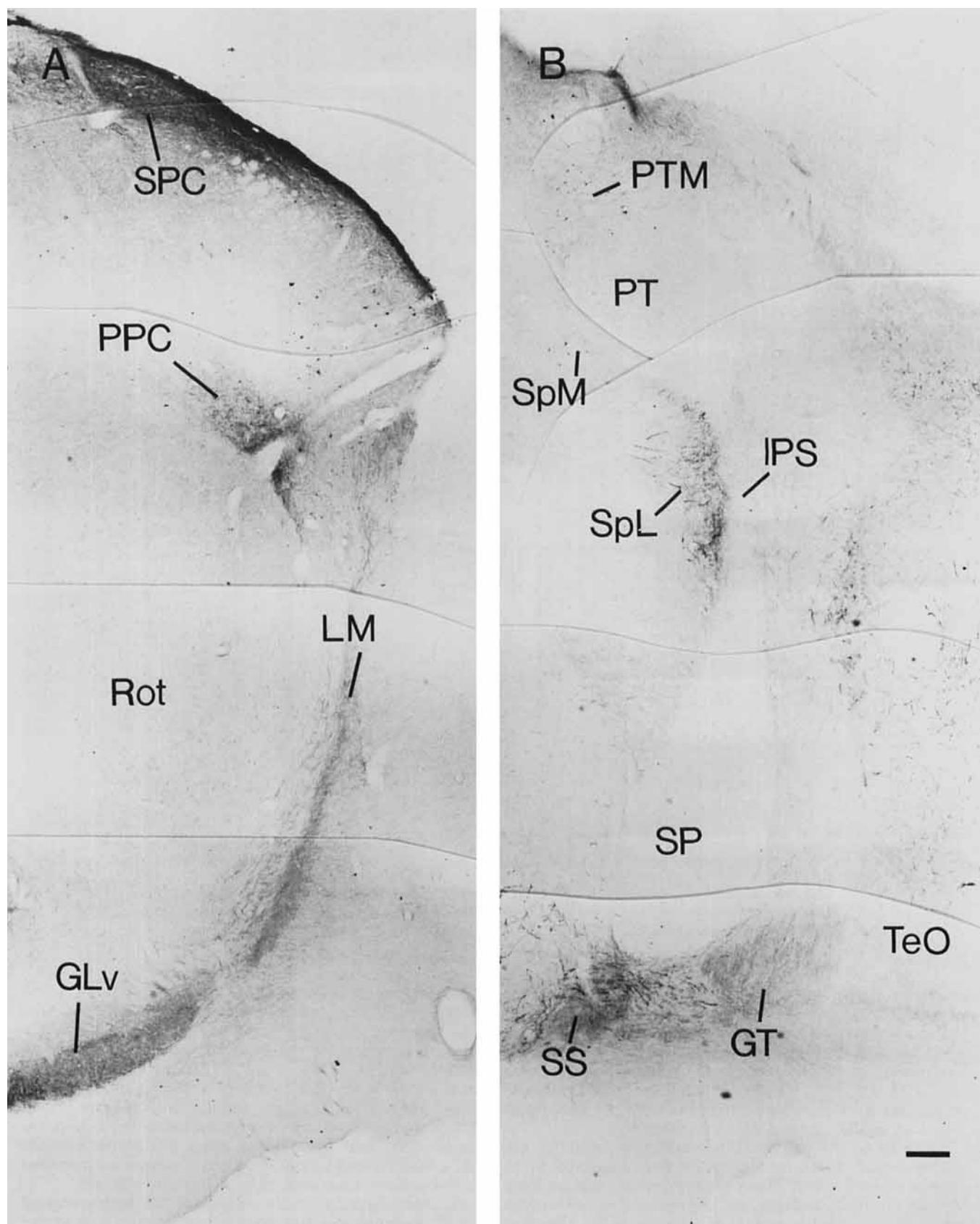


Fig. 16. **A,B:** Photomicrographs showing a dense ChATi innervation in several centers of the pigeon thalamus and pretectum, such as the GLv, SPC, LM, PPC, SS, and SpL. Note also some dispersed ChATi neurons in the PTM and SpM. Scale bar = 200 μm .

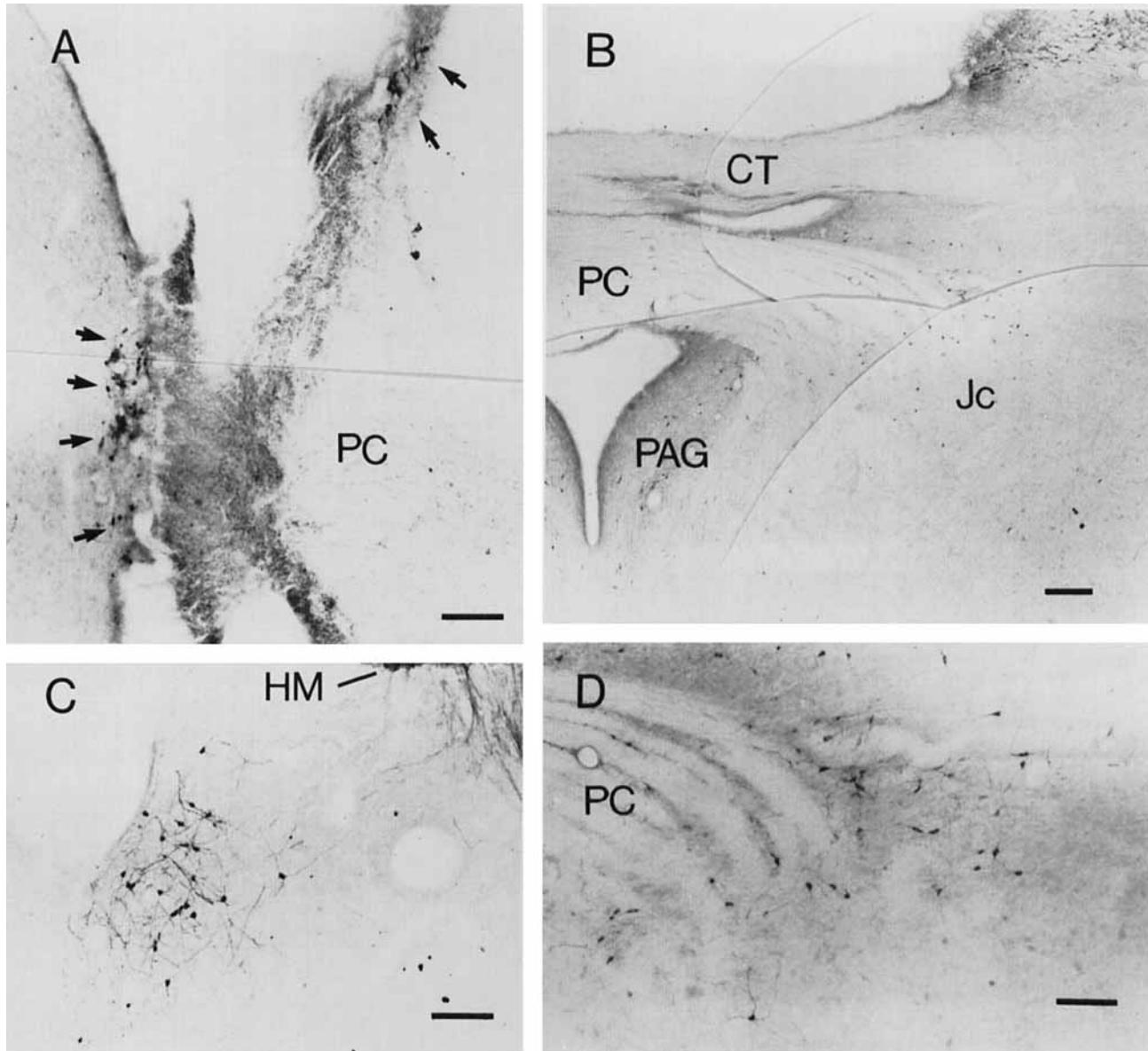


Fig. 17. Photomicrographs of two ChATi cell groups located close to rostral (PCpc; A, C) or caudal (Jc; B, D) levels of the posterior commissure (PC), respectively. Arrows in A indicate ChATi cells of the PCpc. Scale bars = 100 μm in A, C, and D, 200 μm in B.

nucleus and then laterally to exit the brain through the root of the facial nerve. In other vertebrates, the superior salivatory nucleus (visceral part of the facial motor complex) has been shown to contain cholinergic neurons. Since this nucleus has not been clearly identified in birds, we were unable to determine whether this cell group is cholinergic in pigeons. It is possible that part of what we identify as VIIv may be the superior salivatory nucleus.

Adjacent to the fasciculus longitudinalis medialis, the motoneurons of nucleus abducens were observed to be ChATi (Figs. 8, 24A). These motoneurons displayed long, radially arranged dendrites, and their axons coursed ventralwards to leave the brain through the roots of the abducens nerve. Ventrolateral to the nucleus abducens, a group of smaller ChATi neurons was located between the roots of the abducens nerve and the facial motor complex. These

labeled neurons appear to represent the efferent cells of the VIII nerve (VIIIm), based on the descriptions of Whitehead and Morest (1981) (Figs. 8, 23, 24A). The labeled axons of these neurons were observed to course dorsomedially, collect dorsal to the fasciculus longitudinalis medialis, and then course laterally into the root of nerve VIII. Among other pontine cell groups, small ChATi neurons were distributed in the reticular formation, in the corpus trapezoidum (CTz) and the nucleus reticularis pontis caudalis (RP). Finally, small, fusiform ChATi neurons were present in the nucleus linearis caudalis (LC) (Figs. 8, 22, 23).

At more caudal rhombencephalic levels, lightly stained ChATi motoneurons were observed in the glossopharyngeal motor nucleus (IXm) and in the dorsal motor nucleus of the vagus (Xm) (Figs. 9, 25). The labeled axons of the glossopharyngeal and vagal motoneurons were observed to course

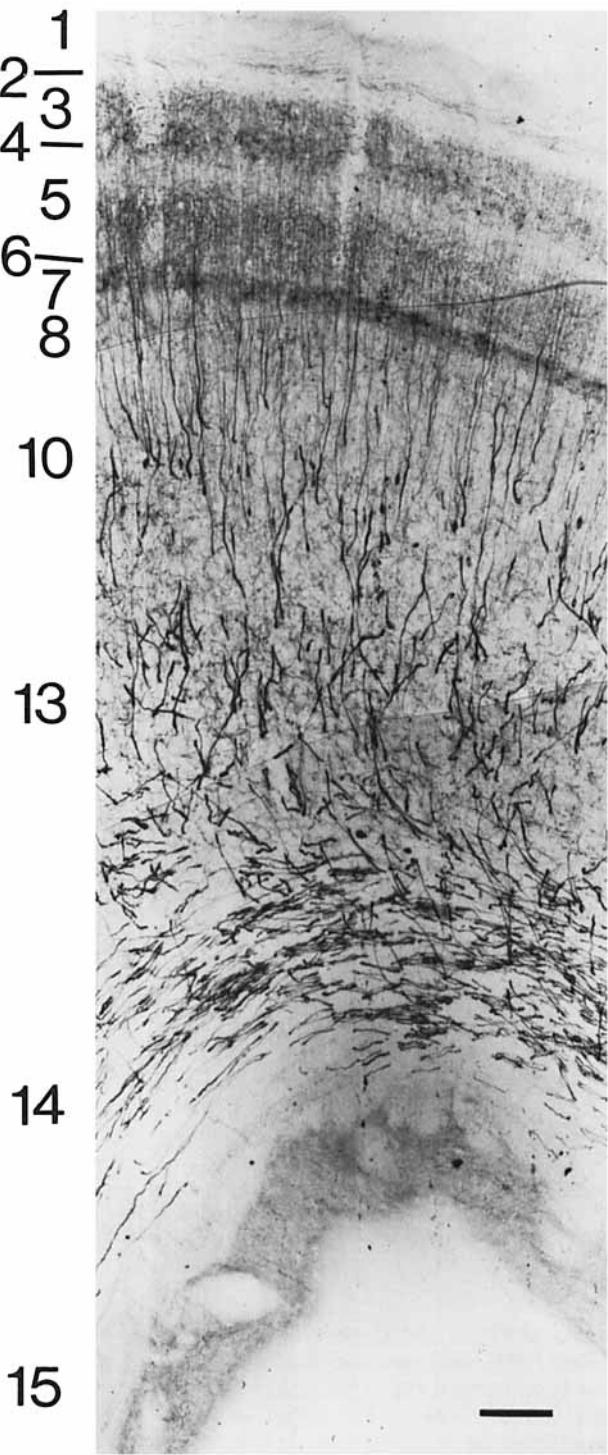


Fig. 18. Photomicrograph through the pigeon midbrain tectum, showing a dense ChATi innervation in several tectal layers (especially layers 3–4, the deeper half of layer 5, and layer 7), as well as a high number of ChATi axons coursing in tectal layer 14. Note the presence of ChATi cell in bodies tectal layer 10. Each cell body has a long, radially arranged, apical process, which ramifies in upper tectal layers. Scale bar = 100 μ m.

laterally to exit the brain through the roots of nerves IX and X, respectively. Other groups of multipolar ChATi motoneurons were present in the ventrolateral floor of the caudal

rhombencephalon (Figs. 9, 24B,C, 25, 26). One of them, located caudal to the VIIv, consisted of a group of large labeled motoneurons of nerve IX, called the retrofacial IX motoneurons (RFa) by Wild (1981). The other group of labeled motoneurons was located caudal to RFa and represented the nucleus ambiguus (Amb). The labeled axons of RFa and Amb motoneurons coursed dorsomedially and then laterally to exit the brain. Another prominent group of large ChATi motoneurons was present at caudal rhombencephalic levels in the hypoglossal nucleus (XIIIm). This cell group was called nucleus intermedius in the atlas of Karten and Hodos (1967) (Figs. 9, 25). The labeled hypoglossal axons coursed ventrally into the hypoglossal nerve.

Small scattered ChATi neurons were also present in the reticular formation of the caudal rhombencephalon (Figs. 9, 24, 25), the nucleus reticularis parvocellularis (Rpc), the nucleus reticularis gigantocellularis (Rgc), the nucleus paragigantocellularis lateralis (PGL), and the nucleus reticularis lateralis (RL). Fusiform ChATi somata were also present in the nucleus paramedianus (PaM) flanking the midline. Finally, sparse ChATi neurons were observed in dorsal areas of the caudal rhombencephalon, such as the VeM, the nucleus cuneatus externus (CE), and the nucleus solitarius (Fig. 9).

Spinal cord. Large ChATi motoneurons were present in the ventral part of the ventral horn, in the so-called nucleus supraspinalis (SSp) (Figs. 9, 25). These labeled neurons showed radially arranged dendrites, and their axons coursed ventrally into the ventral rootlets. More dorsally in the ventral horn, ChATi motoneurons were also present in the spinal part of the Xm and XIIIm. Small ChATi neurons surrounded the large cholinergic motoneurons of the ventral horn. Small ChATi neurons were present in the substantia gelatinosa Rolandi (SG) of the dorsal horn. ChATi neurons were also observed in the nucleus gracilis and cuneatus (GC) at the spinomedullary junction (Figs. 9, 25).

Distribution of ChAT-immunoreactive fibers

Telencephalon

Olfactory bulb. Numerous varicose ChATi fibers were present in the olfactory bulb (OB) (Fig. 1). A dense accumulation of ChATi varicose fibers was observed in the periventricular neuropil surrounding the ventricle, while a moderate to light abundance was present in the granule cell layer (gcl), the internal plexiform layer (ipl), the mitral cell layer (mcl), and the external plexiform layer (epl). A very dense accumulation of thick immunoreactive varicose fibers was observed in the glomerular layer (gl). Caudal to the OB, the nucleus olfactorius anterior (Noa) contained a moderate number of ChATi fibers and varicosities.

Dorsal telencephalon (Wulst, DVR, hippocampal complex, archistriatal complex, and piriform cortex). The dorsal telencephalon contained a moderate to high number of ChATi fibers and varicosities, which were mainly located close to the brain surface. The labeled fibers in the dorsal telencephalon presumably represent mainly axons of extrinsic origin, since no intrinsic ChATi neurons were present in this region (except for the extremely few cells of the CPI and rostromedial neostriatum). Numerous ChATi fibers and varicosities were present in the Wulst [which comprises the hyperstriatum accessorium (HA), the hyperstriatum intercalatum superior (HIS), and the hyperstriatum dorsale (HD)] (Figs. 1–3). The ChATi innervation of the Wulst was mainly confined to its superficial part, and was particularly abundant in the dorsolateral HA, which constitutes part of

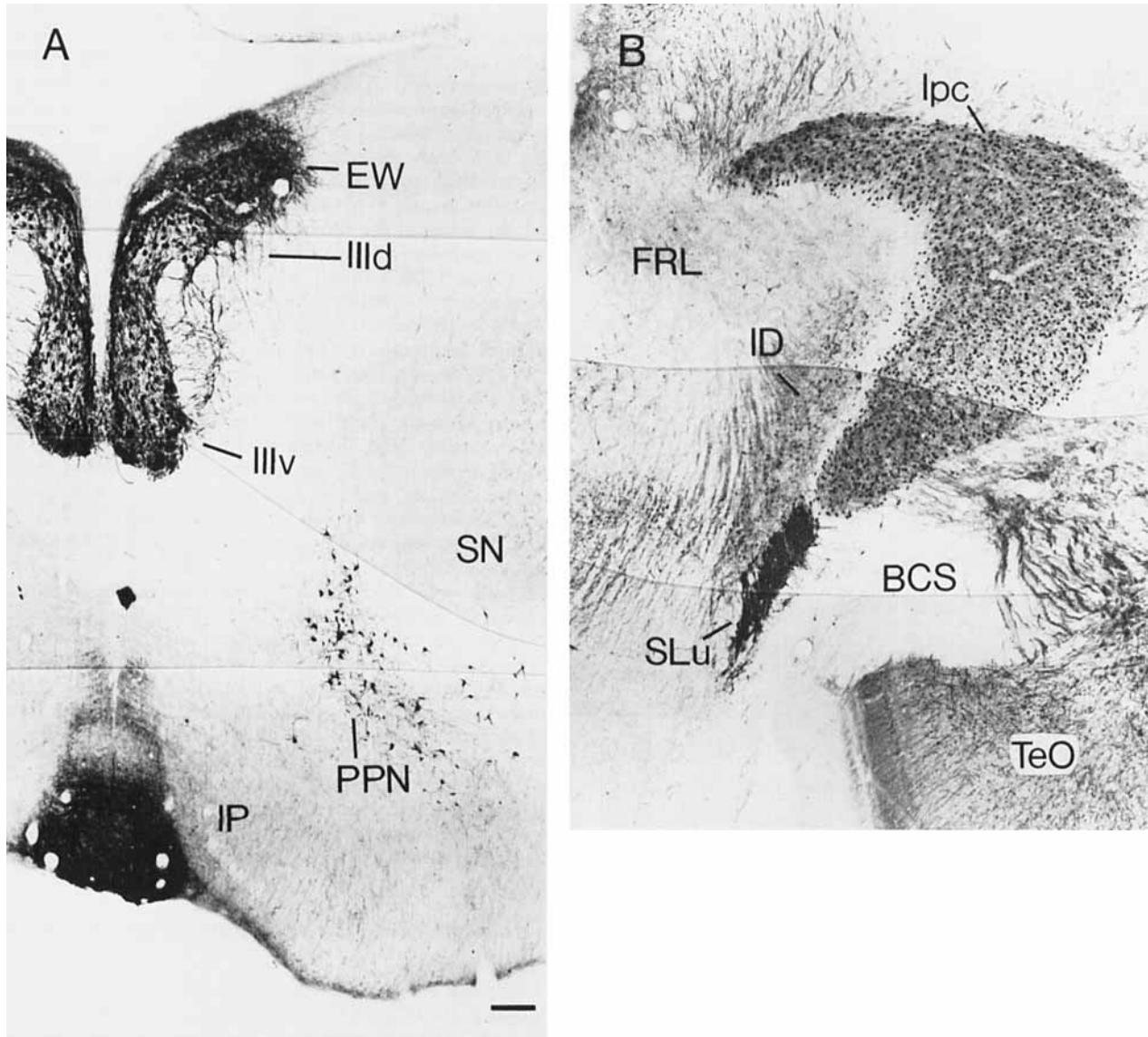


Fig. 19. Photomicrographs of transverse sections through the pigeon mesencephalon and isthmus. **A:** ChATi cell bodies in the oculomotor complex (EW, IIId, IIIv) in the mesencephalon, as well as in the isthmic pedunculopontine nucleus (PPN). Note the extremely

dense ChATi innervation in the interpeduncular nucleus (IP). **B:** ChATi neurons in several isthmic nuclei (Ipc, SLu, and ID). Note the bundle of ChATi axons coursing from Ipc to the tectum. Scale bar = 200 μ m.

what has been termed the intercalated nucleus of the HA (IHA).

Lateral to the vallecula (the telencephalic sulcus marking the boundary between Wulst and DVR), a moderate to high number of ChATi fibers and varicosities were present at the superficial edge of the pallium dorsolateral to the hyperstriatum ventrale (HV), neostriatum (N), and cortex piriformis (CPi) (Figs. 1–5). At caudal levels, this superficial region includes the areas termed the temporo-parieto-occipitalis (TPO) and the area corticoidea dorsolateralis (CDL), while at more rostral levels this region does not have distinct names (Figs. 4, 5). This large superficial region of the DVR was distinguishable from the inner core of the DVR and recognizable as a distinct entity on the basis of its high gamma-aminobutyric acid (GABA) innervation, its connections, and its spatiotemporal pattern of neurogenesis (Tsai

et al., 1981a,b; Rehkämper et al., 1985; Rehkämper and Zilles, 1991; Veenman and Reiner, 1994). This entire region has been termed the pallium externum (PE) by Veenman and Reiner (1994). At caudal telencephalic levels, the archistriatum (which is also a superficial part of the DVR) contained a conspicuously high number of ChATi fibers and varicosities in its dorsal part (Ad), whereas a moderate number was present in the ventral archistriatum (Av) (Fig. 5). Finally, ChATi varicose fibers were present in the hippocampus (Hp) and the area parahippocampalis (APH), which are also superficial pallial structures (Fig. 5). In contrast to the moderate to high ChATi innervation of the superficial dorsal telencephalon, the deeper part of the dorsal telencephalon (i.e., most of hyperstriatum ventrale and neostriatum, and the ectostriatum) was poorly innervated, with the exception of the rostromedial neostriatum,

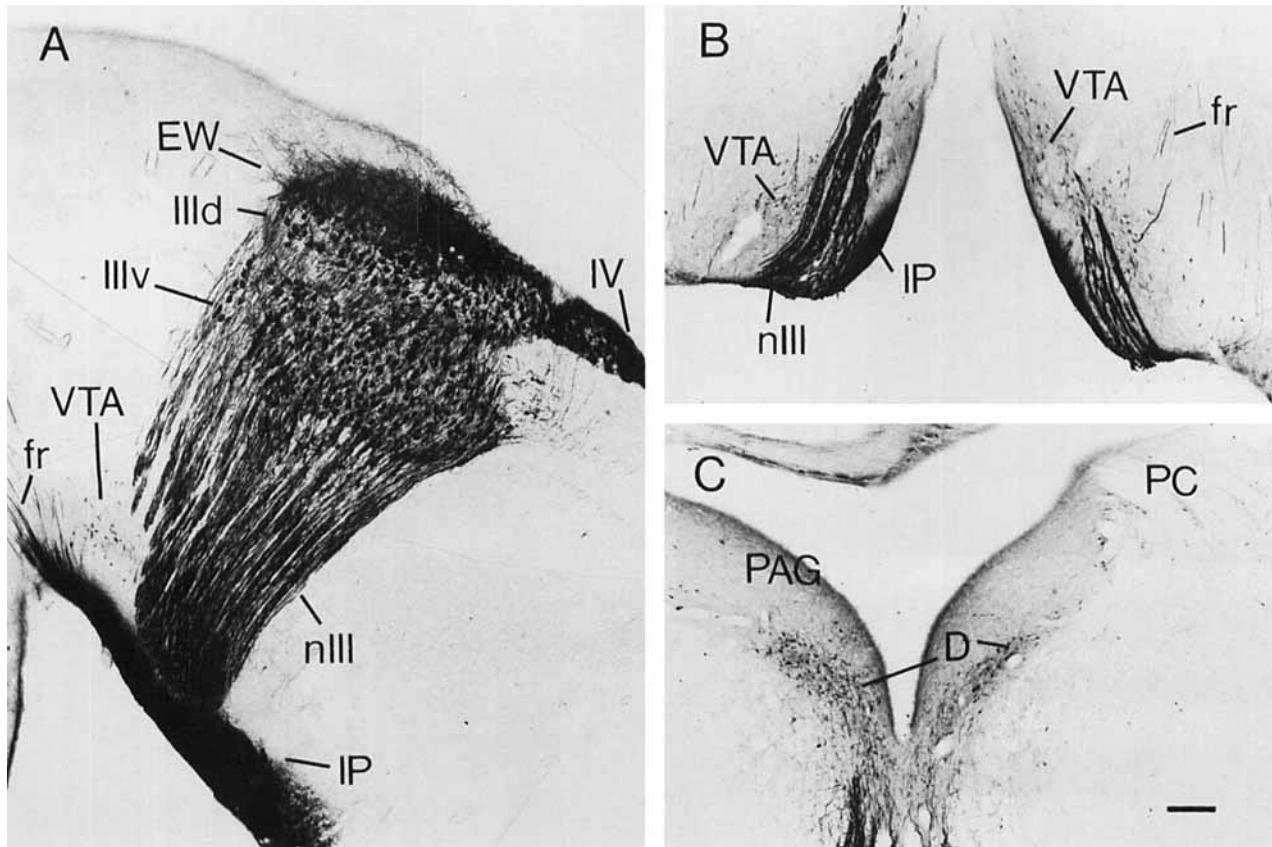


Fig. 20. Photomicrographs of sagittal (A) or transverse (B,C) sections through the pigeon mesencephalon and isthmus. **A:** ChATi neurons in the oculomotor (EW, IIId, IIIv) and troclear (IV) motor nuclei, as well as in the VTA. Note the ChATi oculomotor axons coursing ventrally to exit the brain through the oculomotor nerve (nIII).

Note also the ChATi axons of the fasciculus retroflexus (fr) arriving to the interpeduncular nucleus (IP). **B:** nIII, fr, VTA, and IP as observed in a transverse section. **C:** ChATi neurons in the PAG and D. Scale bar = 200 μ m.

where a moderate number of ChATi fibers and varicosities were present (Figs. 1, 2).

Numerous ChATi nonvaricose fibers, presumably axons, were observed in various telencephalic structures, giving the appearance by their orientation that they were extending from the basal to the dorsal telencephalon. These ChATi axons were observed within: 1) the pallium externum, coursing parallel to the pial surface; and 2) the hyperstriatum ventrale dorsoventrale (HVdv), coursing parallel to the lamina frontalis superior (LFS). Additional populations of ChATi axons also were observed seemingly extending from the basal to the dorsal telencephalon, coursing through HD, through the neostriatum, and along the medial telencephalic wall.

Basal telencephalon. Numerous ChATi fibers and varicosities were present within the basal telencephalon (Figs. 1–5, 10–12). A mat of numerous lightly labeled thin fibers and varicosities was observed throughout the medial (LPO) and lateral (PA) parts of the dorsal striatum. The PA contained, in general, more fibers and varicosities than the LPO. The cholinergic innervation in LPO and PA was homogeneously distributed, although patches of denser ChATi innervation were observed along the dorsal surface of the LPO and PA, adjacent to the lamina medullaris dorsalis. A moderate to high number of ChATi fibers and varicosities were also observed in such ventral striatal

regions as the olfactory tubercle (TO), the lateral and medial olfactory tracts (lot, mot), and the bed nucleus of the stria terminalis. The paleostriatum primitivum of the basal ganglia (PP) contained few labeled fibers and varicosities, although a moderate number of labeled dendritic processes was present in this cell group (Fig. 11). The intrapeduncular nucleus (INP) and the ventral pallidum (VP) contained a high number of fibers and varicosities (Figs. 10B,C, 11, 12C), as well as the dendritic processes of the ChATi neurons in these regions. A moderate to low number of fibers and varicosities were also present in the nucleus of the diagonal band of Broca (Ndb) and in the fasciculus prosencephalicus lateralis (FPL), in addition to the many labeled dendritic processes of ChATi cells (Figs. 2–4, 10B,C, 11). In the septum, numerous fibers and varicosities were observed in the nucleus septalis lateralis (SL) and the nucleus commissurae septi (CoS) (Figs. 3, 4, 11A). Finally, many nonvaricose thin fibers, presumably axons, that appeared to arise from the ChATi cells of the basal telencephalon and course more dorsally were observed throughout the basal telencephalon (Fig. 12C).

Diencephalon

Hypothalamus. At rostral hypothalamic levels, a moderate number of ChATi fibers and varicosities was observed in the POM (Fig. 4). A moderate number of labeled fibers and varicosities was also observed in the nucleus preopticus

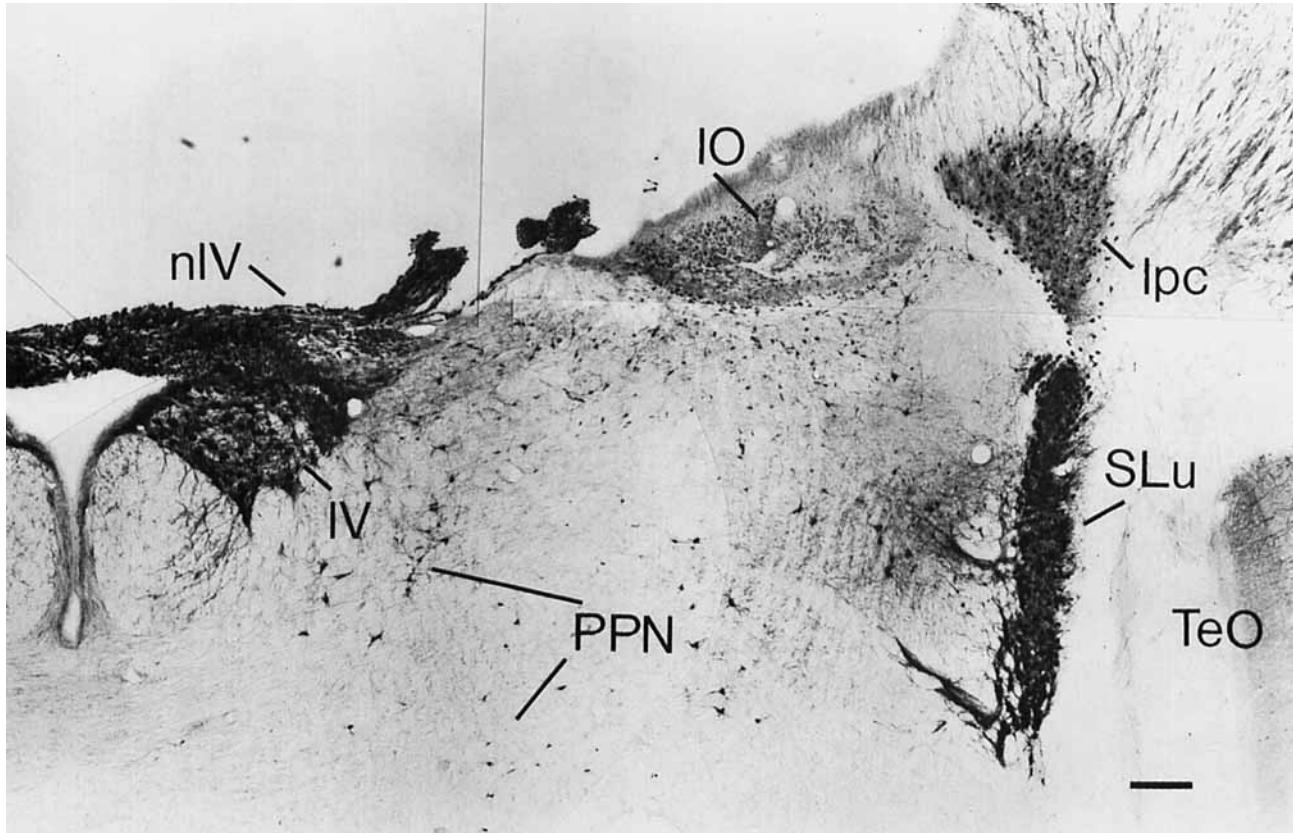


Fig. 21. Photomicrograph of a transverse section through the pigeon isthmus, showing ChATi neurons in the trochlear motor nucleus (IV), the PPN, the SLu, the Ipc, and surrounding the IO. Lightly labeled cell bodies are also observed in the IO. Scale bar = 200 μ m.

anterior and the suprachiasmatic nucleus (SCN) (Fig. 13B). At the level of the supraoptic decussation (DS), a moderate number of immunoreactive processes was observed in PMH, the majority of which were the dendrites of the ChATi PMH neurons (Figs. 4, 5, 13B, 15B). Numerous nonvaricose thin immunoreactive fibers, presumably axons, decussated in the ventral supraoptic decussation (DSV) (Figs. 4, 5). Some labeled axons were also observed in the optic chiasm and tract. In addition, a prominent but loosely collected group of ChATi fibers was observed to course in the lateral hypothalamic region medial to the ventral lateral geniculate nucleus. At rostral levels, this collection of fibers was observed to gather into a tighter bundle and cross in the DS to the corresponding contralateral site. In the lateral hypothalamus, the LHy contained low levels of ChATi fibers and terminals rostrally and moderate levels more caudally (Figs. 4–6, 13B,D, 15). A moderate number of fibers and varicosities was also present in the SCI, the SCE, and the infundibular region. In addition, ChATi fibers that appeared to be axons were observed at the lateral margin of the infundibulum. The periventricular organ (oph) contained numerous fibers and varicosities, which extensively coated the unlabeled perikarya and dendrites of oph cells (Figs. 6, 13D, 15A,C,D). Finally, nonvaricose ChATi fibers, presumably axons, coursed in the lateral part of the FPL, in the ansa lenticularis (AL), and in the medial forebrain bundle (FPM) located medial to AL. These fiber bundles are all located at the dorsolateral edge of the

hypothalamus, in the border zone between hypothalamus and thalamus.

Ventral thalamus, thalamus, and epithalamus. In the ventral thalamus, numerous ChATi fibers and varicosities were observed in the nucleus reticularis superioris pars ventralis (RSv), the nucleus lateralis anterior thalami (LA), and the external part of the nucleus geniculatus lateralis pars ventralis (GLv) (Figs. 5, 6, 13A,B,D, 14A,B, 16A). A moderate number of ChATi fibers was also observed in the internal part of the GLv. Sparse ChATi varicose fibers were present in the nucleus ventrolateralis thalami (VLT) (Figs. 4, 5). In the thalamus, numerous ChATi fibers and varicosities were observed in the DLAmc, DLL, and DLM (Figs. 5, 6, 14A,C). Numerous ChATi fibers and varicosities were also present in the nucleus superficialis parvocellularis (SPC) (Figs. 6, 16A). A moderate number of immunoreactive fibers and varicosities was also present in the nucleus dorsomedialis anterior thalami (DMA), the nucleus subtortundus (SRt) and the perirotundal area (pRot) (Figs. 5, 6, 13D). No immunoreactive fibers were observed in the nucleus rotundus. In the epithalamus, numerous ChATi fibers and varicosities were present in the nucleus habenularis medialis (HM) (Figs. 6, 13C). Two bundles of immunoreactive axons were observed entering or leaving HM. One bundle was the stria medullaris (SMe), which appeared to contain ChATi fibers coursing from the telencephalon to HM. The other was the fasciculus retroflexus (fr), which consisted of ChATi axons arising from HM (Fig. 13C).

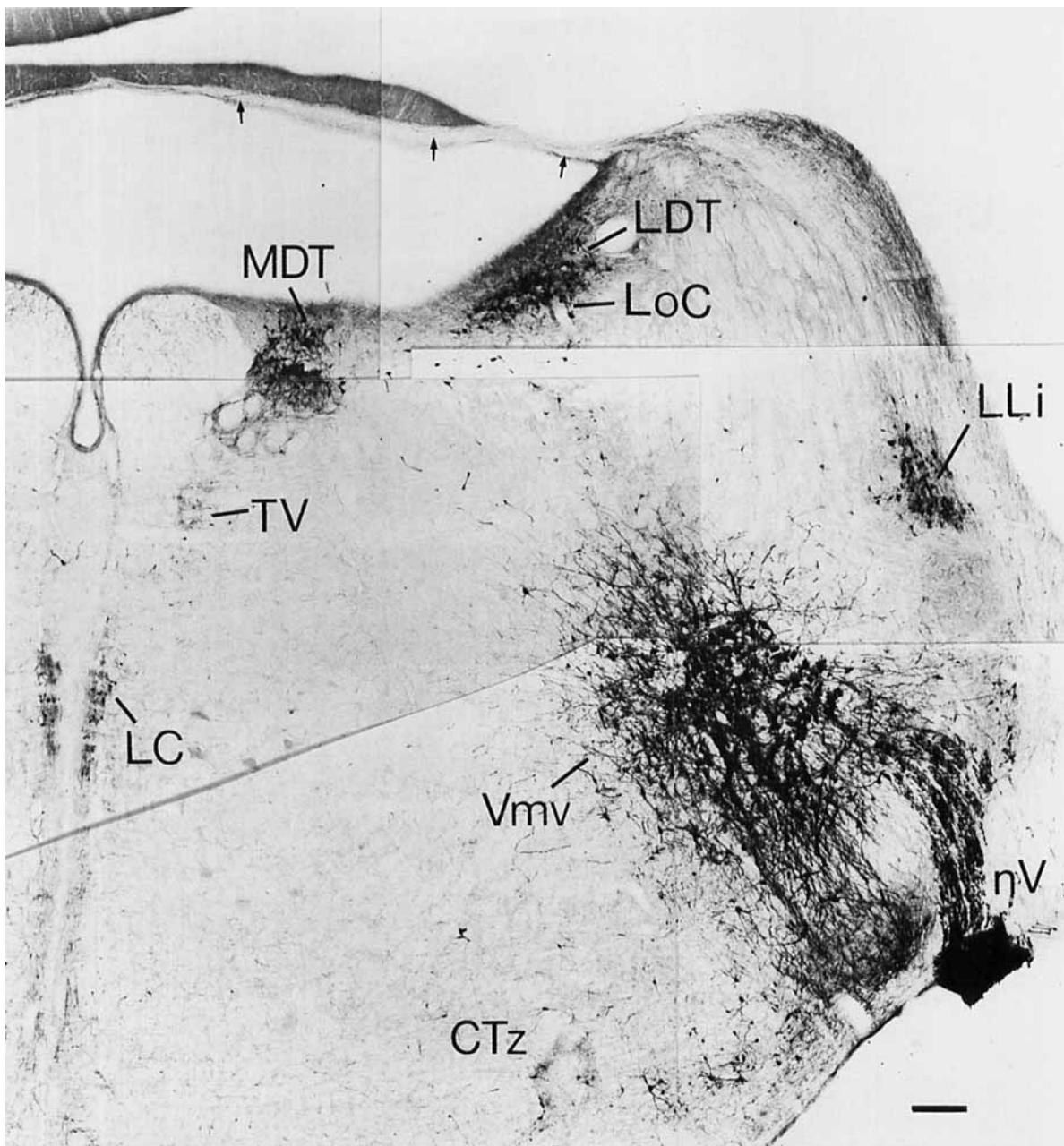


Fig. 22. Photomicrograph of a transverse section through the rostral rhombencephalon of the pigeon, showing ChATi cell bodies in the LDT, LoC, MDT, TV, LLi, LC, and trigeminal motor complex (VmV). Scale bar = 200 μm .

coursing to the interpeduncular nucleus of the isthmic tegmentum (Fig. 20A).

Pretectum. In the pretectum, a moderate to high number of immunoreactive fibers and varicosities was present in the nucleus principalis precommissuralis (PPC), the nucleus superficialis synencephali (SS), the nucleus lentiformis mesencephali (LM), and the nucleus spiriformis lateralis (SpL) (Figs. 6, 13C,D, 16). In SpL, the cholinergic fibers form a dense neuropil dorsolateral to the SpL neurons, with numerous fibers extending from this neuropil into SpL. A low to moderate number of labeled fibers was observed in

the PCpc, Jc, PTM, and SpM, all of which also contain ChATi cell bodies (Figs. 6, 16B, 17). The nucleus pretectalis (PT) and nucleus subpretectalis (SP) did not contain ChATi fibers. At caudobasal levels of the pretectum, a moderate number of ChATi fibers and varicosities was observed in the nucleus of the basal optic root (nBOR).

Mesencephalon

Optic lobe and tectum. In the rostral optic lobe, the nucleus griseum tectalis (GT) contained a moderate number of ChATi fibers and varicosities in its superficial (lateral and medial) stratum (Figs. 6, 13D, 16B). Within the tectum,

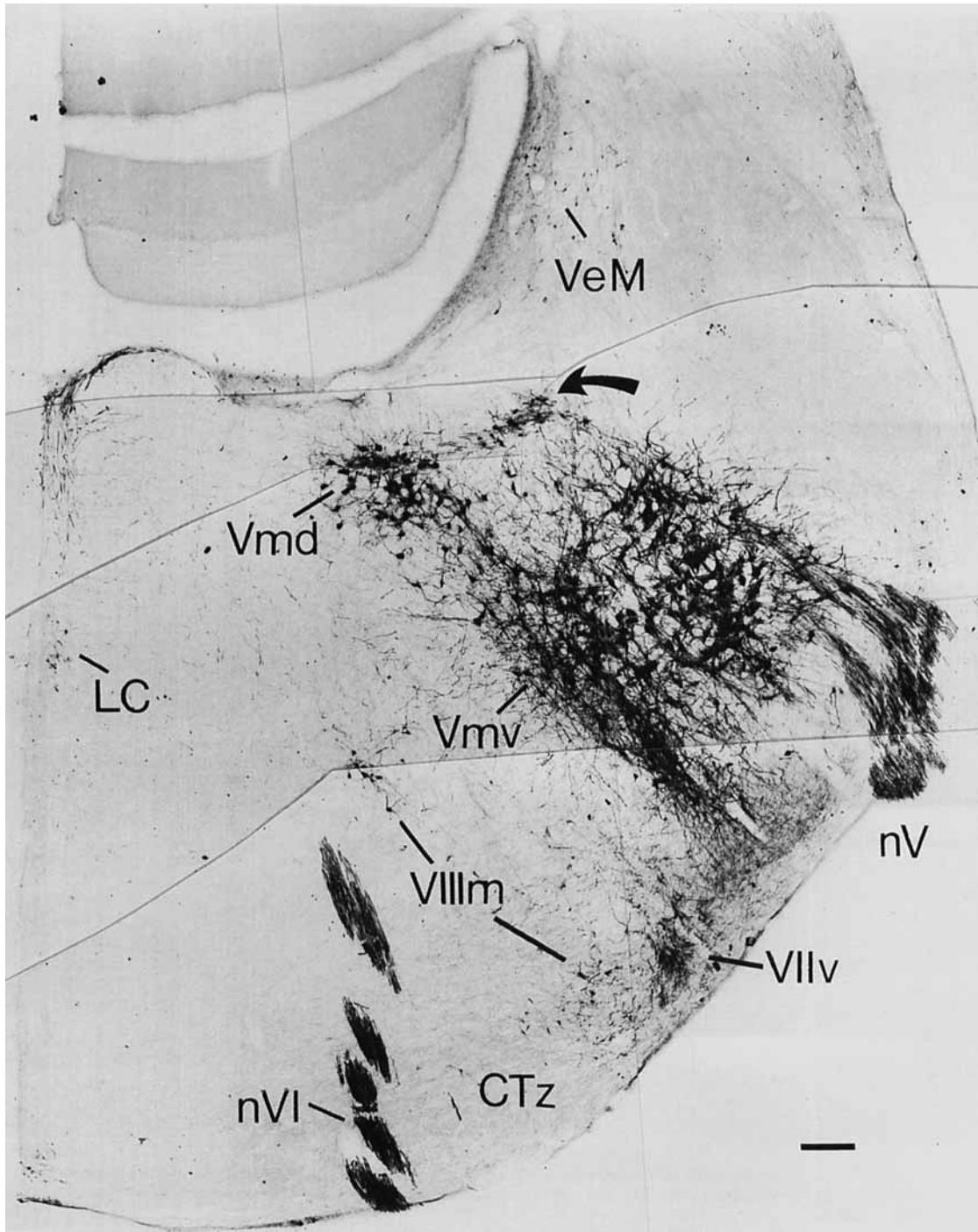


Fig. 23. Photomicrograph of a transverse section through the rhombencephalon of the pigeon, showing the ChATi motoneurons in cranial nerves V, VII, and VIII nuclei. Note that the trigeminal motor complex consists of several subdivisions, which to simplify are designated here as dorsal and ventral nuclei. The curved arrow indicates a

group of small motoneurons that is part of the trigeminal motor complex. Note the ChATi axons coursing into cranial nerves V and VI. ChATi neurons are also present in the VeM and LC. Scale bar = 200 μ m.

most of the few ChATi fibers observed in tectal layer 1 (stratum opticum) and tectal layer 2 appeared to be axons. The remainder of the stratum griseum et fibrosum superficiale (SGFS) of optic tectum (tectal layers 3–12) contained numerous ChATi fibers and varicosities (Figs. 6, 7, 18).

Three particularly dense bands of ChATi fibers were observed in specific tectal layers or sets of layers within the SGFS: 1) tectal layers 3–4; 2) the deeper half of tectal layer 5; and 3) tectal layer 7. Varicose ChATi fibers were observed in lesser abundance in the intervening tectal layers. A

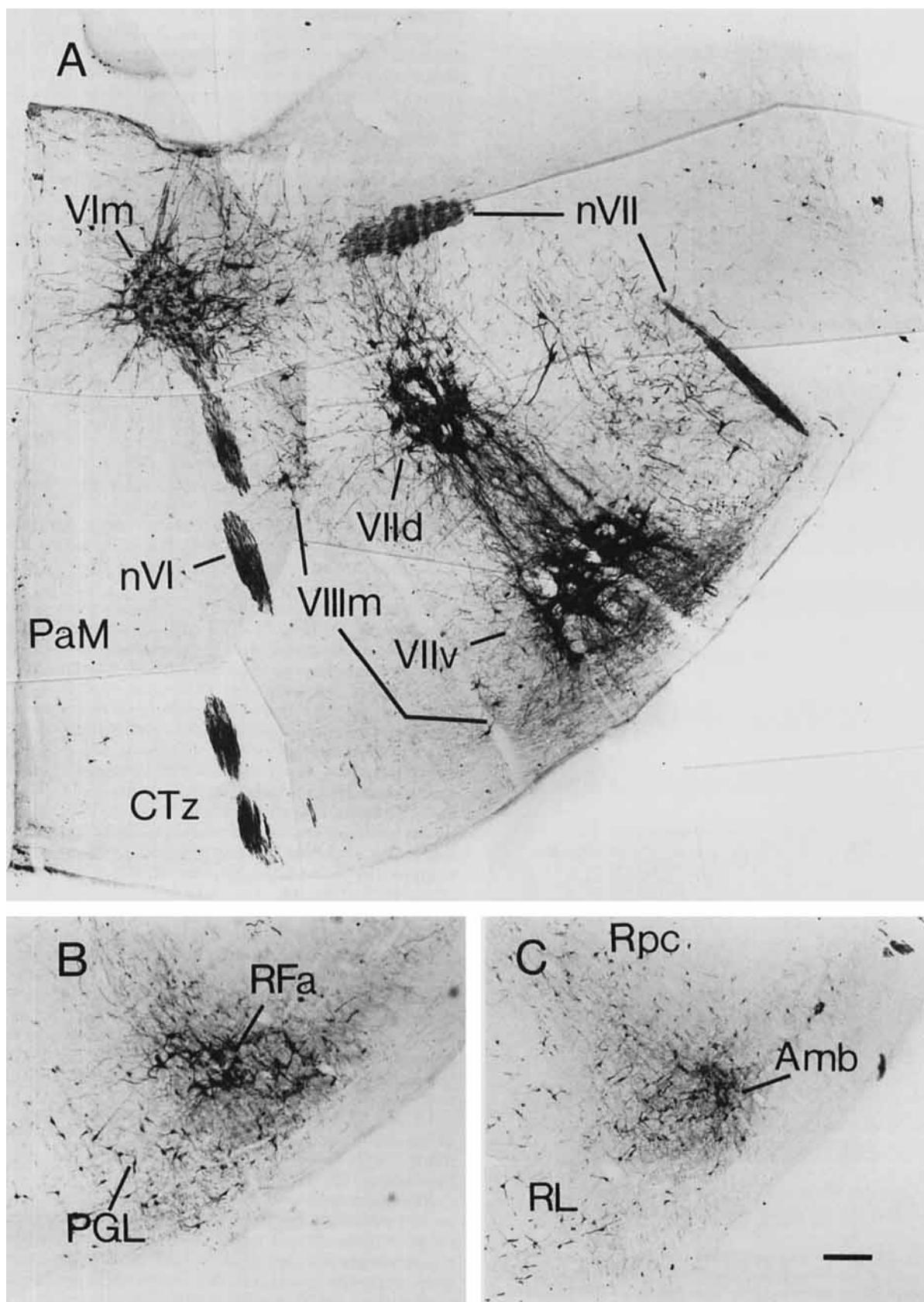


Fig. 24. **A-C:** Photomicrographs of transverse sections through an intermediate level of the rhombencephalon of the pigeon, showing ChATi motoneurons in cranial nerves VI, VII, VIII, IX, and X nuclei. ChATi neurons are also present in the reticular formation. Scale bar = 200 μm .

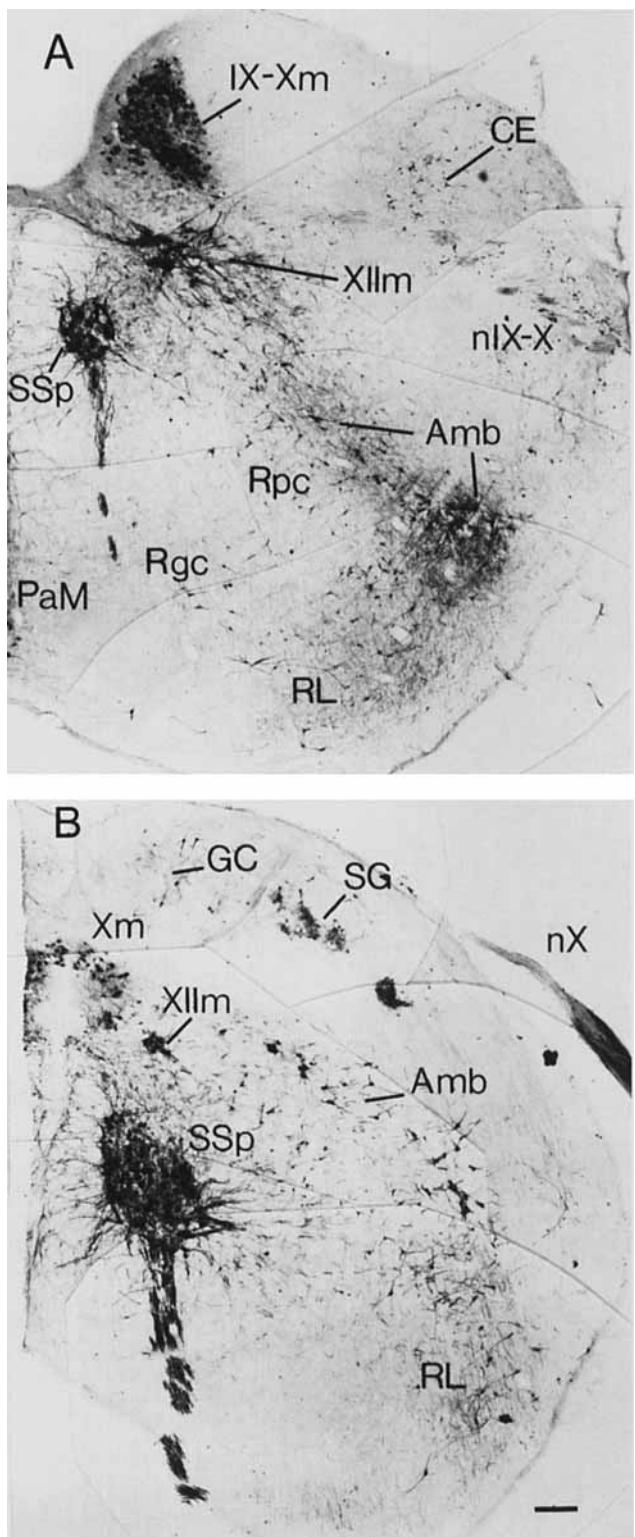


Fig. 25. **A, B:** Photomicrographs of transverse sections through a caudal level of the rhombencephalon of the pigeon, showing ChATi motoneurons in cranial nerves IX, X, and XII nuclei, as well as in the SSp. Scale bar = 200 μ m.

moderate number of varicose ChATi fibers was also present in tectal layers 8, 9, and 10. Long radially oriented ChATi processes were observed to arise from the ChATi cells of tectal layer 10, and traverse the superficial tectal layers (layers 3–9). The stratum griseum centrale (tectal layer 13) contained a moderate number of varicose ChATi fibers. Prominently numerous ChATi axons were observed to course in the stratum album centrale (tectal layer 14), and to turn, traverse layer 13, and ramify in the SGFS. The labeled axons observed in tectal layer 14 could be traced back to Ipc and the other cholinergic cell groups of the isthmic region (Figs. 6, 7, 19). Finally, the stratum griseum periventriculare (layer 15) contained a moderate number of ChATi varicose fibers.

Central gray and tegmentum. A moderate number of ChATi fibers and varicosities were present in the periaqueductal gray (PAG), the nucleus of Darkschewitsch (D), and the nucleus intercollicularis (Ico) (Figs. 6, 7). A small portion of the Ico contained a very high number of fibers and varicosities (Fig. 6). The lateral reticular formation (FRL) contained a low number of ChATi fibers and varicosities (Figs. 6, 7, 19B). A moderate number of ChATi fibers and varicosities were also observed in the ventral tegmental area (VTA) and in the substantia nigra (SN) (Figs. 6, 7, 19A). Finally, the oculomotor complex (EW, IIId, IIIv) contained a few ChATi fibers and varicosities intermingled with the dendrites of the neurons of this complex (Figs. 6, 7, 19A, 20A). Many of the labeled fibers in the complex were the axons of the motoneurons.

Isthmus. Numerous ChATi processes were present in Ipc (Figs. 6, 7, 19B, 21). Many of these were clearly the dendrites of the cholinergic Ipc neurons, while others were clearly the cholinergic axons of the Ipc neurons. We could not rule out the possibility that some of the ChATi processes in Ipc were fibers terminating in Ipc. Some ChATi fibers and terminals were also observed among the numerous ChATi dendrites of the neurons in the disseminated isthmic nucleus, the nucleus isthmi semilunaris (SLu), the medial and lateral dorsal tegmental nuclei (MDT, LDT), and the locus coeruleus (LoC) (Figs. 7, 19B, 21, 22). A moderate number of immunoreactive fibers and varicosities were also observed in the pedunculopontine tegmental nucleus (PPN) and the nucleus tegmenti ventralis (TV) (Figs. 6, 7, 19A, 21, 22). Numerous ChATi axons were observed to arise from Ipc, SLu, and PPN and course into tectal layer 14 (Figs. 6, 7, 19B, 21). Another group of ChATi axons was observed to arise in the isthmic tegmentum (presumably from the PPN and perhaps the MDT and LDT) and course rostrally as a bundle. It is this tract that courses through the ventrolateral hypothalamus internal and parallel to the optic tract and eventually decussates in the ventral supraoptic decussation (Figs. 5–7). The neuropil of the nucleus isthmo-opticus also contained numerous thick, well-labeled varicose ChATi fibers. The neuropil of the trochlear nucleus was rich in the labeled dendrites and axons of its neurons (Figs. 7, 21). Finally, an extremely dense ChATi innervation was observed in the interpeduncular nucleus (IP) (Figs. 19A, 20A,B).

Rhombencephalon. Numerous ChATi fibers were observed extending toward the cerebellum via the brachium conjunctivum (BC). It seems likely that these contribute to the moderate number of fibers and varicosities among the deep cerebellar nuclei [i.e., the processus lateralis cerebelli-ovestibularis (PCV); nucleus cerebellaris intermedius (CbM); and nucleus cerebellaris lateralis (Cbl)] (Fig. 8) and the moderate number of ChATi mossy fibers in the cerebellar

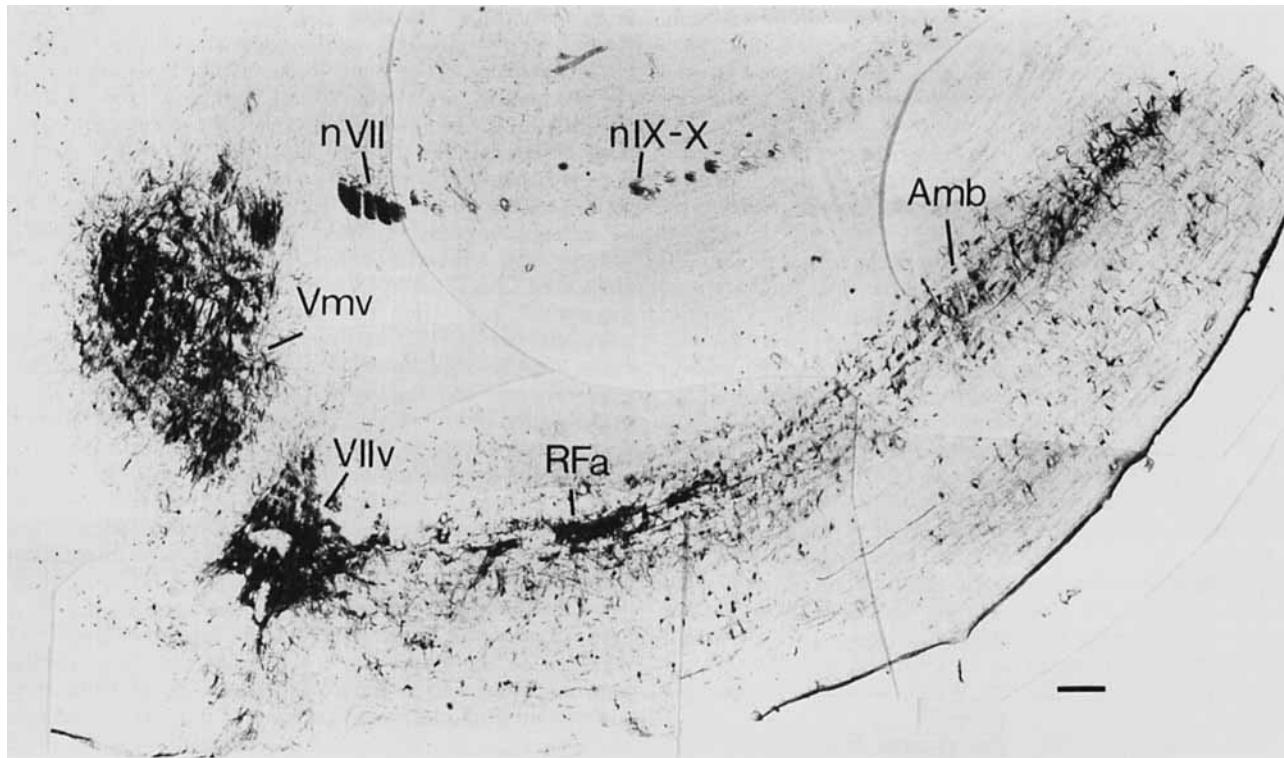


Fig. 26. Photomicrograph of a sagittal section through the rhombencephalon of the pigeon, showing ChATi motoneurons in cranial nerves V, VII, IX, and X nuclei. Scale bar = 200 μm .

granule cell layer (Fig. 22). A moderate number of ChATi fibers and varicosities was present in the nucleus vestibularis medialis (VeM), the nucleus vestibularis dorsolateralis (VDL), and the nucleus vestibularis superior (VS) (Figs. 8, 9, 23). Further caudally in the dorsal rhombencephalon, immunoreactive fibers and varicosities were observed in the nucleus cuneatus externus (CE) (Figs. 9, 25A).

In the ventral rhombencephalon, numerous ChATi fibers and varicosities were observed in intermediate nucleus of the lateral lemniscus (LLi) and in the nucleus linearis caudalis (LC) (Fig. 22). Among cell groups of the reticular formation, ChATi fibers and varicosities were observed in the corpus trapezoideum (CTz), the nucleus reticularis pontis caudalis (RP), the nucleus reticularis parvocellularis (Rpc), the nucleus reticularis pontis caudalis pars gigantocellularis (Rgc), the nucleus paragigantocellularis lateralis (PGL), the nucleus reticularis lateralis (PL), and the nucleus paramedianus (PaM) (Figs. 8, 9, 22–26). ChATi fibers were also observed coursing longitudinally in the ventrolateral rhombencephalon, close to the pial surface (Fig. 26).

Among cranial nerve motor cell groups, a moderate or low number of ChATi fibers and varicosities was present in the dorsal and ventral motor trigeminal nuclei (Vmd, Vmv), the nucleus abducens (Vlm), the dorsal and ventral motor facial nuclei (VIId, VIIv), the retrofacial IX motoneurons (RFa), the nucleus ambiguus (Amb), the glossopharyngeal motor nucleus (IXm), the dorsal vagal motor nucleus (Xm), and the hypoglossal motor nucleus (XIm) (Figs. 7–9, 22–26). Dense plexuses of fibers and varicosities were observed ventrolateral to the Vmv and VIIv, surrounding the ventrolaterally directed dendrites of the motoneurons of the Vmv and VIIv (Figs. 22, 23, 24A). Finally, the roots of

the cranial nerves V, VI, VII, IX, X, and XII contained numerous ChATi fibers representing the labeled axons of the motoneurons of these cell groups (Figs. 8, 9, 22–26). Some ChATi fibers were also observed in the root of cranial nerve VIII (Fig. 9).

Spinal cord. In the dorsal horn, numerous ChATi fibers and varicosities were observed in the substantia gelatinosa Rolandi (SG) (Figs. 9, 25B). ChATi fibers and varicosities were also observed in the nuclei gracilis et cuneatus (GC). In the ventral horn, a moderate number of ChATi fibers and varicosities was present in the nucleus supraspinalis (SSp) (Figs. 9, 25). Numerous ChATi fibers and varicosities were also present in the lateral funiculus. ChATi fibers that appeared to be axons were observed in the ventral funiculus.

DISCUSSION

Organization of cholinergic systems in birds

In this section, we will compare our results with those in previously published studies on the avian brain. The goal will be to provide insight into the organization of the cholinergic systems of the avian brain and into the functional relationship of these systems to other transmitter systems in specific brain regions (such as peptidergic and catecholaminergic systems in the basal ganglia or the midbrain/isthmic tegmentum).

Olfactory bulb. Our observation of moderate to high cholinergic innervation in the olfactory bulb of the pigeon is in agreement with the finding of high levels of muscarinic receptors in the chick olfactory bulb (Wächtler and Ebinger, 1989). The absence of cholinergic neurons in the olfactory

bulb indicates that the cholinergic innervation of the bulb arises outside the bulb. Since studies in mammals indicate a basal forebrain origin and since this region in birds is also rich in cholinergic neurons, we hypothesize a basal forebrain origin in birds too.

Telencephalon—Wulst. Our results corroborate the moderate to high cholinergic innervation found in the pigeon visual Wulst by Shimizu and Karten (1990). In general, the distribution of ChATi fibers in the Wulst (heaviest in the dorsolateral HA, the intercalated nucleus of the HA, and the dorsal parts of the HIS and HD) is consistent with the distribution of muscarinic (Wächtler and Ebinger, 1989) and nicotinic (Shimizu and Karten, 1990; Sorenson and Chiappinelli, 1992) cholinergic receptors in the Wulst. Although several studies (including this one) have noted that cholinergic neurons are absent in the visual Wulst of the avian telencephalon (Shimizu and Karten, 1990; von Bartheld et al., 1991; present results), one study has observed a few ChATi cell bodies in the Wulst of colchicine-injected pigeons (Bagnoli et al., 1992). The cholinergic character of the ChATi cells observed in the avian Wulst by these authors must be considered with caution. We did not observe such ChATi neurons in the Wulst in either our colchicine-treated pigeons or in sections processed with the anti-human ChAT. Thus, there is no definitive evidence that the Wulst contains cholinergic neurons.

Telencephalon—DVR. The present study shows that cholinergic neurons are extremely sparse in the DVR, being limited to a small number of ChATi cell bodies at rostromedial levels of the neostriatum (N) and in the cortex piriformis (CPi). In the present study, however, a moderate to high cholinergic innervation was observed in the rostromedial neostriatum, the pallium externum, the hippocampal complex, and the dorsal archistriatum. All of these areas also contain moderate to high levels of muscarinic receptors (Wächtler and Ebinger, 1989), and low levels of nicotinic receptors (Sorenson and Chiappinelli, 1992). The paucity of cholinergic neurons in the DVR indicates that DVR cholinergic innervation mostly arises outside the DVR. In contrast to the good transmitter-receptor match for the above-noted DVR areas, a poor match occurs in the HV. We found only sparse cholinergic innervation of HV of the pigeon, yet HV has been shown to contain high levels of both muscarinic and nicotinic receptors in chickens (Wächtler and Ebinger, 1989; Sorenson and Chiappinelli, 1992). One possible explanation of this mismatch is that there is an interspecies difference in the cholinergic innervation of the HV. The possibility also exists that cholinergic fibers innervate the HV more extensively than could be detected with our methods. It should be emphasized, however, that neurotensin and its receptors as well as opioid peptides and their receptors also show significant mismatches in HV, with the neurotransmitters being extremely sparse and the receptors extremely abundant (Brauth et al., 1986; Reiner et al., 1984a,b). Thus, mismatches between receptor levels and endogenous ligand levels may be a feature of HV for a number of neurotransmitter systems.

Basal telencephalon: striatal areas. Our results demonstrate a dense cholinergic innervation of the pigeon striatum (LPO and PA), which is in agreement with the observation of high levels of muscarinic receptors in goose striatum (Wächtler and Ebinger, 1989) and low to moderate levels of nicotinic receptors in the LPO (Sorenson and Chiappinelli, 1992). Other striatal cell groups of the basal telencephalon (olfactory tubercle and the bed nucleus of the

stria terminalis) are also rich in cholinergic fibers and terminals. The present results show that many dispersed ChATi neurons are present in the pigeon medial striatum (LPO), and in such striatal cell groups as the olfactory tubercle and the bed nucleus of the stria terminalis. Hodological studies indicate that avian striatal projection neurons are medium-sized cells (7 μm by 11 μm) that contain either substance P (SP), dynorphin (DYN), GABA, or enkephalin (ENK)-GABA (Reiner, 1986; Anderson and Reiner, 1990, 1991; Reiner and Anderson, 1990). Our results show that ChATi cells of the pigeon striatum are larger (11 μm by 19 μm) than the projection neurons. Further, unpublished observations from our laboratory indicate that these ChATi neurons do not project to the tegmental dopaminergic cell fields (K.D. Anderson and A. Reiner, unpublished observations). This indicates that ChATi neurons in the avian striatum must be local-circuit neurons.

Basal telencephalon: pallidum, diagonal band, and septum. Our data reveal that many large, intensely labeled ChATi neurons are present in the paleostriatum primitivum (PP), the ventral paleostriatum (VP), the intrapenduncular nucleus (INP), and the diagonal band of Broca (Ndb). These cholinergic neurons appear to make up a single large field that spans these several cell groups. The ChATi neurons of the basal forebrain appear to be projection neurons since axons that we presume arise from them course into the striatum and through the dorsal telencephalon (see Figs. 3, 12C). It thus seems likely the ChATi neurons of the basal telencephalon contribute to the cholinergic innervation observed in the cortical areas, as suggested by Krebs et al. (1991) and Bagnoli et al. (1992), as well as to a part of the cholinergic innervation of the striatum. Supporting the former idea, the Ndb and the CoS have been shown to project heavily onto the dorsomedial telencephalon (APH and Hp; Benowitz and Karten, 1976; Casini et al., 1986), whereas the INP has been shown to project onto the TPO (Brauth et al., 1978). Our unpublished studies combining ChAT immunolabeling with retrograde Fluorogold labeling indicate that cholinergic neurons of the basal telencephalon do project to Wulst and DVR (L. Medina, C.L. Veenman, and A. Reiner, unpublished observations). In addition, we found that the ventral paleostriatum (VP) and the intrapenduncular nucleus (INP) of pigeon were rich in cholinergic fibers and terminals, while the paleostriatum primitivum (PP) and FPL were poor in cholinergic innervation. These observations agree with the findings showing that PP and FPL possess no or few muscarinic and nicotinic receptors, while VP and INP possessed moderate levels of both (Wächtler and Ebinger, 1989; Sorenson and Chiappinelli, 1992).

The cholinergic cells of the basal telencephalon may also project outside the telencephalon, since ChATi fibers are seen coursing in the outflow tracts of the telencephalon, such as the stria medullaris (SMe) and the fasciculus prosencephalicus medialis (FPM). Cholinergic fibers of the SMe reach the medial habenular nucleus (HM). Since the pallidal regions of the telencephalon project to the lateral habenula and pallidal efferents course via the FPM (Karten and Dubbeldam, 1973; Kitt and Brauth, 1981), the cholinergic fibers of the stria medullaris must arise from cholinergic neurons in the septum or the Ndb. As discussed below, such a pathway has been described in mammals.

With respect to the FPM, a number of studies have shown that the pallidal telencephalic areas project via the FPM into the lateral habenula (HL), posterior nucleus of the ansa lenticularis (ALp), the thalamic dorsointermediate

nucleus (DIP), the pretectal lateral spiriform nucleus (SpL), the VTA and substantia nigra of the mesencephalic tegmentum, and the PPN of the isthmic tegmentum (Karten and Dubbeldam, 1973; Kitt and Brauth, 1981; Reiner et al., 1982a). Among these target areas, the SpL is the only cell group containing numerous ChATi fibers and varicosities. The published data indicate that the pallidal input ends directly in SpL and the cholinergic innervation is richest dorsolateral to SpL (Karten and Dubbeldam, 1973; Kitt and Brauth, 1981; Reiner et al., 1982a). This observation has been confirmed by double-labeling experiments combining biotinylated dextran amine (BDA) injections in the pigeon pallidum and ChAT immunohistochemistry on the same sections (Medina and Reiner, unpublished observations). These observations imply that: 1) pallidal cholinergic neurons do not project to the SpL; and 2) cholinergic projection neurons of the pigeon pallidum are distinct from the GABAergic pallidal neurons that project to the SpL (Reiner, 1986; Reiner et al., 1982b). Similarly, pallidal neurons containing GABA/LANT6 neurons project to VTA and the substantia nigra, while cholinergic pallidal neurons do not (Reiner, 1986; Reiner and Carraway, 1987; Anderson and Reiner, unpublished observations). Thus, the cholinergic projection neurons of the pigeon pallidum do not appear to project to the brainstem, and they represent a population separate from the GABA/LANT6 pallidal projection neurons. The ChATi axons we observed coursing within the FPM may represent afferent fibers projecting to the telencephalon, as will be discussed later.

Finally, numerous small and lightly labeled ChATi neurons were also observed in the lateral septum (SL) with the anti-chicken ChAT. Only a few very lightly labeled neurons were observed in SL with the anti-human ChAT. In contrast to the neurons of the ventromedial septum (CoS), the neurons of the lateral septum do not seem to project to the cortical-like areas of the pigeon brain (Benowitz and Karten, 1976; Brauth et al., 1978; Casini et al., 1986). The lightness of the labeling in these cells with both anti-chicken ChAT and the anti-human ChAT, and the absence of cholinergic cells with similar connections in a similar location in other vertebrate groups, raise the possibility that these ChAT+ neurons may not actually be cholinergic but may possess an antigen similar to chicken ChAT.

Diencephalon—hypothalamus. Our results on the distribution of cholinergic fibers in the avian hypothalamus are consistent with the studies on the location of cholinergic receptors. For example, moderate to high levels of nicotinic receptors have been described in the SCN, LHy, SCE, VMH, and oph (Britto et al., 1992; Sorenson and Chiappinelli, 1992), where we have found a moderate to high cholinergic innervation. Our observation that most of the cholinergic fibers in the PMH are dendrites of the intrinsic ChATi neurons also agrees with the low levels or lack of muscarinic or nicotinic cholinergic receptors observed in this nucleus (Wächtler and Ebinger, 1989; Britto et al., 1992; Sorenson and Chiappinelli, 1992). The present study also demonstrates cholinergic neurons in several regions of the pigeon hypothalamus. For example, cholinergic cell bodies are located in the POM and PMH, which are known to project to the septum (SL, CoS), dorsomedial thalamus, periventricular hypothalamus (including POM and PMH), SCE, tuberomammillary hypothalamus (including SCI), hypophysis (median eminence), VTA, PGA, Ico, and substantia grisea et fibrosa periventricularis of the midbrain tectum (Berk and Butler, 1981). The ChATi neurons of POM and PMH may thus contribute to the cholinergic innerva-

tion observed in these hypothalamic and extrahypothalamic regions. In addition, the present study revealed ChATi neurons in the LHy, SCE, and tuberomammillary hypothalamus (SCI and TU). Cholinergic neurons in the tuberal hypothalamus may contribute to the ChATi axons observed coursing in the lateral margin of the infundibulum. Hodological data indicate that fibers coursing in this zone typically innervate the median eminence (Berk and Butler, 1981).

Diencephalon—thalamus. The present results indicate an abundant cholinergic innervation in all retinorecipient thalamic nuclei (as well as in the retinorecipient SCN of the hypothalamus), corroborating previous results in pigeons and chicks (Sorenson et al., 1989; Güntürkün and Karten, 1991; Bagnoli et al., 1992). Consistent with this, high levels of nicotinic receptors have been described in these retinorecipient nuclei (Britto et al., 1992; Sorenson and Chiappinelli, 1992). High levels of muscarinic receptors, however, are only present in GLv among these retinorecipient thalamic nuclei (Wächtler and Ebinger, 1989). Since retinal ganglion cells do not contain ChAT (Morgan et al., 1981; Sorenson et al., 1989), the retina is unlikely to be the source of the dense cholinergic innervation of the primary visual nuclei of the thalamus. Several nonvisual thalamic nuclei in birds, such as the RSv, DMA, and SRt, are also well innervated by cholinergic fibers and contain moderate levels of nicotinic and muscarinic receptors (Wächtler and Ebinger, 1989; Britto et al., 1992; Sorenson and Chiappinelli, 1992). As discussed below, we believe that the cholinergic neurons of the isthmic tegmentum are likely to be the major source of the cholinergic innervation of these diverse thalamic nuclei.

In the present study, lightly labeled ChATi cell bodies were observed in the ventral (RSv) and dorsolateral (DLAmc, DLL, DLM) thalamus. The cell bodies of the RSv did not label, however, for the anti-human ChAT, and they were not found to be ChATi in the chicken (Sorenson et al., 1989). Therefore, the possibility must be considered that RSv neurons are not truly cholinergic, but contain an antigen similar to chicken ChAT. In contrast, many neurons of DLAmc, DLL, and DLM did label for anti-human ChAT. The DLAmc, DLL, and DLM are the main retinorecipient nuclei of the avian dorsal thalamus. They have been collectively termed the nucleus opticus principalis (OPT) and considered homologous to the mammalian dorsal lateral geniculate nucleus (Repérant, 1973; Meier et al., 1974; Miceli et al., 1975; Ehrlich and Mark, 1984; Watanabe, 1987; Gamlin and Cohen, 1988; Güntürkün and Karten, 1991). In pigeons, the ChATi neurons of the DLAmc and DLL have previously been shown to project to the visual Wulst (Bagnoli et al., 1981; Güntürkün and Karten, 1991), and seemingly contribute to the cholinergic innervation observed in this structure (Vischer et al., 1980). Further, neurons of DLAmc have been shown to label retrogradely following tritiated choline injection into the pigeon Wulst (Bagnoli et al., 1981). Many non-ChATi neurons projecting to the visual Wulst and GABAergic interneurons are also present in the OPT (Güntürkün and Karten, 1991; Veenman and Reiner, 1994). Although we and other authors have observed the DLAmc, DLL, and DLM to contain ChATi neurons that label with anti-chicken ChAT in pigeons (Güntürkün and Karten, 1991; Bagnoli et al., 1992), these cell groups have not been observed to contain ChATi neurons in chicks with this same antisera (Sorenson et al., 1989; Puelles and Martinez, unpublished observations). Thus, although our evidence indicates the ChAT

labeling of OPT neurons in pigeons to be specific, the failure to find similar neurons in other avian species raises the possibility that the ChATi neurons in pigeon OPT are not cholinergic but contain an antigen similar to ChAT.

Diencephalon—epithalamus. The available data show that numerous densely clustered cholinergic cell bodies are present in the medial habenula of pigeons and chickens (Sorenson et al., 1989). The data also clearly show that these cholinergic neurons project to the interpeduncular nucleus via the fasciculus retroflexus, since this fiber bundle can be traced as a collection of cholinergic axons from the medial habenula to the interpeduncular nucleus. In addition, the habenula appears to receive a cholinergic input from the telencephalon via the stria medullaris (SMe). This may be a source of the numerous cholinergic fibers and varicosities we observed in the medial habenula of pigeon. Matching this cholinergic fiber distribution, moderate levels of nicotinic and muscarinic receptors have been found in the HM region (Wächtler and Ebinger, 1989; Britto et al., 1992; Sorenson and Chiappinelli, 1992).

Prepectum. The present results corroborate the presence of a dense cholinergic innervation in the retinorecipient pretectal nuclei of birds (i.e., the SS and LM) observed in previous studies (Sorenson et al., 1989; Güntürkün and Karten, 1991). Consistent with the abundant cholinergic innervation of these nuclei, high levels of nicotinic receptors have also been described in them (Britto et al., 1992; Sorenson and Chiappinelli, 1992), and high levels of muscarinic receptors are also present in some of them (Wächtler and Ebinger, 1989). The source of this innervation is likely to be the same as for the thalamic retinorecipient cell groups. As also described in chicks (Sorenson et al., 1989), the SpL of pigeons is prominently innervated by cholinergic fibers. These fibers form mainly a dense terminal field in a neuropil dorsolateral to the SpL neurons, from which numerous fibers extend into SpL. Consistent with this cholinergic innervation pattern, nicotinic cholinergic receptors in chicks are abundant both in SpL and in the neuropil dorsolateral to SpL (Britto et al., 1992; Sorenson and Chiappinelli, 1992). Finally, the pigeon PPC is densely innervated by cholinergic fibers (present results) and also contains moderate levels of nicotinic and muscarinic receptors (Wächtler and Ebinger, 1989; Britto et al., 1992; Sorenson and Chiappinelli, 1992).

Scattered ChATi neurons were observed in the pigeon prepectum, occupying positions adjacent to the posterior commissure and medial to the SpM. In contrast to these findings in pigeon, cholinergic cell bodies in the prepectum of chicks are densely clustered and clearly confined to the SpM (Sorenson et al., 1989). In addition, while chicken pretectal neurons are intensely ChATi (Sorenson et al., 1989), in the pigeon pretectal neurons are only lightly immunoreactive. Thus, there may be some variation in the organization of the cholinergic cell groups in the prepectum of different birds, and studies on the prepectum of other birds and on the connections of these ChATi cell groups will be needed to analyze further the basis of this apparent variation.

Mesencephalon—optic lobe. As previously described in pigeons and chicks (Sorenson et al., 1989; Bagnoli et al., 1992), the tectal gray (GT) and the tectum are densely innervated by cholinergic fibers. Consistent with these results, high levels of muscarinic and nicotinic receptors have been described in both mesencephalic structures (Wächtler and Ebinger, 1989; Britto et al., 1992; Sorenson

and Chiappinelli, 1992). The distribution of receptors in the tectum shows a laminated pattern, as does that of cholinergic fibers, and there is a good correlation between the density of receptors and the abundance of cholinergic fibers in each tectal layer. For example, the highest tectal levels of cholinergic fibers and nicotinic receptors are found in layer 7. High levels of nicotinic receptors and cholinergic fibers are also observed in tectal layers 3–4, 9–11, and 13. The present findings and previous studies also show that cholinergic neurons are present in the midbrain tectum of pigeons and chickens (Sorenson et al., 1989; Bagnoli et al., 1992). These neurons are located mainly in layer 10. This layer contains the perikarya of neurons projecting to diverse targets, including the ventral geniculate nucleus (GLv), the parvocellular isthmic nucleus (Ipc), the isthmo-optic nucleus, and the lateral pontine nucleus (Hunt and Künzle, 1976a,b; Reiner and Karten, 1982). The morphology of the cholinergic neurons of tectal layer 10 suggests that they may correspond to the tectal neurons projecting to the Ipc and/or to the GLv (Hunt and Künzle, 1976a,b; Reiner and Karten, 1982; Hunt and Brecha, 1984; Woodson et al., 1991). The tecto-Ipc neurons possess small-medium, fusiform cell bodies, each with a single ascending radial dendrite that ramifies in upper tectum (layers 3, 5, and 9) and an axon that exits the tectum through layer 14. The tecto-GLv neurons are also small-medium fusiform cells with a single ascending radial dendrite (ramifying in layer 7) and an axon that exits via tectal layer 1 and has collaterals ramifying in layer 7. We could not determine enough of the detail of the morphology of the ChATi layer 10 neurons of pigeons to identify them unequivocally as either tecto-Ipc or tecto-GLv neurons.

Based on several pieces of data, however, we favor the view that they are mainly tecto-GLv neurons. First, ChATi processes are very abundant in layer 7. These processes do not arise from Ipc (which projects mainly to layers 3 and 5), but tecto-GLv neurons do ramify extensively in layer 7 (as noted above). Secondly, ChATi fibers and terminals are very abundant in the inner layer of GLv, which is the precise part of GLv to which the superficial tectum is known to project (Hunt and Künzle, 1976a,b). Finally, although cholinergic processes are abundant in Ipc, these seem mainly to represent the dendritic processes of the cholinergic Ipc neurons, which are known to ramify as aspiny dendrites within the confines of Ipc (Güntürkün, 1987). Previous studies have suggested that many tecto-GLv neurons may also use GABA as a neurotransmitter (Hunt and Künzle, 1976b; Veenman and Reiner, 1994), as do many tecto-Ipc neurons (Hunt and Künzle, 1976b; Hunt et al., 1977; Veenman and Reiner, 1994). Since the apical dendrites of the cholinergic tectal neurons extend into retinorecipient layers (layers 2–7), it is likely that these cells bisynaptically relay retinal information to their targets.

Finally, consistent with our results on the distribution of cholinergic fibers, moderate to high levels of nicotinic receptors are present in the periaqueductal gray (PAG), the nucleus of Darkschewitsch (D), the intercollicular nucleus, the lateral reticular formation, the ventral tegmental area (VTA), and the substantia nigra of pigeons (Britto et al., 1992; Sorenson and Chiappinelli, 1992).

Isthmus. Our results indicate that all or nearly all neurons in the parvocellular and semilunar isthmic nuclei (Ipc and SLu) are cholinergic, as also observed by other authors (Sorenson et al., 1989; Bagnoli et al., 1992). Using immunolabeling methods, the cholinergic neurons of the Ipc can be seen to have a heavy projection to the tectum,

entering via tectal layer 14 and terminating in upper tectal layers. Immunolabeling methods alone, however, cannot resolve with certainty which of the tectal cholinergic fibers arise from Ipc. Hodological studies have revealed that Ipc neurons project to layer 3 and mainly layer 5 of the tectum (Hunt and Brecha, 1984; Woodson et al., 1991). Thus, much of the cholinergic innervation observed in these tectal layers must arise in the Ipc. The SLu is known to project to layers 8–11 and 13 of the tectum (Hunt and Brecha, 1984). Since available data indicate all neurons of the SLu to be cholinergic (Sorenson et al., 1989), part of the cholinergic innervation observed in tectal layers 8–11 and 13 must originate in this nucleus. In addition, hodological studies reveal SLu to have a heavy projection to SpL and the neuropil dorsolateral to SpL (Reiner et al., 1982a). Since the distribution of cholinergic fibers in this region precisely matches the known projection of SLu to SpL and since seemingly all SLu neurons are cholinergic, it is inescapable that SLu has a cholinergic projection to SpL and is the source of the cholinergic terminals in SpL. In addition to the Ipc and SLu, our results in pigeons also indicate the presence of ChATi neurons in the disseminated isthmic nucleus of pigeons. This region also projects to the tectum, although it is unknown if its cholinergic neurons do (Brecha, 1978). Surprisingly, ChATi cells have not been found in this same region in chickens (Sorenson et al., 1989; von Bartheld et al., 1991).

Our results provide evidence of a dense innervation in the nucleus isthmo-opticus of pigeons (IO). Consistent with a cholinergic innervation of IO, very high levels of nicotinic receptors have been found in chicks on the perikarya of all or nearly all IO neurons and in the neuropil of IO (Britto et al., 1992; Sorenson and Chiappinelli, 1992). In addition, we observed lightly labeled ChATi neurons in IO in some pigeons with our anti-chicken ChAT, but not with the anti-human ChAT. Lightly labeled ChATi neurons have also been reported in the IO of pigeons (Bagnoli et al., 1992), but not in the IO of chickens (Sorenson et al., 1989). Since IO neurons label lightly, if at all, and since they do not label in chickens, the cholinergic character of the ChATi neurons observed in the IO must be considered with caution. Numerous strongly labeled ChATi cells, however, were observed to surround IO in pigeons and may represent ectopic isthmo-optic neurons (Bagnoli et al., 1992). Thus, as suggested by Bagnoli et al. (1992), there may be some cholinergic neurons of the brain that project to the retina. This possibility would be consistent with our observation of cholinergic fibers in the optic tract and chiasm.

One very prominent feature of the isthmic tegmentum of pigeons and chicks is the presence of a distinct group of large cholinergic neurons (Sorenson et al., 1989; von Bartheld and Bothwell, 1992). This cholinergic cell group was not identified previously as a distinct entity. Previous authors have considered the rostral part of this field to lie within the nucleus mesencephalicus profundus pars ventralis and its caudal part within the nucleus subcoeruleus (Karten and Hodos, 1967; von Bartheld and Bothwell, 1992). To simplify, we have termed this cholinergic cell group the pedunculopontine tegmental nucleus (PPN), since its location, cholinergic character, and connections seem comparable to the PPN of mammals (von Bartheld and Bothwell, 1992). Dorsocaudal to PPN, ChATi neurons are also present in the LDT of pigeons and chickens (von Bartheld and Bothwell, 1992), partially intermingled with noradrenergic neurons of LoC (Reiner et al., 1994). The cholinergic neurons of the PPN and LDT may contribute to

the cholinergic innervation of the tectum, since cholinergic axons could be traced from these areas into the optic lobe. In addition, our observations indicate that the cholinergic cells of the PPN and/or LDT may be the main origin of a cholinergic fiber bundle that traverses the midbrain tegmentum, coalesces internal to the optic tract as a distinct bundle at diencephalic levels, and eventually decussates in the ventral supraoptic decussation. Since this fiber bundle appears in a position to give rise to input to a wide variety of midbrain and forebrain cell groups, the PPN and LDT may be major sources of the dense cholinergic innervation in the retinorecipient thalamic and pretectal nuclei, as well as in the VTA, SN, and Ico in the midbrain. In support of this notion, pathway tracing studies have shown that all of these cell groups do receive input from the PPN and LDT (Kitt and Brauth, 1986a). The cholinergic neurons of the PPN and LDT may also contribute to the cholinergic innervation observed in the telencephalon, since projections have been described from these isthmic cell groups to the Wulst (Bagnoli and Burkhalter, 1983; Miceli and Repérant, 1982, 1985; Miceli et al., 1990), the pallium externum of the DVR, the hippocampal complex, the dorsal archistriatum (Benowitz and Karten, 1976; Casini et al., 1986; Kitt and Brauth, 1986a), the striatum, the INP, and the VP (Kitt and Brauth, 1986a,b), which are all moderately or densely innervated by ChATi fibers.

The final noteworthy feature of the isthmic tegmentum is the extremely dense cholinergic innervation in IP, which almost certainly arises from the cholinergic neurons of the medial habenula. Consistent with the dense cholinergic innervation, very high levels of nicotinic receptors (Britto et al., 1992; Sorenson and Chiappinelli, 1992) and moderate levels of muscarinic receptors (Dietl et al., 1988) are present in the interpeduncular nucleus.

Rhombencephalon. In the cerebellum, moderate or high levels of muscarinic receptors are found in the granule cell layer (Wächtler and Ebinger, 1989), where we observed cholinergic mossy-like fibers. The pretectal nucleus SpM is a major source of mossy fibers in the granule cell layer of the cerebellum in birds (Karten and Finger, 1976). Since SpM contains ChATi neurons in chickens and pigeons (Sorenson et al., 1989), cholinergic mossy fibers observed in the avian cerebellum may originate from this nucleus. The present study has provided evidence of several populations of cholinergic neurons in the pigeon brainstem that have not been described previously. These new cholinergic cell groups include those found in the mediodorsal tegmental nucleus [MDT; also called nucleus segmenti dorsalis by Karten and Hodos (1967)], the nucleus segmenti ventralis (TV), the nucleus linearis caudalis raphe (LC), and several vestibular nuclei, as well as dispersed in the reticular formation. Projections from several of these regions (MDT, TV, and LC) to telencephalic areas have been described (Bagnoli and Burkhalter, 1983; Kitt and Brauth, 1986a,b). Thus, cholinergic neurons in these areas may contribute to the cholinergic innervation observed in the avian telencephalon. Finally, our results corroborate the well-known cholinergic character of the motoneurons of the brainstem and spinal cord in birds, and provide a detailed description on the location of the different cranial motor nuclei. Some of these cholinergic motoneuron pools have been described previously in chicks (Sorenson et al., 1989; von Bartheld et al., 1991) and pigeons (Reiner et al., 1991).

Cholinergic cell groups and the segmental plan of brain development

Numerous recent studies have shown that the brain of vertebrates develops in a segmental fashion (reviewed by Noden, 1991). Thus, the hindbrain at an early stage of development consists of seven to eight rhombomeres (rh1–rh8) and the isthmus (sometimes considered part of rhombomere 1); the midbrain consists of one mesomere; and the bulk of the diencephalon consists of three prosomeres (p1–p3) (Bergquist and Källén, 1954; Vaage, 1969, 1973; Keyser, 1972; Puelles et al., 1987; Lumsden and Keynes, 1989; Noden, 1991; Bulfone et al., 1993; Figdor and Stern, 1993). The segmental development of more rostroventral parts of the prosencephalon (i.e., the hypothalamus and the telencephalon) has not yet been fully characterized, but it has been proposed to consist of three more prosomeres (p4–p6) (Bulfone et al., 1993). Each identified segment possesses a regional and serial identity, with specific cell groups (or parts of them) arising within specific segments. The segmental pattern of development leaves its imprint on the adult brain and governs the location and topographic relationships of the various cell groups to each other. In the adult brain, these segmental domains can best be viewed in the sagittal or horizontal plane.

In the present study, we have analyzed the relationship between the cholinergic cell groups of the adult pigeon brain and the segmental domains described in the avian brain. We have done this because we believe a segmental approach for analysing adult brain organization helps clarify the developmental origin of specific cell groups, and because it provides a very concrete framework within which to view evolutionary variation in brain organization. Further, because of the developmental underpinnings of segmental analysis, interspecies differences can be related to potential developmental differences. The organization of the cholinergic cell groups of the adult avian brain according to the segmental plan of brain organization is shown in Figure 27. The boundaries in the midbrain and hindbrain have been identified using the cranial nerves and cranial nerve nuclei as a reference (Vaage, 1969; Lumsden and Keynes, 1989; Noden, 1991), whereas for the forebrain we have followed Puelles's criteria (Puelles et al., 1987; Puelles and Medina, 1994).

It is important to note that the boundaries between segments do not conform necessarily to the conventionally accepted boundaries for the major subdivisions of the avian brain. For example, the pretectum and rostral parts of the tegmentum arise from diencephalic prosomeres (Vaage, 1969; Puelles et al., 1987; Bulfone et al., 1993; Figdor and Stern, 1993). Nonetheless, examination of the segmental organization of the cholinergic cell groups of the avian brain provides a clear means for characterizing the location of each cell group and for characterizing the topographic relationships of specific groups to one another. For example, segmental analysis shows that the PPN of birds is located within the isthmic tegmentum and lies parallel and caudal to the SN, which is located in the mesencephalic tegmentum. This relationship is obscured because the conventional transverse plane employed for birds is not parallel to the plane of division between segments. Rather, the conventional transverse plane tends to include cell groups of different segmental origin. In the discussion below in which we compare cholinergic system organization in birds to that in other vertebrates, we will therefore employ a segmental outlook for many brainstem cell groups.

The segmental approach is particularly useful for the comparative analysis of the cranial motor nuclei (which are ChAT⁺ in all vertebrates) and has revealed some interspecies differences in the abducens and facial motor nuclei. Such interspecific differences may be related to differences in the functions of the facial musculature in the members of the various vertebrate groups.

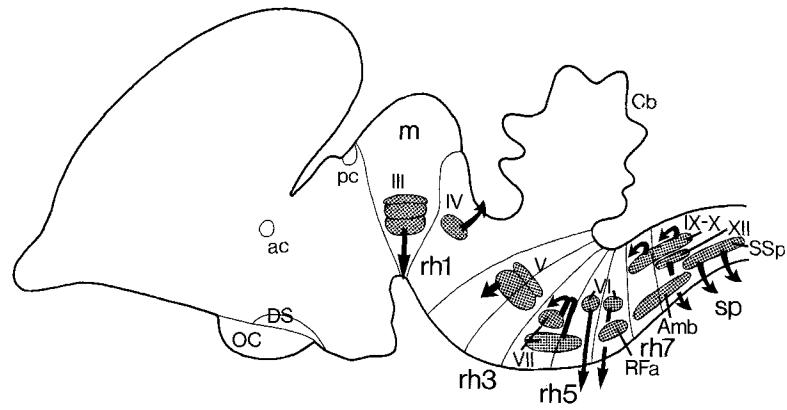
Comparison with other vertebrates and evolutionary considerations

The distribution of ChAT immunoreactivity observed in the avian brain largely resembles that in other vertebrates. Comparable cholinergic cell groups are present in the basal forebrain, the hypothalamus, midbrain tectum, isthmus, and cranial motor nuclei of most vertebrates. However, striking differences are observed in the diencephalon, where several cholinergic cell groups are present in the avian thalamus and pretectum that are not observed in the reptiles or mammals studied thus far. A sagittal schematic of the main ChAT⁺ cell groups observed in the avian brain is shown in Figure 27A and C and compared with those previously described in mammals (represented in Fig. 27B and D).

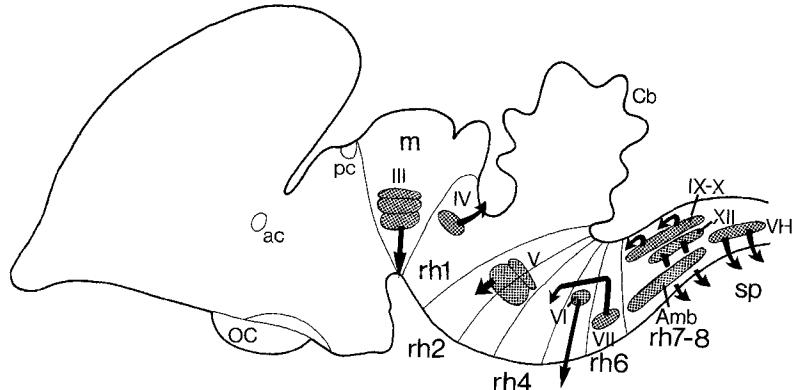
Telencephalon—cortical/pallial areas. ChAT⁺ neurons are present in the cortical areas of mammals (Parnavelas et al., 1986; Hendry et al., 1987; Blaker et al., 1988; Reiner, 1991) and some reptiles (cortex; Medina et al., 1993). In contrast, the cortical areas of other reptiles (Mufson et al., 1984; Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Powers and Reiner, 1993) and the pallial areas of amphibians and fishes (Ekström, 1987; Brantley and Bass, 1988; Ciani et al., 1988) do not contain cholinergic cell bodies. In birds, cholinergic perikarya are nearly entirely absent from pallial areas (i.e., Wulst and DVR), with only a few ChAT⁺ neurons being found in the piriform cortex and rostral neostriatum of pigeons. The ChAT⁺ neurons observed in the DVR of lizards (Hoogland and

Fig. 27. Schematic drawings of sagittal sections of the avian brain (A,C) presenting the main motor and nonmotor cholinergic cell groups and their relationship to the brain segmental domains. A relationship between cranial nerve nuclei and the rhombencephalic (rhombomeres 1–8: rh1–rh8; isthmus included in rh1 by some authors) and mesencephalic (m) segments has been previously described in chicks by Vaage (1969), Lumsden and Keynes (1989), and Noden (1991). Therefore, we have identified the boundaries between rhombomeres and the mesomere in the pigeon brain by using the cranial nerve nuclei and the cranial nerves as a reference (A). Puelles et al. (1987) analyzed the segmentation of the forebrain in chicks, and we have followed their criteria to identify the boundaries between the three prosomeres that they described (p1–p3) in the diencephalon (C). Since the number and arrangement of the segmental domains or neuromeres of the brain is a constant feature in all vertebrates (Vaage, 1969; Puelles et al., 1987; Lumsden and Keynes, 1989), the analysis of the relationship between brain nuclei and segmental domains in different vertebrates represents a powerful tool for comparing the brains of different vertebrates. Therefore, in B and D we present schematics of sagittal sections of mammalian brain, showing the main motor and nonmotor cholinergic cell groups and their relationship to the segmental domains (this relationship was previously described by Keyser, 1972; Noden, 1991; Puelles and Medina, 1994). By comparing schematics A and B for the motor nuclei, it can be concluded that motor nuclei of nerves III, IV, V, X, and XII have a similar location and organization in birds and mammals. Some variability exists, however, in the motor nuclei of nerves VI and VII (see text for more details). By comparing schematics C and D for the nonmotor nuclei, it is apparent that most nonmotor cholinergic cell groups observed in the pigeon brain are also present in identical segments in mammalian brain. Some variation exists in the cortical areas, the thalamus, and the pretectum.

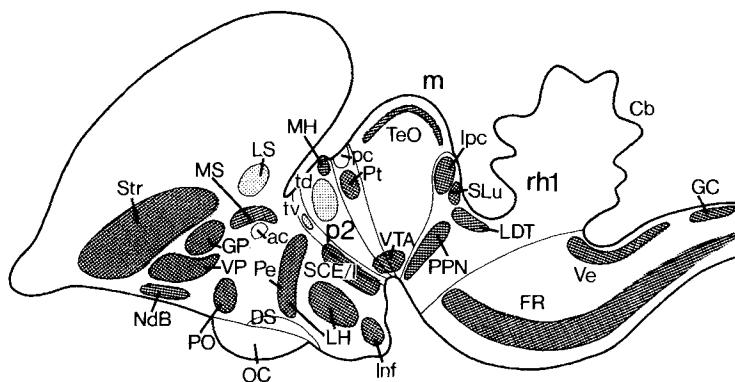
A. avian cholinergic motor nuclei



B. mammalian cholinergic motor nuclei



C. avian cholinergic non-motor nuclei



D. mammalian cholinergic non-motor nuclei

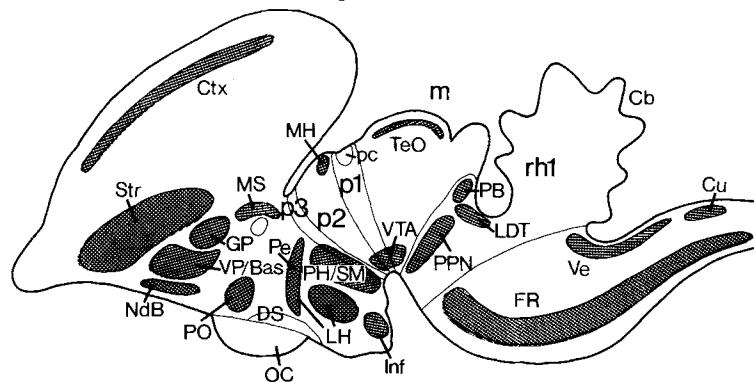


Figure 27

Vermeulen-VanderZee, 1990; Medina et al., 1993) may be comparable to those observed in the pigeon neostriatum, since the neostriatum is part of the avian DVR. The absence of cholinergic cells in the pallial areas of fishes, amphibians, and most reptiles, together with their paucity in pallial areas of birds, suggests that cortical/pallial cholinergic neurons were acquired relatively late during the evolution of vertebrates, as suggested previously (Reiner, 1991; Medina et al., 1993; Powers and Reiner, 1993).

One striking shared feature of the pallium/cortex with respect to the cholinergic system among birds, reptiles and mammals is an extensive cholinergic innervation of these regions by cholinergic neurons of the basal telencephalon. This has been amply demonstrated for mammalian cortex and hippocampal complex (reviewed by Woolf, 1991), and there are data (Medina et al., 1993; Powers and Reiner, 1993) supporting the conclusion that the cortex and DVR of reptiles are similarly innervated by the cholinergic basal telencephalic neurons. Our data presented here strongly support a similar conclusion for birds. Thus, cholinergic innervation of the pallium from basal telencephalon is a conserved feature of the amniote brain.

Telencephalon—striatal regions. The striatum of reptiles and mammals contains cholinergic neurons (*reptiles*: Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993; *mammals*: Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa, 1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Woolf, 1991). These appear to be clearly comparable to the cholinergic neurons of the striatum of birds. The cholinergic cell bodies of the striatum of mammals are local-circuit neurons and have a perikaryal size larger than that of the projection neurons (Woolf and Butcher, 1981; Alheid and Heimer, 1988; Woolf, 1991), as also noted here for birds. The cholinergic neurons of the striatum in reptiles have also been suggested to be interneurons (Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993). The avian dorsal striatum, however, contains cholinergic neurons only in LPO, with the lateral dorsal striatum (i.e., PA) not containing ChATi neurons. This finding is similar to that in reptiles, in whom cholinergic neurons appear confined to the medial part of the dorsal striatum, but different from that in mammals, in whom cholinergic neurons are uniformly distributed throughout the dorsal striatum (Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993).

Telencephalon—pallidal regions, diagonal band, and septum. Large well-labeled cholinergic neurons are observed in pallidal regions (globus pallidus and ventral pallidum), diagonal band, and ventromedial septum of mammals and reptiles (*reptiles*: Mufson et al., 1984; Brauth et al., 1985; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993; *mammals*: Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa, 1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Woolf, 1991). In mammals, the cholinergic cells of the globus pallidus, the ventral pallidum and other parts of the substantia innominata (including the nucleus basalis of Meynert), the diagonal band, and the ventromedial septum form a single large complex that is referred to as either the rostral cholinergic column or the basal forebrain cholinergic system (Satoh and Fibiger, 1985; Woolf, 1991). Part of the cholinergic cells of the ventral pallidum, INP, PP, and lateral forebrain

bundle in birds may be comparable to the mammalian nucleus basalis of Meynert. Previous authors have suggested that the cholinergic cells located in these same regions in reptiles might also be comparable to the nucleus basalis of Meynert (Medina et al., 1993; Powers and Reiner, 1993).

Cholinergic neurons are restricted in their distribution in the septum in mammals, being located only in the ventromedial septum near the anterior commissure. ChATi cell bodies are also scarce in the septum of turtles and crocodilians (Brauth et al., 1985; Powers and Reiner, 1993), and in lizards they are confined to ventrocaudal areas around the anterior commissure (Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993). In contrast to the restricted distribution of cholinergic neurons in the septum in mammals and reptiles, the ChATi neurons in the avian septum are much more numerous and widespread. Although cholinergic neurons are found in the ventromedial septum in birds (as they are in mammals and reptiles), birds also possess many ChATi in the lateral septum. As noted previously, it is uncertain if these lateral septal ChATi neurons in pigeons are truly cholinergic since they only label lightly with anti-chicken ChAT and rarely with anti-human ChAT. Further, the lateral septum does not project to the dorsal telencephalon in birds (Benowitz and Karten, 1976; Casini et al., 1986). Thus, the ChATi neurons in the lateral septum are unlike other basal telencephalic cholinergic neurons. Finally, the absence of cholinergic neurons in the lateral septum of mammals and reptiles also suggests caution in interpreting the lateral septal ChATi neurons in birds as being cholinergic.

The present study reinforces the idea that the cholinergic neurons of the avian basal forebrain project to dorsal telencephalic areas (Wulst, DVR, and hippocampal complex). Projections from the cholinergic neurons of the basal forebrain to the dorsal telencephalon have also been demonstrated in mammals (Woolf, 1991) and are strongly suggested for reptiles by hodological, receptor binding, and immunohistochemical data (Medina et al., 1993; Powers and Reiner, 1993). In addition, the cholinergic neurons of the mammalian basal forebrain are known to project via the stria medullaris to the medial habenula (Woolf and Butcher, 1985; Woolf et al., 1990; Woolf, 1991), as our data appeared to suggest for birds also. In mammals, some axons of the stria medullaris continue into the fasciculus retroflexus to innervate the interpeduncular nucleus (Woolf, 1991). We cannot rule out that this is also the case in birds. The basal telencephalon of teleost fishes contains cholinergic neurons that may be comparable to the basal forebrain system of birds, reptiles, and mammals (Ekström, 1987; Brantley and Bass, 1988). Thus, the cholinergic system of the basal forebrain might have originated as early in the evolution of vertebrates as the divergence between rayfinned and lobe-finned fish. The cholinergic system of the basal forebrain might thus represent a shared primitive trait among jawed vertebrates.

Diencephalon—hypothalamus. The distribution of cholinergic cell bodies in the avian hypothalamus largely resembles that described in mammals (Tago et al., 1987). In both groups of vertebrates, there is a system of cholinergic neurons located adjacent to the ventricle (including the infundibulum), whereas another system of cholinergic cell bodies is located laterally, mainly along the border with the ventral thalamus. In reptiles, the periventricular cholinergic system (including in the infundibulum) is present, but

cholinergic neurons are scarce in the lateral hypothalamus (Medina et al., 1993; Powers and Reiner, 1993).

Diencephalon—thalamus. The retinorecipient areas of the pigeon dorsal thalamus (which are comparable to the mammalian dorsal geniculate nucleus) have been shown to contain numerous ChATi neurons that project to the visual Wulst of the telencephalon (which is comparable to mammalian primary visual cortex) (Güntürkün and Kartén, 1991; Bagnoli et al., 1992). ChATi neurons have not, however, been observed in the dorsal lateral geniculate nucleus of chicks, reptiles, or any mammal species studied thus far (Sorenson et al., 1989; Woolf, 1991; Medina and Smeets, 1992; Medina et al., 1993; Powers and Reiner, 1993). This fact further supports the concern that the ChATi neurons in pigeon OPT are not truly cholinergic. One approach for resolving this would be to employ *in situ* hybridization histochemistry to determine if OPT neurons contain ChAT mRNA. A cholinergic cell group termed the nucleus of the tractus rotundus has been identified in a cyprinid teleost fish by Ekström (1987). This cell group lies lateral to the periventricular nucleus of the posterior tuberculum (TPp), and Ekström identified it as being located in the dorsal thalamus. From examination of this cell group in the sagittal plane (Ekström, 1987) and study of the comments of other authors on the diencephalon of teleosts (Striedter, 1990a,b; Bradford and Northcutt, 1983), it is uncertain whether this cell group is thalamic or pretectal in location. A retinal input has been traced to the general region lateral to the TPp in several species of rayfinned fishes, including several teleost fishes (Striedter, 1990a,b; Butler and Saidel, 1991; Northcutt and Butler, 1991; Saidel and Butler, 1991; Butler and Northcutt, 1992), so that it is possible that the cholinergic neurons of the nucleus of the tractus rotundus receive retinal input. The uncertainty, however, about the region of the fish brain to which these ChATi neurons belong makes it difficult to compare them with the ChATi neurons of the pigeon OPT.

Diencephalon—epithalamus. The present study and previous studies make it clear that cholinergic neurons are present in the habenula of mammals (Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa, 1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Tago et al., 1989; Woolf, 1991), birds (Sorenson et al., 1989), lizards (Medina et al., 1993), turtles (Powers and Reiner, 1993), and probably amphibians (Ciani et al., 1988). In these vertebrates, habenular cholinergic cells project via the fasciculus retroflexus to the interpeduncular nucleus. In contrast, the habenula of rayfinned fish has not been found to contain cholinergic neurons (Ekström, 1987; Brantley and Bass, 1988). It is uncertain if this is also true of lobe-finned and cartilaginous fish. If so, this would suggest that cholinergic cell bodies in the medial habenula first appeared in early amphibians and were retained in modern land vertebrates.

Pretectum. Several groups of cholinergic cell bodies are present in the pretectum of birds (Sorenson et al., 1989) and teleost fishes (Ekström, 1987; Wullmann and Roth, 1992). In teleost fish, these cholinergic neurons are located in the nucleus pretectalis superficialis, pars magnocellularis (PSm), which does not receive retinal input, and in the nucleus corticalis (C), which receives retinal input (Butler et al., 1991). In birds, the cholinergic pretectal cell groups do not receive retinal input (Gamlin and Cohen, 1988). In contrast to teleost fish and birds, cholinergic cell bodies are not present in the pretectum of mammals (Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa,

1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Woolf, 1991) or reptiles (Medina et al., 1993; Powers and Reiner, 1993). The absence of cholinergic cell bodies in the pretectum of reptiles suggests that the cholinergic nuclei of the pretectum of birds and those of the pretectum of fishes evolved independently.

Mesencephalon—tectum. Cholinergic neurons are present in the midbrain tectum of teleost fishes, amphibians, birds, and some mammals (*teleosts*: Ekström, 1987; Brantley and Bass, 1988; Zottoli et al., 1987; *anurans*: Ciani et al., 1988; *chicks*: Sorenson et al., 1989; *pigeons*: present study; Bagnoli et al., 1992; *rats*: Tago et al., 1989; *cats*: Vincent and Reiner, 1987). These neurons contribute to the cholinergic innervation of the tectum. In contrast, the midbrain tectum in other mammalian species and in reptiles has not been found to contain cholinergic neurons (*turtles*: Powers and Reiner, 1993; *crocodiles*: Brauth et al., 1985; *lizards*: Medina and Smeets, 1992; Medina et al., 1993; *baboon*: Satoh and Fibiger, 1985a; *macaque*: Mesulam et al., 1984; *guinea pig*: Maley et al., 1988). Thus, the evolutionary history of tectal cholinergic neurons in vertebrates is currently unclear.

Mesencephalon—central gray and tegmentum. As in birds, ChATi neurons are present in the central gray and the ventral tegmental area in mammals (Tago et al., 1989). Cholinergic neurons do not seem to be present in the midbrain central gray and the VTA in other vertebrates (Ekström, 1987; Brantley and Bass, 1988; Medina et al., 1993; Powers and Reiner, 1993).

Isthmus. An isthmic nucleus that contains cholinergic neurons and is reciprocally connected with the tectum is present in birds (Sorenson et al., 1989; Bagnoli et al., 1992), as well as in mammals, reptiles, amphibians, and fishes (*mammals*: Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa, 1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Tago et al., 1989; Woolf, 1991; *reptiles*: Brauth et al., 1985; Medina and Smeets, 1992; Medina et al., 1993; Powers and Reiner, 1993; *amphibians*: Ciani et al., 1988; *fishes*: Ekström, 1987; Brantley and Bass, 1988; Zottoli et al., 1988). In birds, this nucleus is the Ipc, and it is known to be reciprocally connected with the tectum in a point-to-point fashion (Hunt et al., 1977). The homologous cell group in reptiles has been traditionally called the magnocellular isthmic nucleus (Medina and Smeets, 1992; Medina et al., 1993; Powers and Reiner, 1993), but more recently Powers and Reiner (1993) have suggested that the term Ipc be used for the cholinergic isthmic cell group reciprocally connected with the tectum in reptiles as well. The homologous cell group to the sauropsid Ipc in mammals is termed the parabigeminal nucleus, while in amphibians and rayfinned fish it is termed the isthmic nucleus. As in birds and reptiles, the parabigeminal-isthmic nuclei of mammals, amphibians, and fishes also project to the midbrain tectum (Ricciuti and Gruber, 1985; Beninato and Spencer, 1986; Zottoli et al., 1988). In all species studied, this cell group is situated in the alar portion of the isthmic neuromere (sometimes included in rh1). The presence of a comparable cholinergic isthmic nucleus in mammals, birds, reptiles, amphibians, and fishes indicates that this probably represents a primitive feature of the brain of vertebrates that has been highly conserved during evolution.

In contrast, an SLu has only been unequivocally identified in birds. A possible reptilian homologue to SLu has, however, been proposed (Medina et al., 1993; Powers and Reiner, 1993). In birds, the SLu is characterized by its

prominent cholinergic projection to SpL in the pretectum. Reptiles possess a homologue of SpL called the nucleus of the posterior commissure. The possibility that the proposed homologue of SLu in reptiles has been accurately identified is supported by the observation of an apparent rostrally directed cholinergic projection from this nucleus and a dense cholinergic innervation dorsolateral to the nucleus of the posterior commissure in lizards (Medina et al., 1993). Pathway tracing studies are required to evaluate further the merits of this proposed homology.

The major cholinergic cell groups in the isthmic tegmentum of birds (i.e., PPN and LDT) resemble the mammalian pedunculopontine tegmental nucleus (PPN) and laterodorsal tegmental nucleus (LDT), respectively (Houser et al., 1983; Mesulam et al., 1984; Satoh and Fibiger, 1985; Kasa, 1986; Vincent et al., 1986; Vincent and Reiner, 1987; Maley et al., 1988; Tago et al., 1989; Woolf, 1991). These avian and mammalian cell groups contain cholinergic cell bodies, are located in similar areas of the brain (the isthmus), and seem to have similar projections to the midbrain tectum, retinorecipient thalamic and pretectal nuclei, and telencephalon (Sofroniew et al., 1985; Beninato and Spencer, 1986; Kasa, 1986; Woolf and Butcher, 1986; Dolabela De Lima and Singer, 1987; Hallanger et al., 1987; Fitzpatrick et al., 1988; Woolf, 1991). Comparable cholinergic cell groups have also been described in reptiles and termed the reticular isthmic nucleus and LDT in lizards (Medina and Smeets, 1992; Medina et al., 1993) and the reticular isthmic nucleus and LoC in turtles (Powers and Reiner, 1993). The segmental location of these cell groups further supports these proposed homologies. For example, in all amniotes studied, the cholinergic neurons of the PPN (as seen in the sagittal plane) are located in the isthmic tegmentum (isthmic neuromere; sometimes included in rhombomere 1) just caudal to the dopaminergic neurons of the substantia nigra and retrorubral area of the midbrain tegmentum (mesomere 1) (Medina et al., 1993).

Similar cell groups have also been reported in teleost fish and termed the nucleus reticularis superior and nucleus a (Ekström, 1987; Brantley and Bass, 1988; Zottoli et al., 1988). Although the connections of these cholinergic isthmic tegmental cell groups in reptiles and fish have not been analyzed in detail, immunohistochemical studies suggest that they have ascending cholinergic projections that decussate in the ventral supraoptic commissure of reptiles and the postoptic commissure of fish. Thus, the known projections of these cell groups largely resemble those observed in birds. This finding supports the notion that reptiles and teleost fish possess homologues of the cholinergic TPP-LDT systems in mammals and birds.

Motoneurons of the cranial nerve nuclei and spinal cord.

The motoneurons of the cranial nerve nuclei and spinal cord in all vertebrates are cholinergic (*fishes*: Ekström, 1987; Brantley and Bass, 1988; *amphibians*: Ciani et al., 1988; Gonzalez et al., 1993; *reptiles*: Medina et al., 1993; Powers and Reiner, 1993; *birds*: present study; *mammals*: Houser et al., 1983; Satoh and Fibiger, 1985; Vincent and Reiner, 1987; Maley et al., 1988; Tago et al., 1989). This seems to represent another primitive retained feature of the cholinergic systems of the brain of vertebrates. Although the cranial nerve nuclei seem to be highly conserved in different vertebrates, a detailed analysis using the criteria of segmental origin and final adult segmental position shows that some small interspecies differences are present in the location of specific motoneuron pools, indicating that some changes have occurred during evolution to modern vertebrates.

As noted above, the cranial nerve motor nuclei are segmentally arranged (Lumsden and Keynes, 1989; Noden, 1991; Medina et al., 1993). Since the number and arrangement of the segmental domains or neuromeres of the brain are constant features in all vertebrates (Vaage, 1969; Puelles et al., 1987; Lumsden and Keynes, 1989), the analysis of the relationship between cranial motor nuclei and segmental domains in different vertebrates represents a powerful tool for studying their development (Stern et al., 1991; Lumsden et al., 1991; Guthrie and Lumsden, 1992) and for comparing them among vertebrates (Díaz and Puelles, 1992a,b; Medina et al., 1993; Puelles and Medina, 1994).

The relationship between cranial motor nuclei and brain segments or neuromeres has been investigated in different vertebrates (Lumsden and Keynes, 1989; Baker et al., 1991; Noden, 1991; Medina et al., 1993), and is summarized in Figure 27 for birds and mammals. Comparison of the segmental position of motor nuclei among different vertebrates leads to the following observations. First, the third cranial nerve motor nuclei are located in the basal region of the mesencephalon (derived from the mesomere) in embryos and adults of all vertebrates. Second, the fourth cranial nerve motor nucleus is located in the basal region of the isthmic neuromere (sometimes included in rhombomere 1) in embryos and adults of all vertebrates. Third, the fifth cranial nerve motor nuclei are found in the basal region of rhombomeres 2 and 3 (rh2, rh3), with the nerve root located in rh2, in embryos and adults of all vertebrates. Fourth, the sixth cranial nerve motor nuclei are located in the basal region of rhombomeres 5 and 6 (rh5, rh6), with nerve roots in rh5 and rh6, in embryos and adults of birds and reptiles. The situation in mammals and fish may be more complicated for the sixth cranial nerve nucleus. Distinct rostral and caudal nuclei of nerve VI are observed in adult teleost fishes (Ekström, 1987; Brantley and Bass, 1988), suggesting their possible location in two rhombomeres. The nerve VI cell bodies are found, however, only in rh6 in skate and shark embryos, and only in rh5 in mouse and ferret embryos. One parsimonious interpretation of this interspecies variation in the segmental location of the nerve VI motor nucleus is as follows. The primitive pattern may be the possession of nerve nucleus VI motoneuron groups in both rh5 and rh6. This pattern is observed in bony fish, reptiles, and birds. From this interpretation, the presence of VI motoneurons only in rh5 in mammals and only in rh6 in cartilaginous fish would be derived. Further data on additional species in diverse classes of vertebrates are needed, however, to determine definitively the evolution of nerve nucleus VI motoneurons.

Fifth, in avian and mammalian embryos and in adult avians, the seventh cranial nerve motor nuclei are located in the basal region of rh4 and rh5, whereas the nerve root is located in rh4. The facial nerve root is also located in rh4 in adult reptiles. However, in the brain of adult reptiles and mammals, the nerve VII motor nuclei occupy positions that are caudal to the nerve root in rh4. In mammals (and seemingly also reptiles), this caudal migration of the motoneurons during development produces the prominent genu of the facial nerve (Windle, 1933; Kimmel, 1940). The arrangement of the nerve VII motor nuclei in teleost fishes seems to be similar to that in adult reptiles and mammals (Ekström, 1987; Brantley and Bass, 1988) and might also involve a migration of the motoneurons from the original segmental position during development to a location caudal to the exit of nerve VII. Finally, the motoneurons of nerve XII occupy a position in rh8 in all vertebrates.

Medullary cholinergic cell groups. As in birds, cholinergic neurons are dispersed in the medullary reticular formation of mammals, reptiles, and fishes (Brauth et al., 1985; Ekström, 1987; Brantley and Bass, 1988; Tago et al., 1989; Woolf, 1991; Medina et al., 1993; Powers and Reiner, 1993). In addition, cholinergic cell bodies have been described in some vestibular nuclei in mammals, reptiles, birds and fishes (Ekström, 1987; Brantley and Bass, 1988; Tago et al., 1989; Medina et al., 1993; Powers and Reiner, 1993).

Concluding remarks

The distribution of ChATi cell bodies and fibers in the brain of birds largely resembles that in mammals and reptiles. Comparable cholinergic cell groups are observed among amniotes in the basal forebrain, hypothalamus, epithalamus, midbrain tectum, isthmic region, cranial motor nuclei, and reticular formation (see Fig. 27). A striking difference between birds and mammals, however, is the general absence of cholinergic neurons from dorsal telencephalic areas in birds and their abundance in these presumably comparable areas in mammals.

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