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Distribution of Choline Acetyltransferase Immunoreactivity in the Brain of the Lizard *Gallotia galloti*

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ABSTRACT

The aim of the present study is to provide a complete description of the distribution of choline acetyltransferase (ChAT) immunoreactivity (i) in the brain of the lizard *Gallotia galloti*, on the basis of two different primary antisera: rat anti-ChAT and rabbit anti-chicken ChAT. Considering that the brain is a segmented structure, we have analysed our data with respect to transverse segmental domains (or neuromeres), which have been previously described by several authors in the brain of vertebrates.

In the telencephalon, ChATi neurons are seen in the cortex, anterior dorsal ventricular ridge, basal ganglia, diagonal band, and bed nucleus of the stria terminalis. Further caudally, ChATi cell bodies are located in the preoptic area, hypothalamus, habenula, isthmus, and all motor efferent centers of the brainstem and spinal cord.

Plexuses of ChATi fibers are observed in the areas containing cholinergic cell bodies. In addition, distinct plexuses are found in the cortex, the posterior dorsal ventricular ridge, the neuropiles of all primary visual centers of the diencephalon and mesencephalon, and several non-visual nuclei of the brainstem.

The distribution of ChAT immunoreactivity in the brain of *G. galloti* resembles in many respects that of other vertebrates, and differences are mainly observed in the pretectum and midbrain tectum. Transverse segmental domains were identified in the brainstem and forebrain of *Gallotia* when the cranial nerve roots and fiber tracts were used as a reference, and most cranial motor nuclei were found to occupy the same segmental positions as have been reported in the chick. © 1993 Wiley-Liss, Inc.

Key words: acetylcholine, nervous system, segmental domains, reptiles, comparative neuroanatomy

The production of antibodies against choline acetyltransferase (ChAT), an enzyme directly responsible for Ach synthesis, has provided an accurate method to localize cholinergic elements in the brain. By means of these antibodies, distributional maps of the CNS are available for several mammalian species (e.g., rat: Houser et al., '83; cat: Kimura et al., '81; Vincent and Reiner, '87; guinea pig: Maley et al., '88; primates: Mesulam et al., '84; Satoh and Fibiger, '85a), as well as for some non-mammalian vertebrates, e.g., frogs (Ciani et al., '88), teleosts (Brantley and Bass, '88; Ekström, '87), and cyclostomes (Wächtler, '83). These studies have shown many common aspects in the cholinergic systems of the brain of mammals, amphibians, and fishes, such as the presence of cholinergic neurons in the basal telencephalon, habenula, midbrain tectum, and cranial motor nuclei, and the presence of numerous cholinergic fibers innervating the optic neuropiles or coursing in the fasciculus retroflexus. Data on the distribution of cholinergic fibers and perikarya in the whole brain of other groups of vertebrates, such as reptiles and birds, are needed before concluding whether these aspects are common to all vertebrates, or before suggesting evolutionary trends in the distribution of cholinergic elements in the brain. However, only partial mappings of ChAT immunoreactivity are available in the brain of birds (Sorenson et al., '89) or reptiles (Mufson et al., '84; Brauth et al., '85; Hoogland and Vermeulen-VanderZee, '90; Medina and Smeets, '92). The

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aim of the present study is to provide a complete description on the distribution of ChAT immunoreactivity in the brain of a reptile, the lizard *Gallotia galloti*. Our results were compared with those obtained in other vertebrates, in order to gain insight into the evolution of the cholinergic systems of vertebrates. As the brain seems to be a segmentally organized structure (anatomical evidence: Rendahl, '24; Bergquist and Kallen, '54; Vaage, '69; Keyser, '72; Puelles et al., '87; genetic evidence: Keynes and Lumsden, '90; Noden, '91; Price et al., '91), the use of segmental (or

Abbreviations

ac	anterior commissure
Acc	nucleus accumbens
ADVR	anterior dorsal ventricular ridge
Amb	nucleus ambiguus
IX Amb	nucleus ambiguus, glossopharyngeal part
X Amb	nucleus ambiguus, vagal part
Amc	nucleus centralis amygdalae
Ame	nucleus externus amygdalae
Aml	nucleus lateralis amygdalae
Apol	area preoptica lateralis
AT	area triangularis
Bst	bed nucleus of the stria terminalis
cc	central canal
Cerb	cerebellum
CP	cell plate of Unger
csv	commissura supraoptica ventralis
csd	commissura supraoptica dorsalis
Cxd, CxD	cortex dorsalis
CxL	cortex lateralis
Cxla	cortex lateralis anterior
Cxlad	cortex lateralis anterior, pars dorsalis
Cxlav	cortex lateralis anterior, pars ventralis
Cxlp	cortex lateralis posterior
Cxml	cortex medialis, large-celled part
Cxms	cortex medialis, small-celled part
dh	dorsal horn
Dlh	nucleus dorsolateralis hypothalami
Dll, Dlm	nucleus dorsolateralis thalami, large-celled part or pars magnocellularis
Dlp	nucleus dorsolateralis thalami, small-celled part or pars parvocellularis
Dm	nucleus dorsomedialis thalami
EW	nucleus of Edinger-Westphal
flm	fasciculus longitudinalis medialis
fr	fasciculus retroflexus
Gld	nucleus geniculatus lateralis, pars dorsalis
Glv	nucleus geniculatus lateralis, pars ventralis
GP	globus pallidus
GT	griseum tectale
h	hypothalamus
gl	granular cell layer
gVII	genu of the facial nerve
Hl	nucleus lateralis habenulae
Hm	nucleus medialis habenulae
i	isthmus
Ic	nucleus intercalatus
IIId	nucleus oculomotorius dorsalis
IIIv	nucleus oculomotorius ventralis
inf	infundibulum
Ip	nucleus interpeduncularis
Isd	nucleus isthmi diffusus
Ism	nucleus isthmi magnocellularis
Isp	nucleus isthmi parvicellularis
Iss	nucleus isthmi semilunaris
IV	nucleus trochlearis
IXm	nucleus motorius nervi glossopharyngei
Jm	nucleus juxtacommissuralis medialis
Lc	locus coeruleus
LDT	laterodorsal tegmental nucleus
lim	tractus limitans
lfb	lateral forebrain bundle
Lm	nucleus lentiformis mesencephali
Lte	nucleus lentiformis thalami, pars extensa
Ltp	nucleus lentiformis thalami, pars plicata
m	mesencephalon
Mp	nucleus medialis posterior
Mt	nucleus medialis thalami
nBOR	nucleus of the basal optic root
NdB	nucleus of the diagonal band of Broca
Ncp	nucleus of the posterior commissure
n III–XII	cranial nerves III–XII
Noa	nucleus olfactorius anterior
Nsa	nucleus septalis anterior
Nsi	nucleus septalis impar
Nsl	nucleus septalis lateralis
Nsm	nucleus septalis medialis
Ov	nucleus ovalis
oph	organon periventriculare hypothalami
OC	optic chiasm
OT	optic tract
pc	posterior commissure
Pd	nucleus posterodorsalis
PDVR	posterior dorsal ventricular ridge
Ph	nucleus periventricularis hypothalami
pl	Purkinje cell layer
pa	parencephalon anterior
Pall	pallidum
pp	parencephalon posterior
Prd	nucleus pretectalis dorsalis
Prmc	nucleus profundus mesencephali, pars caudalis
pRot	perirotundal belt
Prv	nucleus pretectalis ventralis
ps	secondary prosencephalon
RA8	retrorubral area (reptilian A8)
Ras	nucleus raphes superior
rh 1–8	rhombomeres 1–8
Ric	reticular isthmic nucleus, pars centralis
Ris	reticular isthmic nucleus, pars superficialis
rIV–rXII	roots of the cranial nerves IV–XII
Rot	nucleus rotundus
s	synencephalon
S	septum
SA	striato-amygdaloid area
Sc	nucleus subcoeruleus
SCN	nucleus suprachiasmaticus
si	nucleus salivatorius inferior
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
sp	spinal cord
Sr	substantia reticular
ss	nucleus salivatorius superior
Str	striatum
T	tectum
TO	tuberculum olfactorium
tol	tractus olfactorius lateralis
tom	tractus olfactorius medialis
Torc	torus semicircularis, nucleus centralis
Torl	torus semicircularis, nucleus laminaris
Vdso	nucleus descendens nervi trigemini, pars oralis
Veds	nucleus vestibularis descendens
vh	ventral horn
VIa	nucleus abducens accessorius
VIIIm	nucleus motorius nervi octavi
VIImd	nucleus motorius nervi facialis, dorsal part
VIImv	nucleus motorius nervi facialis, ventral part
VIp	nucleus abducens principalis
Vh	nucleus ventromedialis hypothalami
Vlt	nucleus ventrolateralis thalami
VmT, Vm	nucleus ventromedialis thalami
Vmd	nucleus motorius dorsalis nervi trigemini
Vmvc	nucleus motorius ventrocaudalis nervi trigemini
Vmvr	nucleus motorius ventrorostralis nervi trigemini
vp	ventral peduncle of the lateral forebrain bundle
VP	ventral pallidum
Xm	nucleus motorius nervi vagi
Xmd	nucleus motorius dorsalis nervi vagi
Xmv	nucleus motorius ventralis nervi vagi
XIm	nucleus motorius nervi spinalis
XIIm	nucleus motorius nervi hypoglossi

neuromeric) criteria for analysing the distribution of immunoreactivity in the adult brain must represent a powerful tool to understand its organization better and to compare different vertebrates. Therefore, in the present study we have made an effort to analyse the relationship between ChAT immunoreactive cell groups and fibers in the brain of *Gallotia* and the segmental domains of the forebrain and brainstem, which have been described previously in birds and lizards (Vaage, '69, '73; Martinez-de-la-Torre, '85; Puelles et al., '87).

MATERIALS AND METHODS

The brains of eight adult lizards, both male and female, of the species *Gallotia galloti* (Reptilia, Lacertidae) were used. Four animals were anaesthetised with ethyl ether and perfused successively with: 1) saline solution; 2) 4% paraformaldehyde, 15% picric acid, 0.1% glutaraldehyde in 0.1 M, pH 7.4 phosphate buffer (PB); 3) 4% paraformaldehyde in PB, with increasing quantities of sucrose (5%, 10%, 15%); and 4) finally, 20% sucrose in PB for 60 minutes. The brains were dissected and cut at 30–40 μm thickness on a freezing microtome in the transverse or corrected horizontal (parallel to the optic tract) plane. Sections were collected serially in PB, rinsed three times in 0.05 M Tris-buffered saline (TBS), pH 7.6, and incubated in a rat anti-ChAT antiserum (INCSTAR), diluted 1:100 in TBS containing 0.5% Triton X-100, for 3 days, at 4°C and under constant agitation. The sections were rinsed three times in TBS, and then incubated in a rabbit anti-rat serum (CLB, Amsterdam), diluted 1:50 in TBS containing 0.5% Triton X-100 during 1 day at 4°C and under constant agitation. Following the secondary antibody incubation, sections were rinsed three times in TBS and immersed in a peroxidase anti-peroxidase complex (rat PAP, Vector Laboratories, USA), diluted 1:400 in TBS with 0.5% Triton X-100 during 1 hour at room temperature and under constant agitation. After the last incubation, sections were rinsed two times in TBS and one time in PB, and treated with 0.05% diaminobenzidine (DAB) and 0.01% H_2O_2 intensified with 0.04% ammonium nickel sulfate for 10 minutes. Finally, sections were rinsed in 0.05 M Tris-HCl buffer, pH 7.6, mounted (0.3% gelatine solution in Tris-HCl buffer), dehydrated, and coverslipped.

The other four animals were anaesthetised with ethyl ether and perfused with 4% paraformaldehyde in PB. The brains were dissected, immersed in 30% sucrose in PB overnight, and cut at 30–40 μm thickness on a freezing microtome in corrected horizontal or sagittal planes. Sections were rinsed in PB, preincubated in a goat serum albumin, and incubated in a polyvalent rabbit anti-serum against chicken ChAT (antiserum 1465, kindly donated by Dr. Miles Epstein, University of Wisconsin, Madison, WI; diluted 1:3,000) overnight, at room temperature under constant agitation. Subsequent steps in the immunoprocedure involved the following incubations: 1) biotinylated goat anti-rabbit antibody (1:100, Vector) for 1 hour; 2) avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector); and 3) 0.05% DAB solution in 0.1 M Tris-HCl buffer, pH 7.6, with 0.01% hydrogen peroxide. Control sections were processed without primary antiserum. In most cases, adjacent sections were processed immunohistochemically for other markers, such as substance P, Leu-enkephalin and tyrosine-hydroxylase (for methods, see Medina and Smeets, '92), identifying cell groups and fiber tracts.

In order to facilitate the visualisation of the diencephalic neuromeric domains, we have chosen the corrected-horizontal (parallel to the optic tract) as well as the sagittal planes of section for this study, instead of the more frequently used transverse sections. We have followed the criteria of Puelles et al. ('87) and Vaage ('69) for the segmentation of the forebrain and brainstem, respectively. The nomenclatures of Smeets et al. ('86a) and Ten Donkelaar et al. ('87) are followed for the forebrain and brainstem grisea, respectively.

RESULTS

When sections were incubated without the first antiserum no labeling was observed. The two primary antisera employed in this study produced essentially the same immunoreactivity pattern, except for some cortical and infundibular neurons that were immunoreactive only with the rabbit anti-chicken ChAT (see below). Accordingly, a single distribution of ChAT immunoreactivity is described, reporting the exceptions with it. The distribution of ChAT immunoreactive (ChATi) neurons and fibers is depicted in a series of transverse sections from rostral (a) to caudal (z) levels in the brain of *Gallotia galloti* (Figs. 1–5). Asterisks indicate cell bodies that are only immunoreactive with rabbit anti-chicken ChAT antibody.

Distribution of ChATi cell bodies

Telencephalon. The most rostrally located immunoreactive cell bodies are seen in the olfactory structures. The nucleus olfactorius anterior contains round, small ChATi somata, whereas larger immunoreactive cell bodies are present in the olfactory tubercule (Figs. 1a–c, 6A, 7A,B). Interstitial ChATi cell bodies are also seen within the tractus olfactorius lateralis and medialis, adjacent to the brain surface (Figs. 1a,d, 6A,B, 7A–C).

The cortical areas show immunoreactive neurons that are only positive with the rabbit anti-chicken ChAT primary antibody (Figs. 1a–d, 2e–g, 3h, asterisks). These ChATi neurons are mainly located in the medial part of the lateral cortex, as well as in the cell plate of Unger in the dorsal cortex (Figs. 1a–d, 2e–g, 3h, 6E,F). Additional ChATi neurons are present in the cortical plate of the dorsal cortex, and in the rostral part of the large- and small-celled medial cortex (Figs. 1a,b, 6E).

The anterior dorsal ventricular ridge contains ChATi somata, located mainly in its medial part, which appear to be more numerous and better stained with the rabbit anti-chicken ChAT primary antibody (Figs. 1a–d, 2e–g, 3h, 6G). The amygdaloid complex, on the contrary, does not contain ChATi cell bodies.

The largest number of telencephalic ChATi cell bodies is found in the basal ganglia. The nucleus accumbens and striatum contain dispersed immunoreactive somata (Figs. 1a–d, 2e, 6A–C, 7A,B, 8A,B, 15A, 16A). Small ChATi cells are also seen at rostral levels of the bed nucleus of the medial forebrain bundle or ventral pallidum (Figs. 1d, 6B, 7C). Further caudally, more densely clustered bipolar ChATi somata are observed in the nucleus of the diagonal band of Broca (Figs. 2e,f, 6C,D, 7C,D, 8D, 15A, 16A). Laterally, ChATi neurons are also present within and around the lateral and medial forebrain bundle. By comparison with

adjacent sections stained for substance P and enkephalin, some of the latter immunoreactive somata are located within the globus pallidus (Figs. 2e,f, 6C,D, 7C, 8C), as described by Russchen et al. ('87). However, most of them belong to a large group located within the medial forebrain bundle, in the caudal portion of the ventral pallidum (Figs. 2e,f, 6C,D, 7D, 8D, 15A,B, 16A). Small, lightly stained neurons are present adjacent to the anterior commissure, i.e., in the bed nucleus of the stria terminalis (Figs. 2g, 6D).

Preoptic and hypothalamic areas. Caudal to the cholinergic cells of the ventral pallidum, dispersed ChATi somata are located in the area preoptica lateralis (Figs. 2f, 15A,B, 16A). They possess long processes that are arranged parallel to the ventricular surface.

At the level of the supraoptic commissure, faintly stained cells are present in the ventral part of the periventricular hypothalamic nucleus (1 in Figs. 2g, 10A,B). These ChATi somata are replaced caudally by a group of larger, more densely stained fusiform cells (2 in Figs. 3h, 10A). A third ChATi cell group appears in the most caudal part of the periventricular hypothalamic nucleus, formed by a few, disperse neurons (3 in Figs. 3i, 10A,B). In addition, cell bodies, only positive with the rabbit anti-chicken ChAT primary antibody, are present in the infundibular region (asterisks, Fig. 3i,j).

Thalamus, epithalamus, and pretectum. Only the epithalamus contains ChATi somata (Figs. 3j, 4k, 9B, 15A). The cells are located in the ventral part of the medial habenular nucleus, where they are densely clustered. Their axons course ventrally into the fasciculus retroflexus.

Mesencephalon. ChATi cell bodies are restricted to the mesencephalic basal plate, where numerous immunoreactive neurons are seen in the oculomotor complex, formed by the Edinger-Westphal nucleus and the dorsal and ventral parts of the nucleus oculomotorius (Figs. 4n,o, 15A). The Edinger-Westphal neurons show some long dendrites directed dorsolaterally, whereas their axons course ventrally, together with the axons of the dorsal oculomotor subnucleus. Many axons arising from the ventral part of the nucleus oculomotorius cross the midline, and enter the contralateral oculomotor nerve root.

Isthmus: alar plate. Tightly clustered, small immunoreactive neurons are present as a subpopulation in the nucleus isthmi pars magnocellularis (Ism), mixed with other non-immunoreactive cells (Figs. 4o, 5p,q, 11B,C, 12A,B, 15C, 16B). Additionally, a group of ChATi neurons is observed rostral and medial to the magnocellular isthmic nucleus (Figs. 4o, 11B, 12A, 15C, 16B). On the basis of its loose arrangement, this group is labeled by us as the nucleus isthmi diffusus (Isd).

Immediately behind these isthmic nuclei, a dense cluster of small, intensely immunoreactive cells is found. These neurons are characterized by long dendrites that are directed towards the lateral surface of the brain, where they fan out in a strong ChATi neuropile (Figs. 4o, 11B, 12B,C, inset, 15C, 16B). On the basis of its position with respect to the n. isthmi magnocellularis, this group of cells is considered homologous to the semilunar isthmic nucleus of birds (Sorenson et al., '89), and accordingly labeled in the present study (Iss).

Medial to this nucleus, other large groups of immunoreactive neurons are present in the isthmic reticular formation (Ri). The main group consists of tegmental multipolar ChATi neurons, showing very long dendrites oriented towards the ventrolateral surface of the brain (Figs. 4n,o, 11A,B, 12B–D, inset, 15B, 16C). Within this large group of immunoreactive reticular neurons, it is possible to distinguish central and superficial subgroups (Ric, Ris). The latter contains smaller ChATi neurons that are placed more ventrally.

Dorsocaudally to the Ric, and adjacent to the floor of the lateral horn of the fourth ventricle, a group of small bipolar ChATi neurons appears in the griseum centrale (Figs. 5p, 11C, 12B, inset, 15B, 16C). On the basis of its position we have called this group nucleus laterodorsalis tegmenti (LDT). ChATi neurons of this group lie within the confines of the locus coeruleus. Another small group of immunoreactive neurons is located dorsocaudal to the LDT, around the lateral edge of the ventricle, adjacent to the superior cerebellar peduncle (Fig. 5q).

Isthmus: basal plate. The basal plate of the isthmus contains the ChATi neurons of the nucleus trochlearis (Figs. 4o, 11B, 12C). These neurons are large, and their axons arch laterally and dorsally towards the dorsal midline (Fig. 5p), exiting from the brain through the contralateral trochlear nerve.

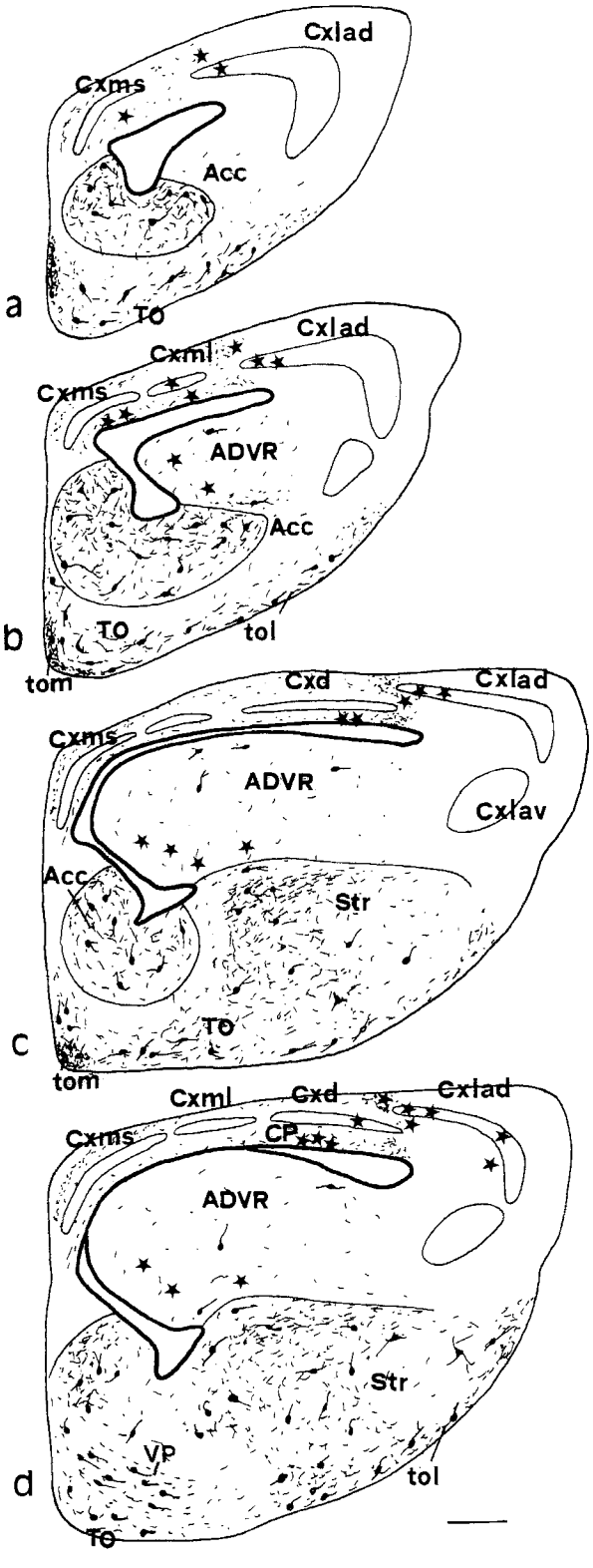
Rhombencephalon. At the level of the trigeminus, the trigeminal motor complex consists of three separate clusters of ChATi cells, viz., a small dorsal subnucleus (Vmd) and two ventral subnuclei, named pars ventralis rostralis (Vmvr) and pars ventralis caudalis (Vmvc), respectively (Figs. 5q,r, 13A, 14A,B, 15B,C, 16B,C). The dorsal and ventrorostral portions belong to the second rhombomeric domain, marked by the trigeminal root, whereas the ventrocaudal portion lies in the third rhombomeric domain. The neurons of the ventrorostral and ventrocaudal subnuclei have long dendrites that extend ventrolaterally to a superficial ChATi neuropile (Figs. 5q,r, 13A, 14).

An additional group of small ChATi neurons is present adjacent to the Vmvr and Vmvc. These cells do not belong to the trigeminal motor complex, since their axons seem to course caudalwards within the lateral tegmentum. We consider this cell group as a subpopulation of the rostral portion (subnucleus oralis) of the nucleus descendens nervi trigemini (Figs. 5r, 13A).

A small number of tiny immunoreactive neurons is present within the nucleus reticularis superior lateralis (Figs. 4o, 5p, 14A,B, 15C, 16B).

Further caudally, ChATi cells are observed in the nucleus abducens principalis (VIp), just lateral to the fasciculus longitudinalis medialis (Figs. 5r, 13A, 14A, 15B). The neurons of the abducens have long dendrites directed laterally, whereas their axons course ventrally into the rootlets of the abducens nerve. Ventrolateral to the VIp, a small group of large ChATi cell bodies is present in the tegmentum, which corresponds to the nucleus abducens accessorius (VIa). Their axons course first towards the principal nucleus (VIp), where they do a hairpin turn and leave the brain together with the axons of the VIp.

Several groups of immunoreactive neurons are observed in the basal plate of the medulla oblongata. The rostral half of the medulla is characterized by the presence of ChATi facial motoneurons, which are arranged in two groups (Figs. 5s,t, 14B). Large, fusiform ChATi neurons are seen in the dorsal part of the nucleus motorius nervi facialis (VIImd). Their axons course first dorsomedialwards into the genu of the facial nerve, ascend within it across the segments containing the nucleus abducens, and finally turn laterally into the root of the facial nerve. Close to the ventrolateral surface of the brain, another distinctive group



Figs. 1–5. (Continue on following pages.) Drawings of a series of transverse sections (**a–y**) through the brain of the lizard *Gallotia galloti*, showing the distribution of choline acetyltransferase (ChAT) immunoreactive (ChATi) cells and fibers. Asterisks represent cells observed only with the anti-chicken ChAT antibody. Scale bar = 2 mm.

of large ChATi neurons appears, which seems to represent a ventral part of the nucleus motorius nervi facialis (VIImv). These neurons have dendrites directed ventrolaterally, reaching the surface where they ramify and form a plexus. The axons of these neurons course medialwards to the genu, and exit from the brain through the root of the facial nerve. Dorsal to the VIImv, small, ChATi neurons are observed in the nucleus salivatorius superior (ss).

Another group of ChATi neurons which is topographically related to the facial complex lies near the point where facial motor fibers collect into the genu, just caudal to the VIp (Figs. 5s, 13A, 14A). These neurons characteristically have processes that cross the midline, and represent efferent cell bodies of the VIIIth cranial nerve (Barbas-Henry and Lohman, '88), through which their ChATi axons exit the brain.

Caudal to the facial-octaval complex, faintly stained cell bodies are observed in the glossopharyngeal motor nucleus (IXm) and, further caudally, in the dorsal motor nucleus of the vagus (Xmd). The ventral motor nucleus of the vagus contains strongly immunoreactive neurons (Xmv; Fig. 5u–y). The axons of the glossopharyngeal motoneurons course first rostralwards, and then laterally to their exit through the IXth nerve root, whereas the axons of the vagal motoneurons exit the brain laterally through several rootlets. There are also immunoreactive neurons in other two efferent centers related to the IXth nerve (Figs. 5u,v, 14A,B). One of them is the nucleus salivatorius inferior (si), which contains small ChATi neurons. The other is the glossopharyngeal part of the nucleus ambiguus (IXAmb), represented by large, dispersed ChATi neurons located ventrolaterally in the tegmentum.

Another prominent basal ChATi cell group observed in the medulla is formed by very large immunoreactive neurons of the nucleus hypoglossus (XIIm). These neurons have long dendrites that extend laterally and axons that course directly into the ventral roots of the nerve (Figs. 5w,x, 13B, 14A). More ventrally, the vagal part of the nucleus ambiguus (XAmb) also contains ChATi cell bodies. Sparse, small immunoreactive neurons are seen dispersed throughout the lateral reticular formation of the medulla oblongata (Figs. 5r–x, 14A,B). Larger ChATi reticular neurons are found within the medial reticular formation.

Finally, we observed a small group of ChATi neurons in the caudal part of the nucleus vestibularis descendens (Veds: Figs. 5v, 15A).

Spinal cord. The ventral horn of the spinal cord contains three separate groups of ChATi motoneurons (Fig. 5y). Two of them are composed of large, fusiform ChATi neurons that have long lateral dendrites. The ventralmost motoneurons possess medial dendrites that cross the midline. The third group of motoneurons is located in the dorsal portion of the ventral horn, and consists of smaller, round immunoreactive cells (Fig. 5y). The axons of most of these neurons seem to exit from the neural tube through the ventral roots. Small ChATi interneurons are also seen medially in the ventral horn of the spinal cord. A number of small, fusiform immunoreactive neurons are observed in the base of the dorsal horns of the spinal cord. These cells emit axonal and dendritic branches that cross the dorsal gray commissure, entering the contralateral dorsal horn.

Distribution of ChAT immunoreactive fibers

Telencephalon. Immunoreactive fibers and varicosities are observed in most olfactory structures of the telencepha-

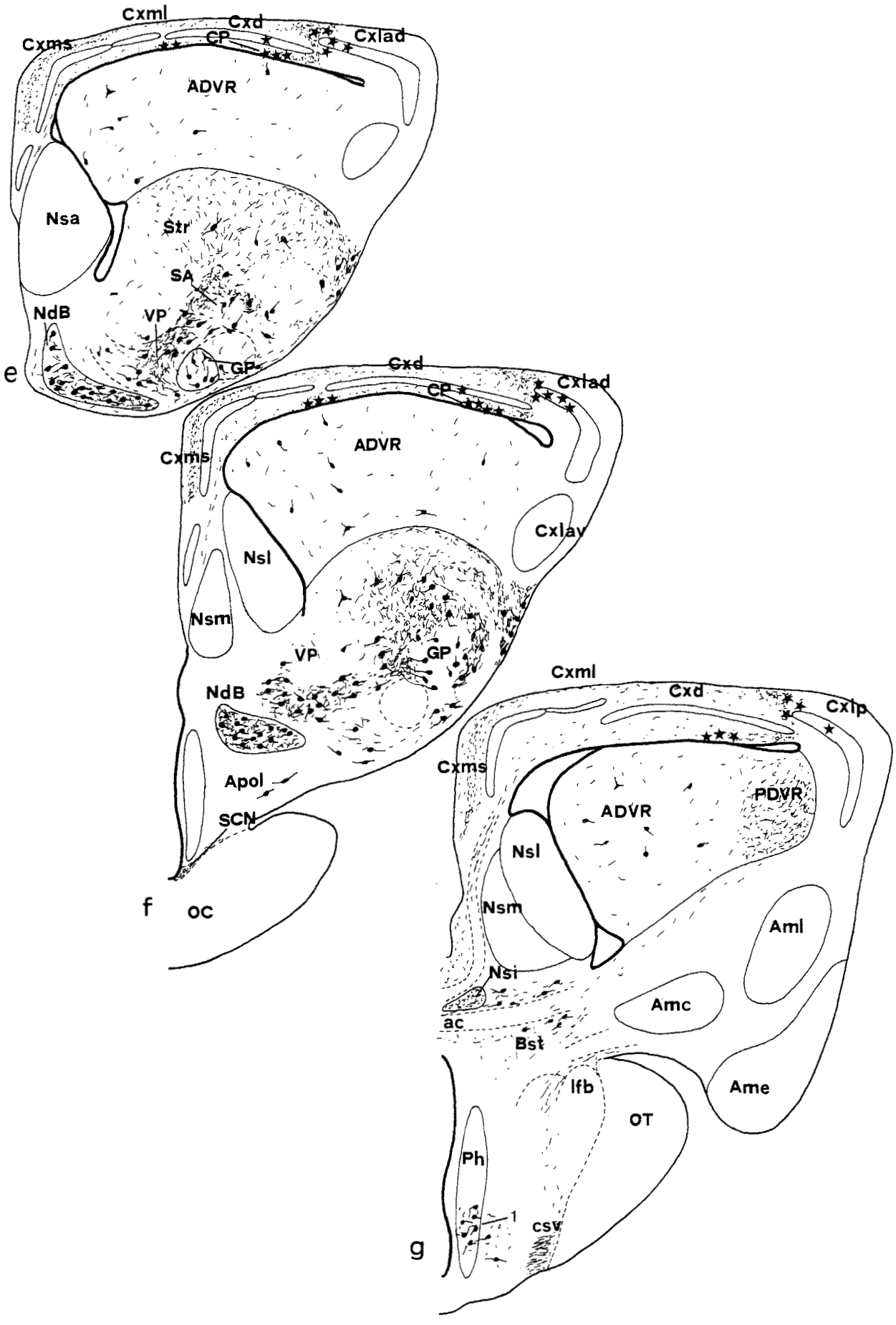
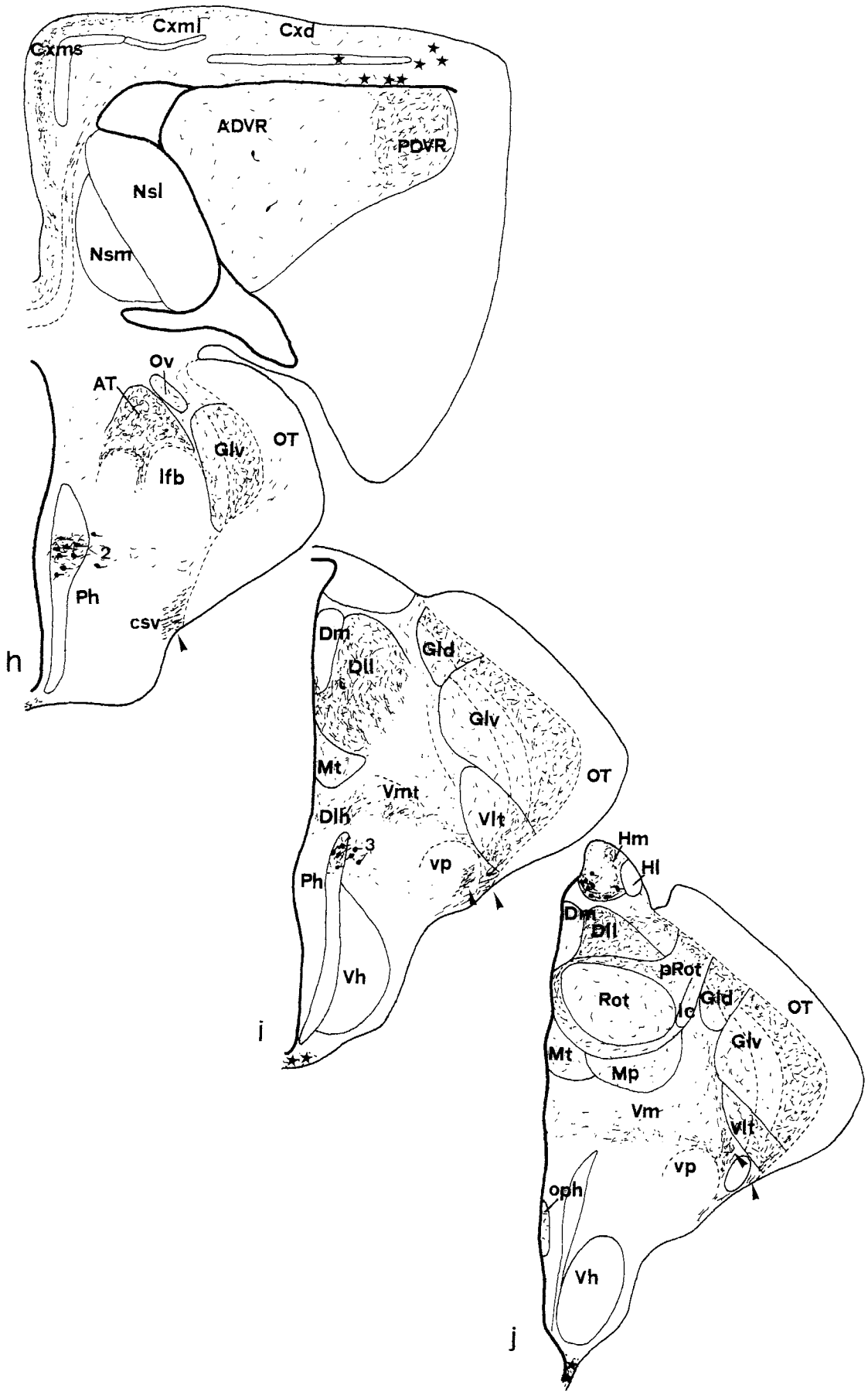


Figure 2 (See legend, p. 265)



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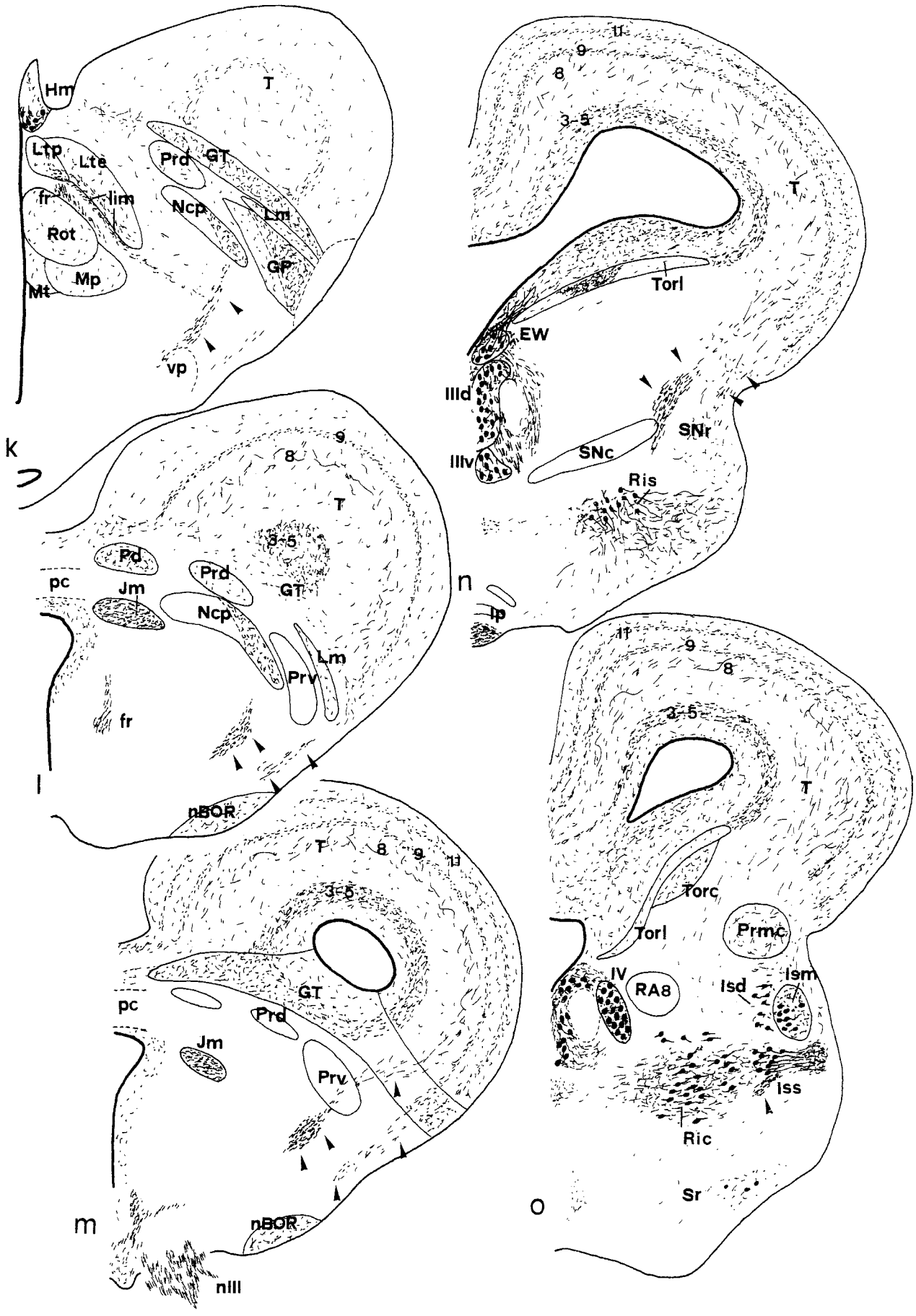

Figure 4 (See legend, p. 265)

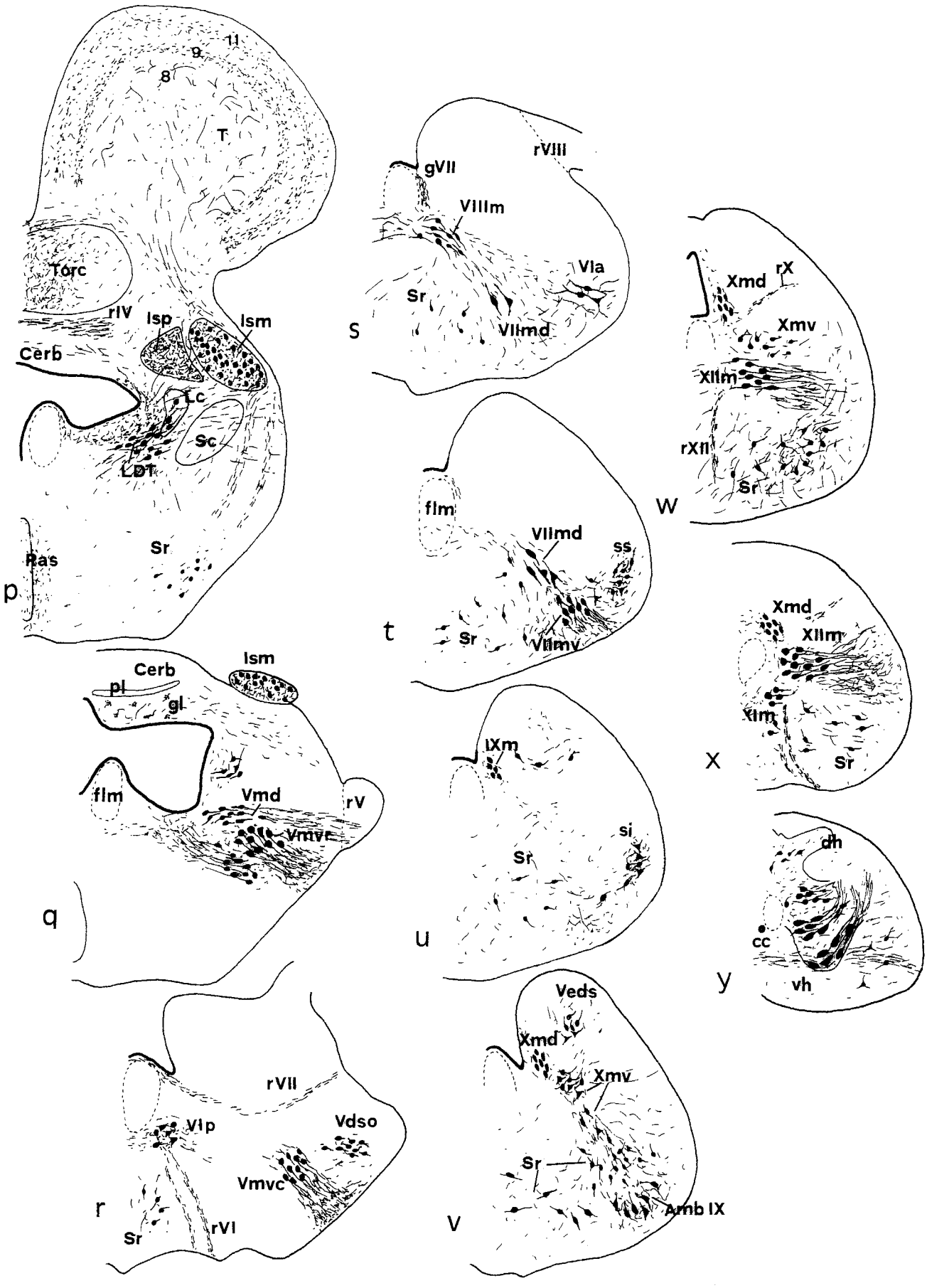

Figure 5 (See legend, p. 265)

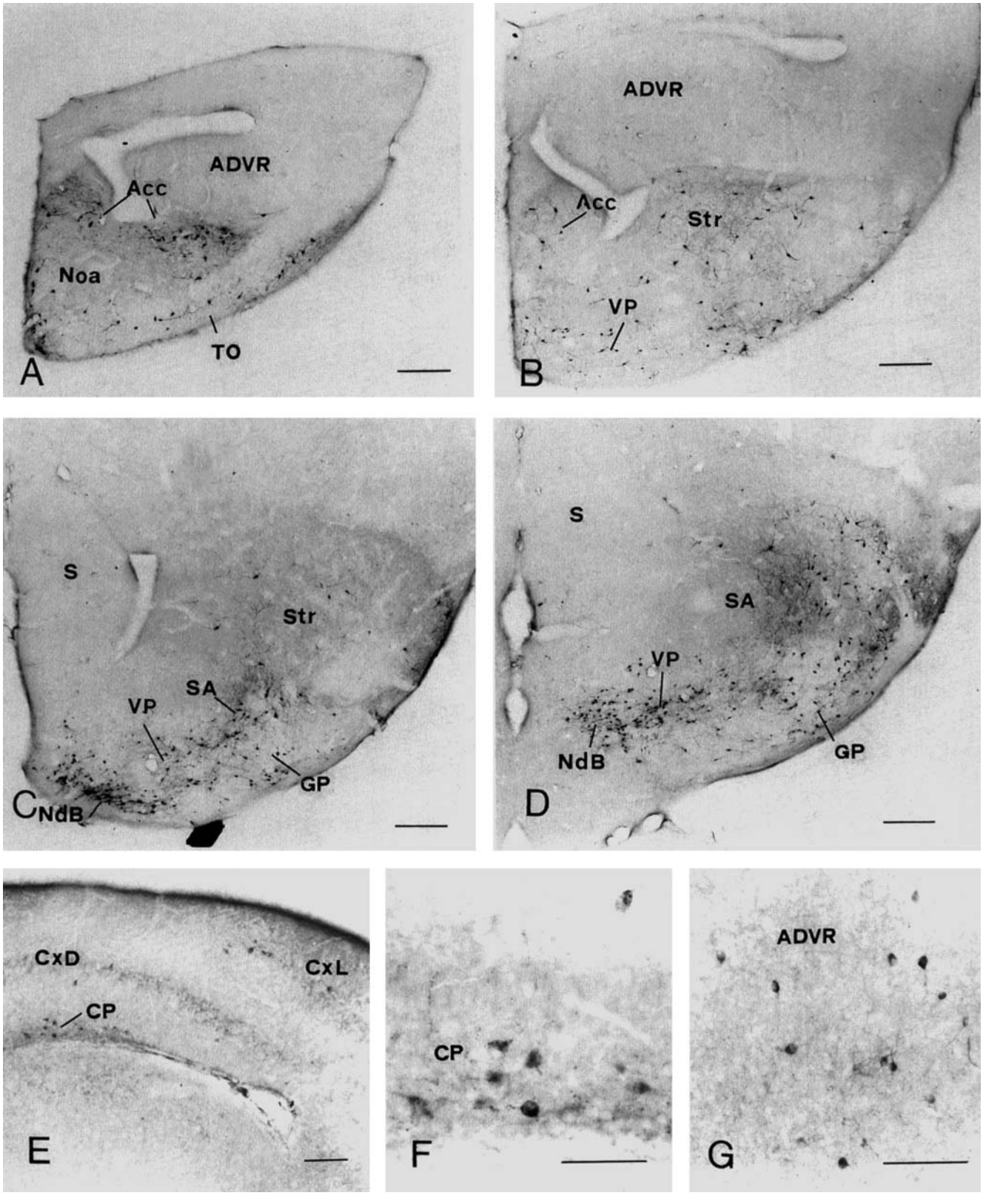

Fig. 6. Photomicrographs of transverse sections through the telencephalon of *Gallotia galloti*, showing ChATi cell bodies and fibers at several rostrocaudal levels. **A–D:** Sections at successive rostrocaudal levels of the basal forebrain. **E,F:** Neurons in the dorsal and lateral cortices that were only immunoreactive with the rabbit anti-chicken ChAT antibody. **G:** ChATi neurons in the anterior dorsal ventricular ridge. Scale bars: A–D = 200 μm; E,G = 100 μm; F = 50 μm.

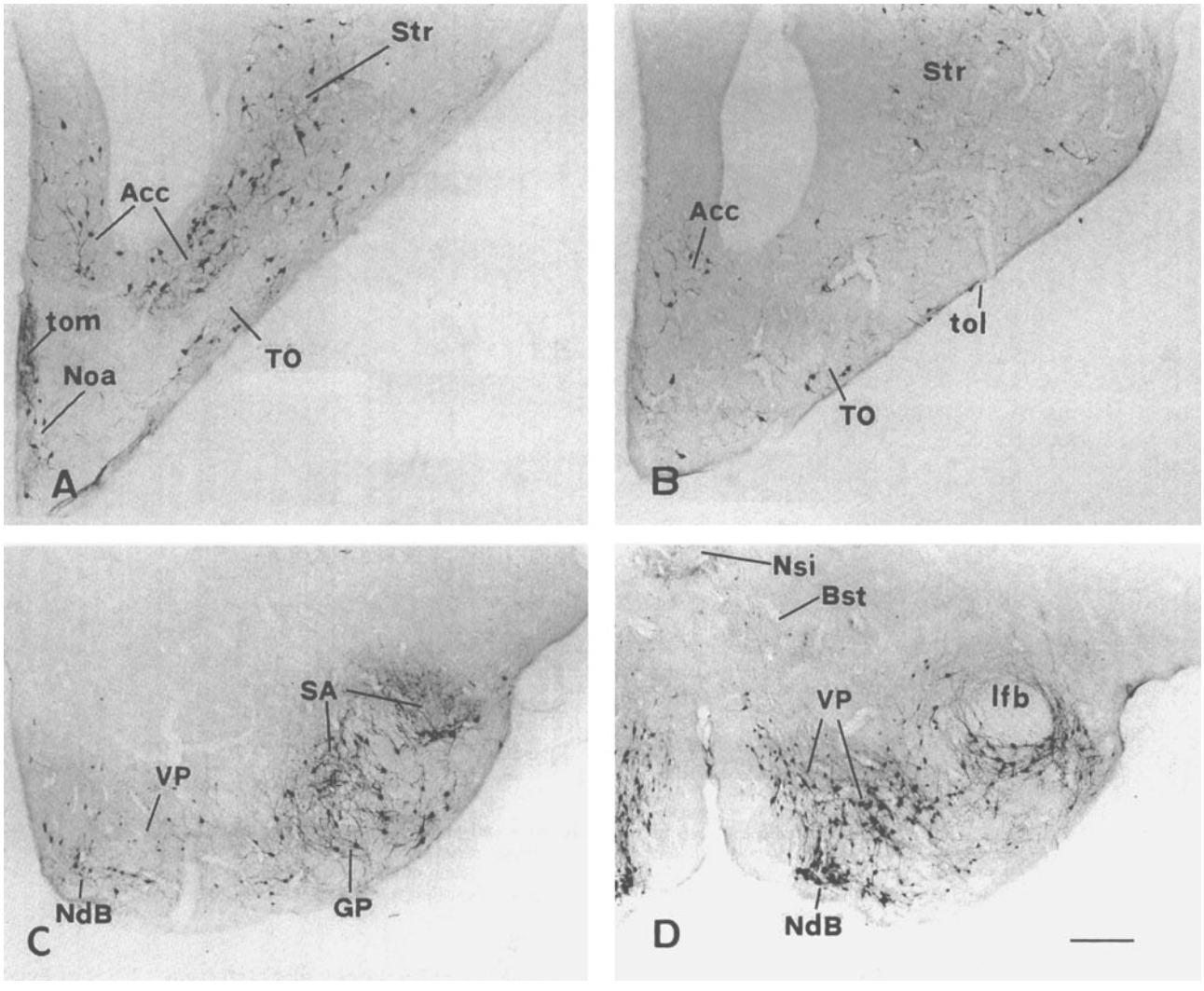

Fig. 7. **A–D:** Photographs of corrected-horizontal sections through the telencephalon of *Gallotia galloti*, showing ChATi cell bodies and fibers at successive rostrocaudal levels of the basal forebrain. Scale bar = 200 μm.

lon (Figs. 1a–d, 2e,f, 6A–D, 7A) and in the olfactory bulb. Smooth ChATi fibers course in the lateral olfactory tract. A very dense plexus of varicose fibers is observed in the nucleus olfactorius anterior and the medial olfactory tract. Further caudally, the nucleus of the lateral olfactory tract is densely innervated by cholinergic fibers, whereas the olfactory tubercule contains a moderate number of ChATi varicose fibers probably representing processes of the intrinsic immunoreactive neurons (Figs. 1a–d, 6A, 7A,B, 8A).

Smooth ChATi fibers are observed in the alveus and can be traced to cortical areas (Figs. 1a–d, 2e–g, 3h). A dense lamina of immunoreactive varicose fibers is present in the intermediate part of the outer plexiform layer of the medial cortex. A distinctive plexus is observed in the outer plexiform layer of the lateral part of the dorsal cortex. A few ChATi fibers are seen in the inner plexiform layer of the medial and dorsal cortices, as well as in the cortical plate of Unger. The lateral cortex is devoid of ChATi fibers.

A moderate innervation by ChATi fibers is observed in the anterior dorsal ventricular ridge, but a much denser plexus is found in its posterior portion (Figs. 1a–d, 2e–g, 3h).

Immunoreactive fibers are observed in the nucleus accumbens, striatum, ventral pallidum, and nucleus of the diagonal band of Broca (Figs. 1a–d, 2e,f, 6A–D, 7A–D, 8A–D, 15A, 16A). At caudal striatal levels distinctive, dense plexuses of ChATi varicose fibers surround the lateral forebrain bundle (lfb). These plexuses are related to processes of ChATi cell bodies located close to them, also around the lfb. Topographically, part of them may be correlated with the so-called striato-amygdaloid transitional area (Figs. 2e,f, 6C,D, 7C, 8C).

In the septum, a dense immunoreactive plexus is observed in the nucleus septalis impar (Figs. 2g, 7D). ChATi fibers are also observed around the anterior commissure, i.e., the bed nucleus of the stria terminalis (Figs. 2g, 7D).

Preoptic and hypothalamic areas. Smooth ChATi fibers are present in the area preoptica lateralis. A moderate plexus of ChATi varicose fibers is observed in the nucleus suprachiasmaticus with the anti-chicken ChAT antibody

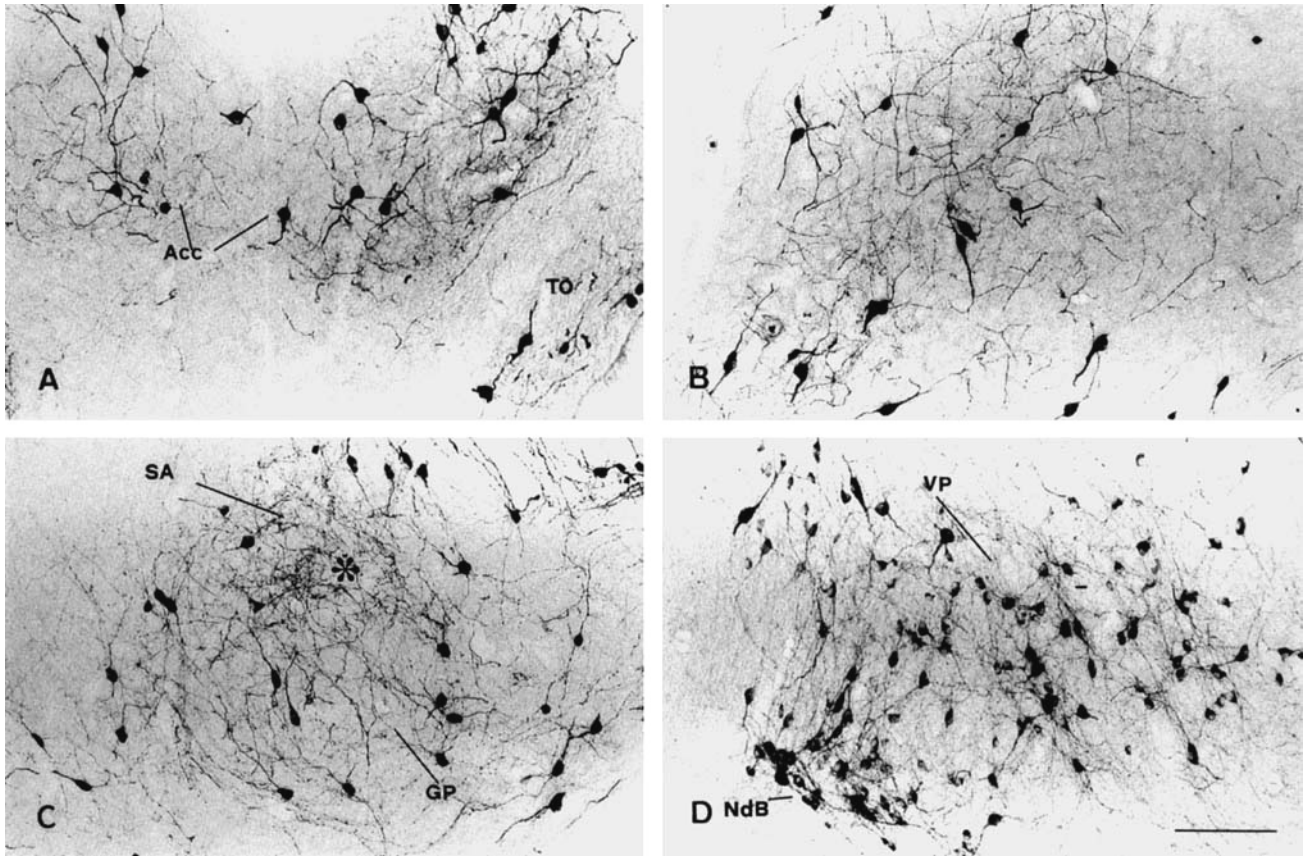

Fig. 8. High magnification photographs of ChATi neurons and fibers in the basal forebrain. **A, B:** Immunoreactivity in the nucleus accumbens and the rostral striatum, respectively. **C, D:** ChAT immunoreactivity at caudal telencephalic levels. Note the dense plexus of ChATi fibers in the striato-amygdaloid area (C, asterisk), with most cell bodies surrounding it. Scale bar = 100 μm.

(Fig. 2f). In the hypothalamus, moderate to dense plexuses of varicose immunoreactive fibers are seen in the three groups of cholinergic cell bodies of the nucleus periventricularis hypothalamicus (Figs. 2g, 3h,i, 10A,B). These ChATi plexuses are connected with those on the other brain side by a ChATi bundle of fibers, which cross the midline through the dorsal supraoptic commissure. The ChATi hypothalamic cells also seem to be the origin of the few varicose fibers observed in the area lateralis hypothalami. A number of ChATi varicose fibers are also seen in the nucleus dorsolateralis hypothalami and around the unstained cell bodies of the periventricular hypothalamic organ (Fig. 3i,j). A moderate plexus of fibers is present lateral to this organ. Further caudally, there is a small path of intensely immunoreactive varicose fibers in the infundibular region (Fig. 3h–j). These fibers surround the infundibular cell bodies that are only immunoreactive with the rabbit anti-chicken antibody.

Finally, numerous ChATi fibers decussate in the commissura supraoptica ventralis, coursing then as two longitudinal bundles of fibers internal and parallel to the optic tract (arrowheads in Figs. 2g, 3h–j, 4k–o, 10A,B, 15A–C, 16B). These two bundles can be traced caudally, across the diencephalon and mesencephalon, to the isthmic region, where they originate. At the level of the supraoptic commissure, a few ChATi fibers leave these bundles and course into the contralateral optic nerve.

Thalamus, epithalamus, and pretectum. The two longitudinal bundles of ChATi fibers referred above travel through the diencephalon, giving off collaterals into the ventral thalamus, the dorsal thalamus, and the pretectum (arrowheads in Fig. 10A,B). These three segmental domains (Puelles et al., '87, '91) are differentially innervated by the ChATi fibers, so that they can be easily distinguished one from another (Figs. 10A,B, 15A–C). The dorsal thalamic domain generally contains less ChATi fibers than the ventral thalamic and pretectal domains. In addition, two bundles of fibers underscore the pretecto-thalamic boundary, collecting adjacent to it (Figs. 10A,B, 15A,C). One of them is composed of the axons of the ChATi habenular neurons, which course within the fasciculus retroflexus (fr). The other group of fibers collects in the foremost portion of the precommissural pretectum, spreading in a sheet along the intermediate and superficial levels of the pretecto-thalamic limit. We shall refer to it as the tractus limitans (lim).

In general, a large number of immunoreactive fibers and varicosities is present in the diencephalon of *Gallotia*. Particularly dense plexuses are observed in all the retinorecipient neuropiles (Figs. 3h–j, 4k–m, 9A,B, 10A,B, 15A–C). In the ventral thalamus, these are represented by the nucleus ovalis, nucleus ventrolateralis, and nucleus geniculatus lateralis pars ventralis, whose superficial neuropiles

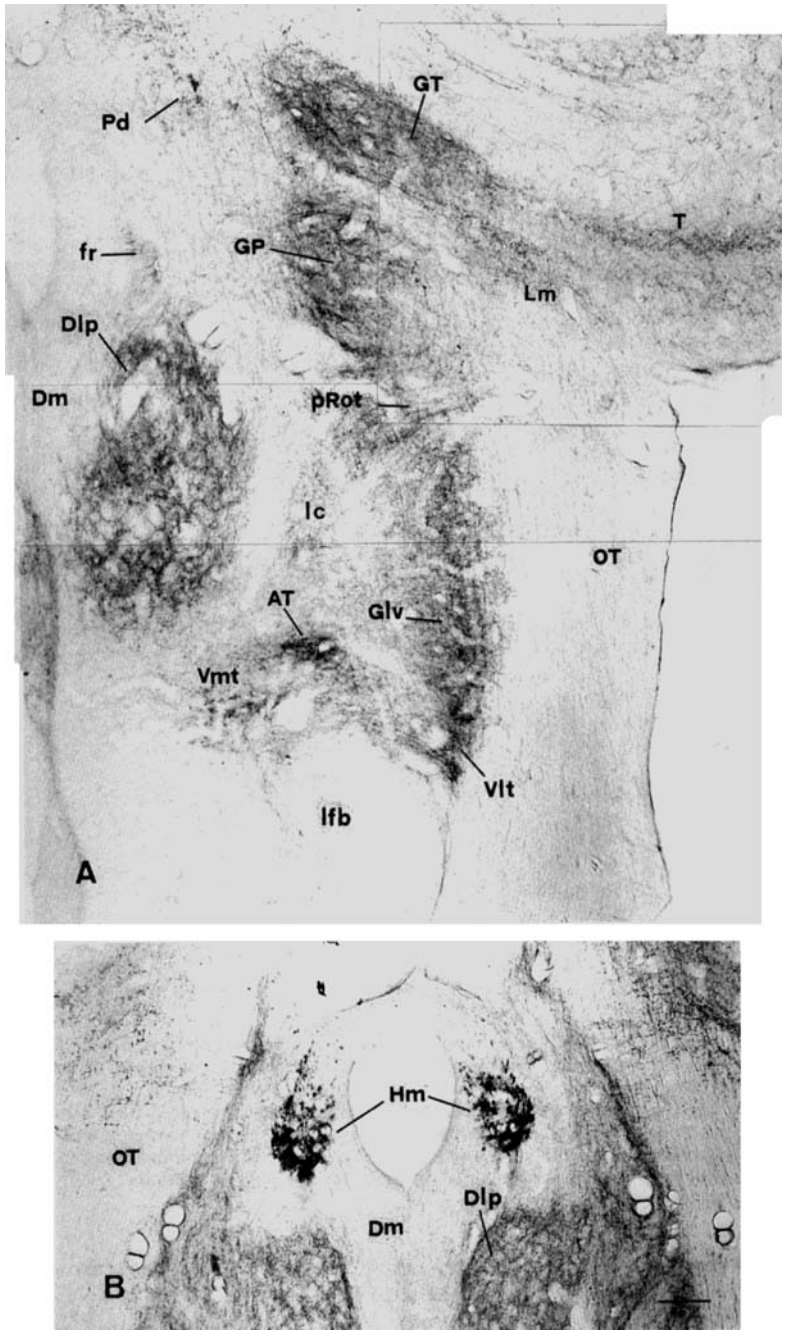

Fig. 9. Photographs of corrected-horizontal sections through the diencephalon and mesencephalon of *Gallotia galloti*. **A:** Note the dense plexuses of ChATi fibers in the superficial neuropiles of the primary visual centers, as well as in the area triangularis, nucleus ventromedialis, and nucleus dorsolateralis of the thalamus. **B:** ChATi cell bodies in the medial habenular nucleus. Scale bar = 100 μm.

have a dense innervation by immunoreactive fibers. Moderate innervation is also observed in the cellular plates and medial neuropiles of the latter two nuclei. Another ventral thalamic center containing a dense plexus of ChATi terminals is the area triangularis. A moderate plexus of terminals is observed in the nucleus ventromedialis.

The dorsal thalamus contains a dense plexus in the retinorecipient neuropiles of the nucleus geniculatus lateralis pars dorsalis and perirotundal belt, as well as in the nucleus dorsolateralis (Figs. 3i,j, 4k, 9A,B, 10A,B, 15A–C). With the anti-chicken ChAT antibody, the neurons of the perirotundal belt appear to be surrounded basket-like by immunoreactive terminals. Moderate- to light plexuses of ChATi fibers are found within the nucleus intercalatus, nucleus rotundus, nucleus medialis thalami, and nucleus medialis posterior. The medial habenular nucleus also contains moderate to dense plexuses of fibers that surround the cholinergic cell bodies or constitute a separate, densely stained neuropile in the dorsal part of the habenula (Figs. 3j, 4k, 9B).

Retinorecipient neuropiles of the pretectum, represented by the lateral neuropile of the nucleus geniculatus pretectalis, nucleus lentiformis mesencephali, and nucleus posterodorsalis, are densely innervated by ChATi fibers (Figs.

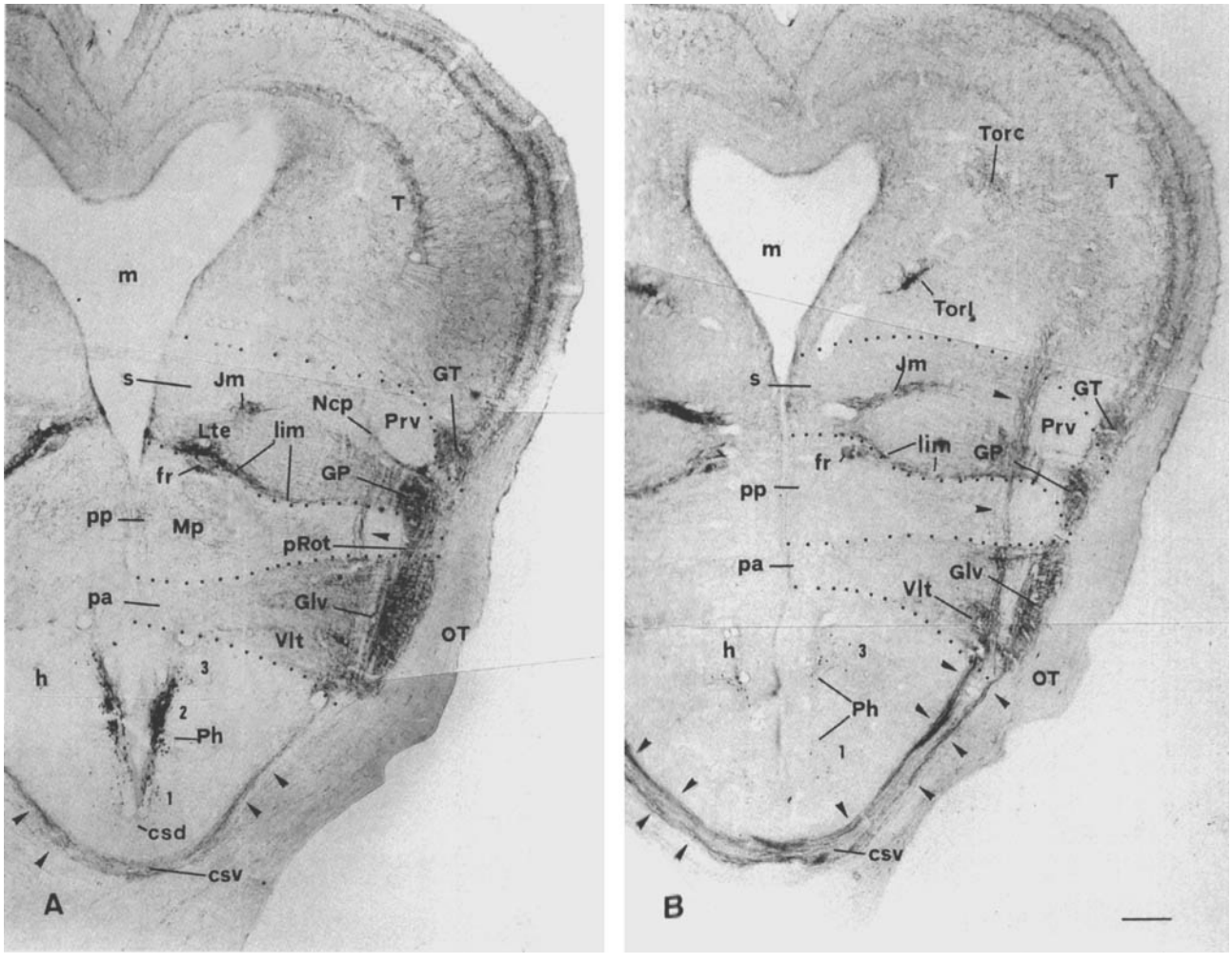

Fig. 10. **A,B:** Photographs of corrected-horizontal sections through the hypothalamus (h), diencephalon (pa, pp, s), and mesencephalon (m) of *Gallotia galloti*. Three different subpopulations of ChATi cell bodies are seen in the periventricular hypothalamic nucleus (1, 2, 3). Note the dense plexuses of ChATi fibers in the superficial retinorecipient neuropiles of the diencephalon and mesencephalon. Two longitudinal bundles of immunoreactive fibers cross the brain at this level (arrowheads) and converge in the ventral supraoptic commissure (csv). These longitudinal fiber bundles emit collaterals into the parencephalon anterior (pa), parencephalon posterior (pp), and synencephalon (s), producing a characteristic pattern of innervation that changes at the limits of these three segmental domains (separated by dot lines in A and B). The tractus limitans (lim) is a large group of such collaterals that underscores the pretecto-thalamic limit. Scale bar = 200 μm.

4k–m, 9A, 10A,B, 15B,C). The cellular plate and medial neuropile of the nucleus geniculatus pretectalis contain moderate plexuses of ChATi fibers. The nucleus lentiformis thalami—pars plicata and pars extensa—shows a dense innervation by ChATi fibers in a zone adjacent to the tractus limitans which is apparently due to terminal arborisation of its fibers (Figs. 4k, 10A,B). This band of terminals extends to the ventricular lining, where a dense ChATi patch appears (Fig. 10A). The nucleus juxtacommissuralis medialis, which lies just caudal to the nucleus lentiformis thalami pars plicata, also shows a dense ChATi plexus (Figs. 4l,m, 10A,B, 15B). A moderate innervation was observed in the neuropile of the nucleus pretectalis dorsalis, the lateral neuropile of the nucleus of the posterior commissure, and the nucleus of the basal optic root (Figs. 4k–m, 10A).

Mesencephalon. A laminar organization of ChATi fibers is observed in the griseum tectale (GT) and the optic tectum (T). Very dense plexuses of ChATi varicose fibers are present in the superficial tectal layers 9 and 11, which are known to receive optic afferents (Medina and Smeets, '92), as well as in layers 3 and 5 of the tectum (Figs. 4k–o, 5p, 9B, 10A,B, 15B,C). Dense plexuses are also seen in the superficial retinorecipient layers and the periventricular layers of the GT (Figs. 4m, 9A). Moderate innervation is observed in the stratum griseum centrale and other superficial strata of both alar mesencephalic structures. Smooth ChATi fibers are seen in the tectal layer 8.

A very dense plexus of ChATi varicose fibers is observed rostrally in a small area within the nucleus laminaris of the torus semicircularis (Figs. 4n, 10B, 15B). ChATi fibers and varicosities are also present around the cells of the Edinger-Westphal and oculomotor nuclei (Figs. 4n,o, 15A). The nucleus centralis of the torus semicircularis, the nucleus profundus mesencephalicus pars caudalis, and the substan-

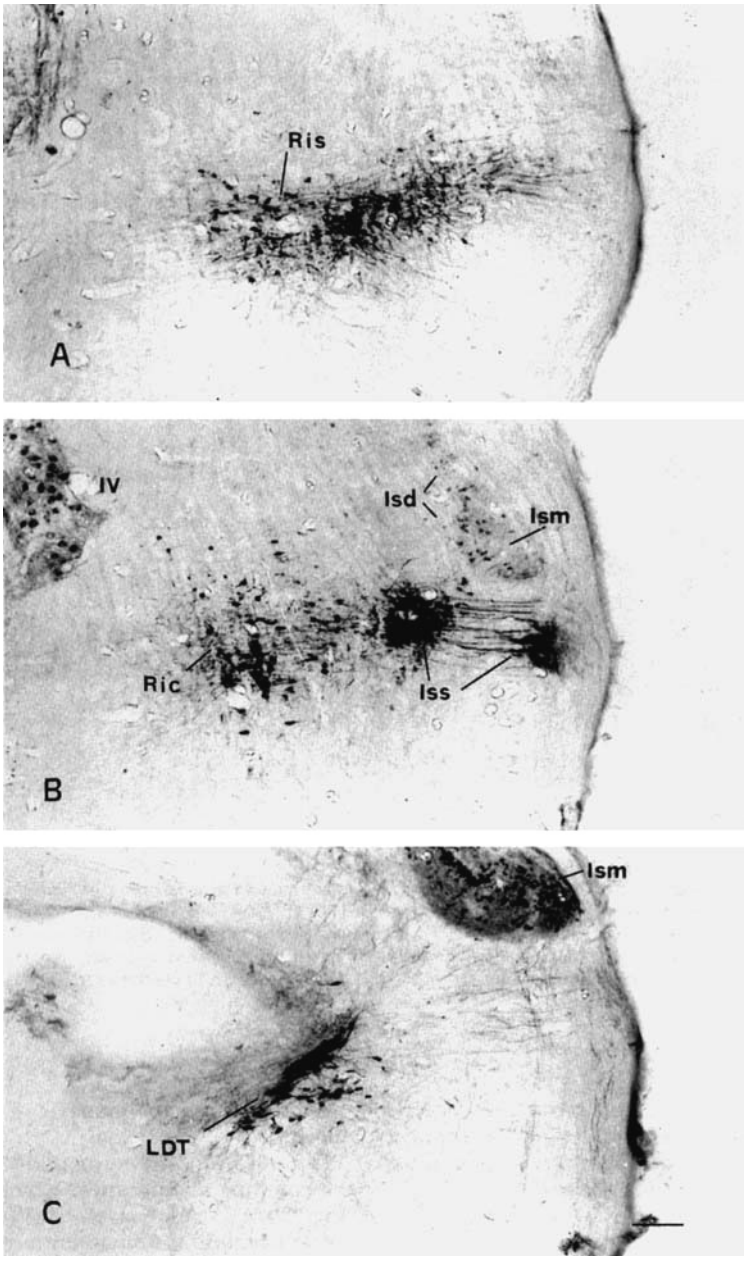

Fig. 11. Photographs of transverse sections through the isthmus of *Gallotia galloti,* going from rostral (**A**) to caudal (**C**) levels. Note the long lateral dendrites of the ChATi neurons of the semilunar isthmic nucleus (**B**). Scale bar = 100 μm.

tia nigra pars reticulata contain a moderate number of ChATi varicose fibers (Figs. 4n,o, 5p, 10B).

Isthmus and cerebellum. A large number of ChATi fibers and varicosities is seen in the isthmic alar plate (Figs. 4o, 5p,q, 11A–C, 12A–D, 15C, 16B,C), particularly in the magnocellular, parvicellular, and diffuse isthmic nuclei. Just caudal to the main isthmic nuclei, a number of immunoreactive varicose fibers surround the ChATi neurons of the Iss, Ris, and Ric nuclei, as well as those of the LDT nucleus. A dense plexus of varicose fibers is seen in the periventricular neuropile deep to the LDT and the locus coeruleus. All these nuclei seem to be the origin of the inner longitudinal tract of thick ChATi fibers that courses through the mesencephalon and diencephalon, and decussates rostrally in the supraoptic commissure. The decussated fibers may partially contribute to the superficial longitudinal tract described above.

In the cerebellum (Fig. 6p,q), a number of thick ChATi fibers are seen in the granule cell layer. These fibers produce clusters of thick varicosities, shaped like mossy terminals.

In the basal plate, a plexus of ChATi varicose fibers is seen around the neurons of the trochlear nucleus (Figs. 4o, 11B, 12C).

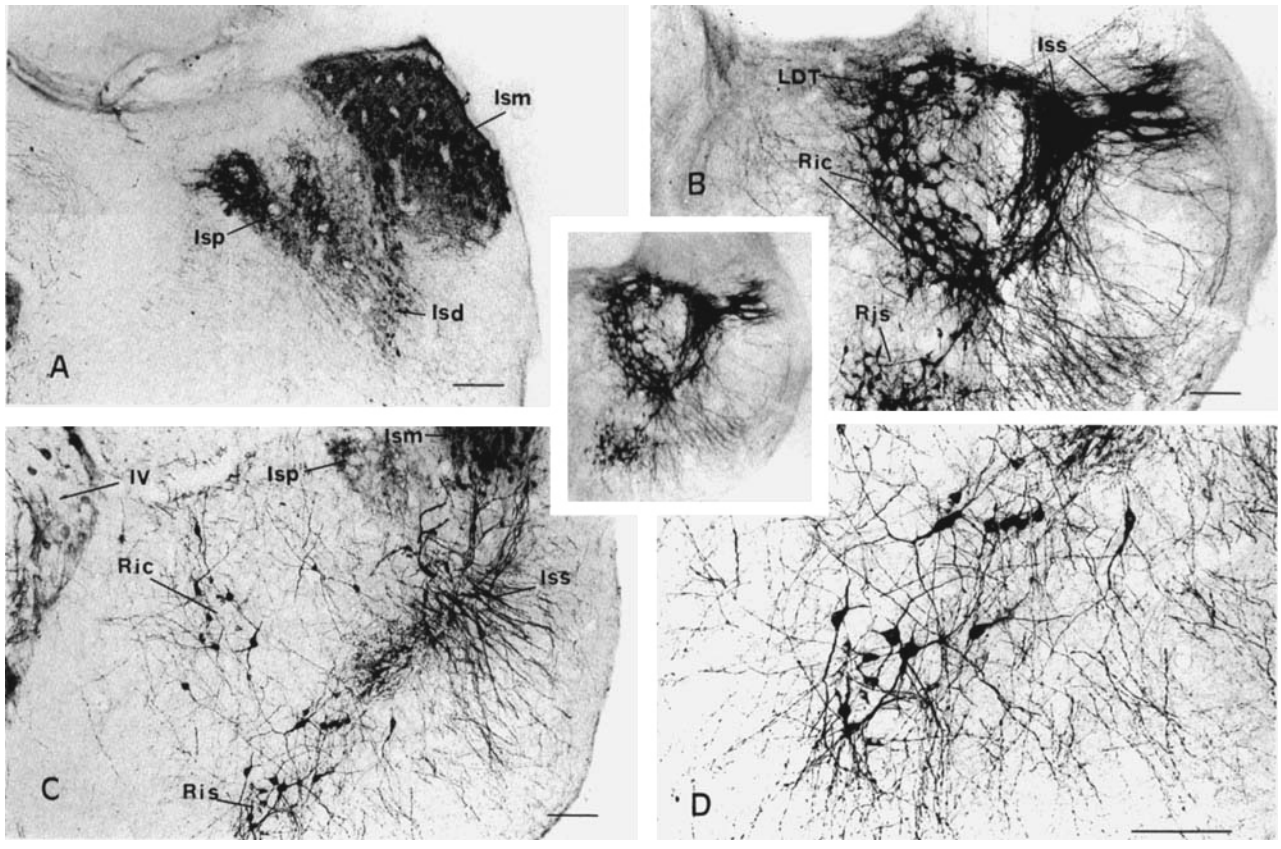

Fig. 12. Photographs of corrected-horizontal sections through the isthmus of *Gallotia galloti*. **A:** Immunoreactive cells (Ism, Isd) and dense plexuses of ChATi fibers (Isp, Ism) in the rostral isthmus. **B:** Strong immunoreactivity of the neurons of the reticular isthmic nucleus (Ric, Ris), semilunar isthmic nucleus (Iss), and laterodorsal tegmental nucleus (LDT) in the caudal isthmus (**inset,** panoramic view). **C:** Section taken at an intermediate level between A and B. **D:** Detail of ChATi neurons in the Ris. Scale bar = 100 μm.

Rhombencephalon. The roots of all branchial (V, VII, IX, X) and somatic (III, IV, VI, XII) motor nerves contain ChATi fibers, as expected (Figs. 4m, 5p–r,w,x). The root of the VIIIth cranial nerve occasionally showed some ChATi fibers.

Varicose ChATi fibers are seen surrounding the cell bodies and dendrites of most motoneurons (Figs. 5q–x, 13A,B, 14A,B, 15B,C, 16B,C). Distinctive plexuses are observed coinciding with the ventrolateral dendritic fields of the trigeminal and facial nuclei, as well as with the medial dendritic field of the ventrocaudal trigeminal nucleus. Other dense plexuses are present at the superior and inferior salivatory nuclei, the nucleus ambiguus, and the hypoglossal nucleus.

A moderate number of ChATi fibers surround the immunoreactive neurons of the reticular substance, and the nucleus vestibularis descendens (Figs. 4o, 5p,r–x, 13A, 14A,B, 15A). Other ChATi fibers innervate zones adjacent to the isthmic and rhombencephalic midline which may correspond to raphe nuclei (Figs. 4o, 5p, 14A,B). Several immunoreactive axon bundles course longitudinally through the rhombencephalic tegmentum. Most of them seem to have their origin in the ChATi cells of the reticular isthmic tegmentum. Others seem to arise from the small ChATi cells of the oralis subnucleus of the descending trigeminal nucleus.

Spinal cord. Numerous ChATi varicose fibers surround the columns of immunoreactive cells in the ventral horn (Fig. 5y). A number of ChATi varicose fibers are also present around the small immunoreactive cell bodies of the dorsal horn. Axons and dendrites of immunoreactive motoneurons cross the spinal cord radially. In addition, smooth immunoreactive fibers are observed to course longitudinally through the spinal cord.

DISCUSSION

In the present account, the distribution of acetylcholine was studied in the brain of *Gallotia galloti* by using two different antibodies against ChAT: a monoclonal rat anti-ChAT and a polyclonal rabbit anti-chicken ChAT. In general, both antibodies yielded a consistent staining pattern of cell bodies and fibers, with the exception of some cortical and infundibular immunoreactive neurons that stained only with the polyclonal rabbit anti-chicken ChAT. The specificity of both primary antibodies has been demonstrated previously in non-mammalian vertebrates (Levey et al., '83; Johnson and Epstein, '86; Sorenson et al., '89), and

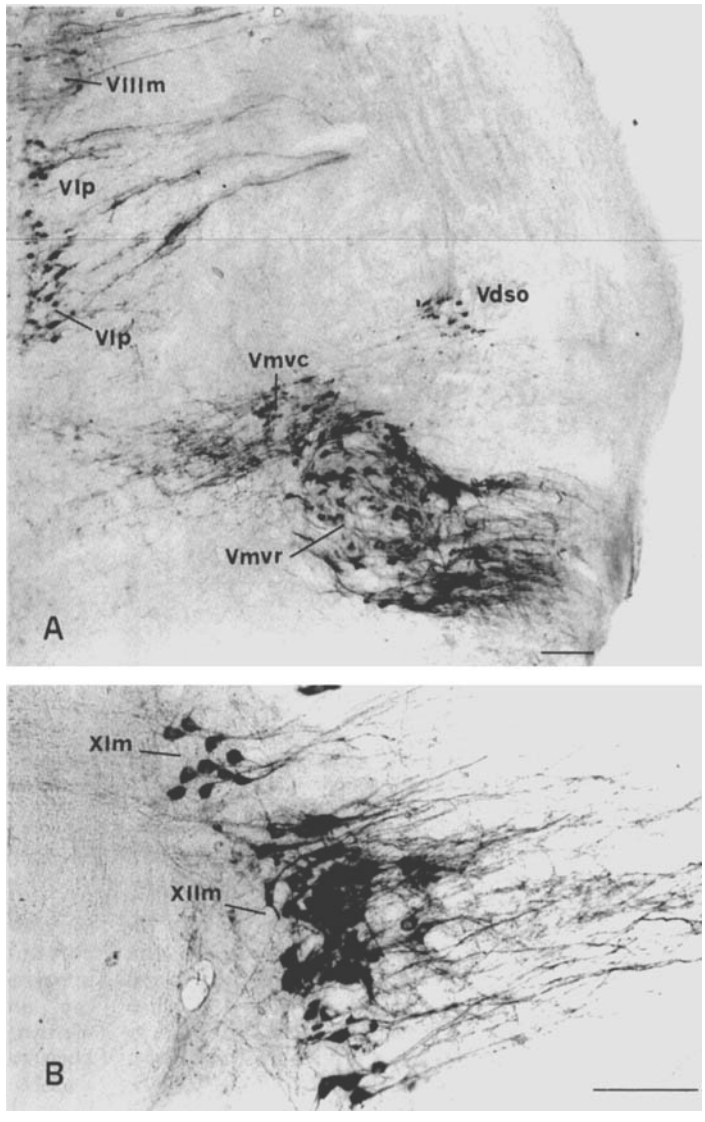

Fig. 13. **A,B:** Photographs of corrected-horizontal sections through the rhombencephalon of *Gallotia galloti*. ChATi neurons are observed in the motor centers of nerves V, VI, VIII, XI and XII. In addition, immunoreactive cell bodies are seen in the oral descending trigeminal nucleus (Vdso). Scale bar = 100 μm.

method specificity was checked here by controls omitting the first antibody. The observation of immunoreactive neurons in cortical and infundibular areas only with the rabbit anti-chicken ChAT may indicate a higher sensitivity of this antibody to reptilian ChAT, although a nonspecific cross-reaction cannot be ruled out.

A segmental analysis of choline acetyltransferase immunoreactivity in the brain of *Gallotia*

The presence of longitudinal subdivisions (alar, basal, and paramedian plates) in the neural tube is an accepted feature of the morphology of the CNS of vertebrates (Bergquist and Källén, '54; Vaage, '69; Keyser, '72; Puelles et al., '87). In addition to the longitudinal subdivisions, morphological transverse segmentation of the rhombencephalon and forebrain has been repeatedly postulated in vertebrates (Bergquist and Källén, '54; Vaage, '69; Keyser, '72; Puelles et al., '87). This has been based on the observation of proliferative discontinuities in the neural tube wall during development, which resulted in the appearance of transverse bulges or neuromeres in the neural axis. Recently, the existence of transverse segments or neuromeres in the brain has been largely accepted after the discovery of homeobox genes that show a segmented expression pattern in the brain which coincides with the morphological limits (see reviews by Keynes and Lumsden, '90; and Noden, '91 for the rhombencephalon; Price et al., '91, for the prosencephalon). Since the brain seems to be a segmentally organized structure, in order to understand better the organization of the cholinergic systems in the brain of *Gallotia*, in the present account we have tried to analyse our results with respect to segmental domains. In the next paragraphs we shall describe the segments that have been reported previously in the brain of different vertebrates, and we shall try to identify the corresponding segments in the brain of the lizard *Gallotia*.

Different authors agree on the description of transverse segments in the rhombencephalon (rhombomeres) and mesencephalon (Vaage, '69, '73; Puelles et al., '87; Lumsden and Keynes, '89; Noden, '91). The rhombomeres can be easily identified in the brains of different vertebrates, when

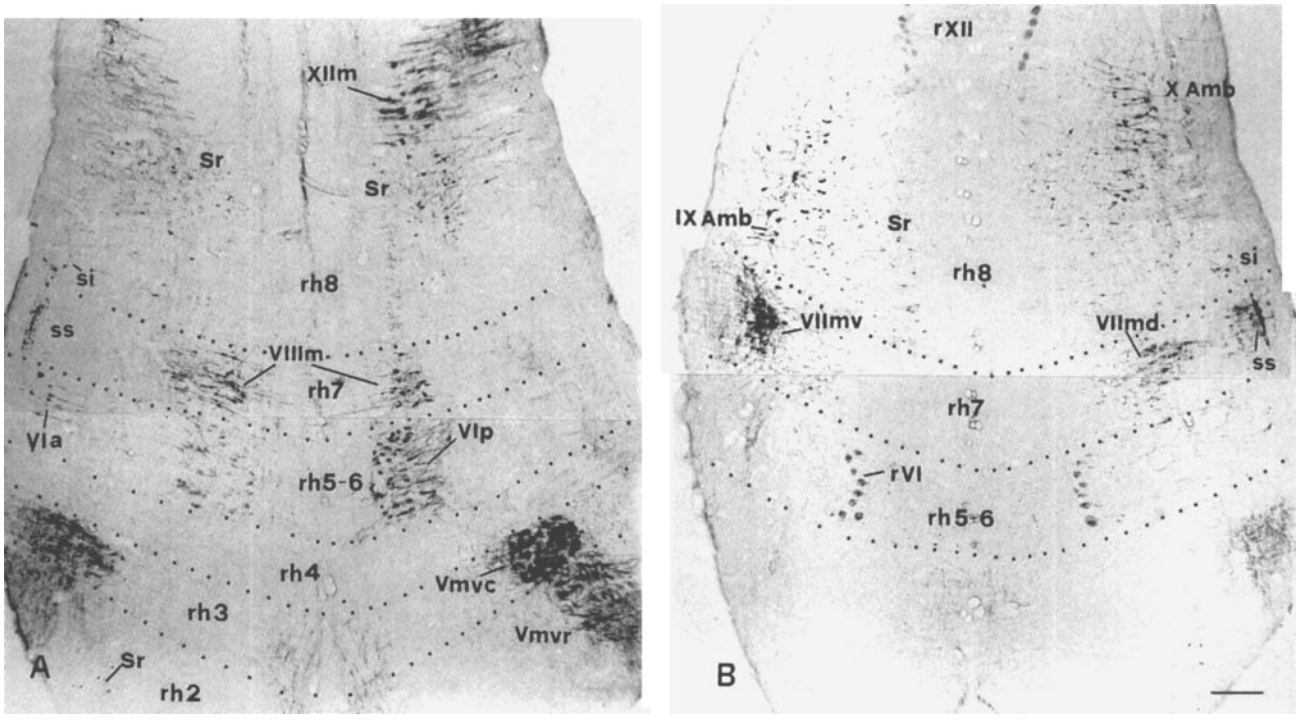

Fig. 14. **A,B:** Photographs of corrected-horizontal sections through the rhombencephalon of *Gallotia galloti*, showing ChATi neurons in the reticular formation and the motor nuclei of nerves V–XII (A is dorsal to B). The ChATi cell groups are arranged within different segmental domains (rh1–8; limits are drawn with dot lines). Dendrites and axons course within these domains parallel to the limits. Note the dense plexuses related to the lateral dendrites of the trigeminal motoneurons (A). Scale bar = 200 μm.

the cranial nerves are used as a reference (Vaage, '69, '73; Lumsden and Keynes, '89; Noden, '91). In reptiles, the correlation between cranial nerve roots and transverse segmental domains is the same as that described in other vertebrates (Puelles, unpublished observations in embryos), and we have followed the same criteria in identifying the rhombomeres in the present study (see Figs. 14A,B, 17A). Except for the VIIth and IXth efferent neurons, the various somatomotor, branchiomotor, and preganglionic efferent ChATi cell groups of the cranial nerves of lizards occupy the expected basal segmental positions, as in chick embryos (Lumsden and Keynes, '89). The facial and glossopharyngeal motoneurons occupy positions that are caudal to their segmental origin, marked by the nerve roots (Figs. 14A,B, 17A). This suggests that these motoneurons in reptiles migrate caudalwards during development, as has also been reported for mammals (Windle, '33; Kimmel, '40). Our mapping of the nucleus abducens across rhombomeres 5 and 6 (Fig. 17A) is based on embryonic data showing the roots of the VIth nerve emerging from these two neuromeres (Puelles, unpublished observations), a condition similar to that found in chick embryos.

General agreement also exists with respect to the description of transverse neuromeres in the diencephalon (Rendahl, '24; Bergquist and Källén, '54; Keyser, '72; Puelles et al., '87, '91). Three transverse neuromeres have been described in the diencephalon, named synencephalon (s), posterior parencephalon (pp), and anterior parencephalon (pa) (see review by Puelles et al., '87). Observations of embryological material indicate that the same three transverse neuromeres are also present in the diencephalon of reptiles (Puelles, unpublished observations). The dorsal portions of the three diencephalic neuromeres consist of the pretectum (s), the epithalamus (pp), the dorsal thalamus (pp), and the ventral thalamus (pa) region, whereas the ventral portions are comprised of tegmental areas. Both the horizontal (Fig. 10A,B) and sagittal (Fig. 15A–C) ChAT-stained sections through the diencephalon of the lizard show these three well-delimited, transverse neuromeres (Fig. 17B), which are further recognisable by the presence of ChATi fiber bundles coursing adjacent to the limits (fasciculus retroflexus and tractus limitans; Figs. 10A,B, 15A,B), and by a different density of ChATi fibers between them (Figs. 10A,B, 15C). The delineation of these three diencephalic neuromeres in the present study coincides with that described by Martinez-de-la-Torre ('85) in several reptilian species on the basis of material stained with cresyl violet or for acetylcholine esterase activity.

No conclusive evidence for transverse segments or neuromeres has been shown in the prosencephalon so far (reviewed by Puelles et al., '87), and the rostralmost transverse neuromeric domain, referred to as secondary prosencephalon (ps), contains the telencephalon, preoptic region, and hypothalamus. Thus, in the present study the ChATi cell bodies observed in these areas of the brain appear within a single segment in Figure 17B.

The acknowledgement of transverse segments in the neural tube and their use in studying the brain, as in the present account, imply reconsideration of a number of anatomical concepts that are usually misunderstood and misemployed, such as which is the longitudinal axis of the brain, or what is dorsal and ventral in the brain. The

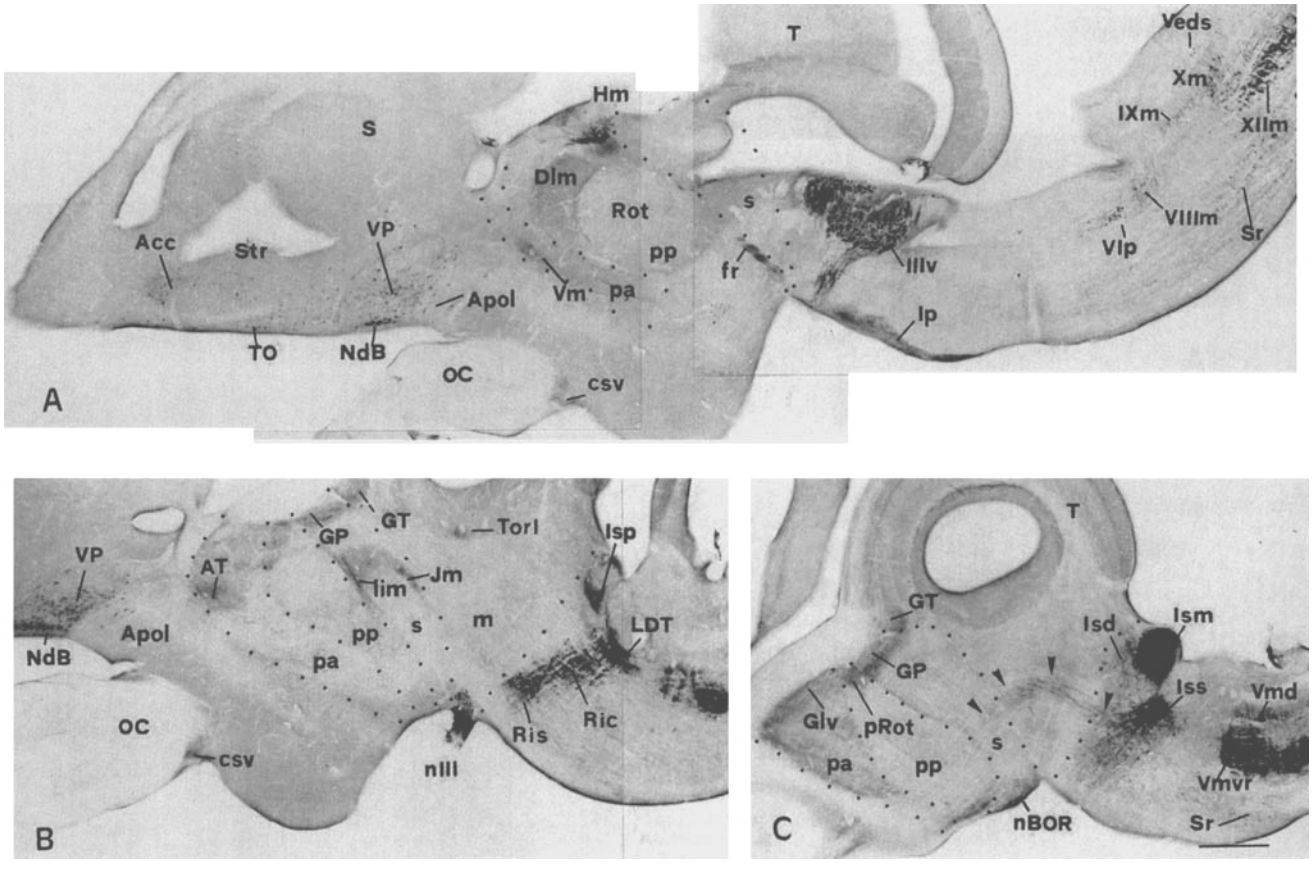

Fig. 15. Photographs of sagittal sections through the brain of the lizard *Gallotia galloti*, at medial (**A**), intermediate (**B**), and lateral (**C**) levels, showing ChATi structures. Dot lines mark the intersegmental limits of the diencephalon (pa, pp, s) and mesencephalon (m). Note the large number of ChATi cell bodies in the caudobasal telencephalon (Bas, NdB) and in the isthmic region (Ric, Ris, LDT, Ism, Iss, Isd). A bundle of ChATi fibers emerges from the isthmus and courses rostralwards into the diencephalon (arrowheads in C). Scale bar = 2 mm.

present study provides evidence on the longitudinal encephalic axis, mainly through observations of the cholinergic tract that courses rostralwards from the isthmus, following a bent trajectory that reproduces the shape of the cephalic flexure and the longitudinal axis of the brain postulated by the neuromeric theory (Figs. 15C, 16B, 17B). Rostrally, this tract becomes incorporated into the medial aspect of the ventral supraoptic commissure, where many of its fibers cross the midline and continue caudalwards contralaterally as a lateral component of the ventral supraoptic commissure (Fig. 10) reaching the optic tectum (crossed isthmotectal pathway). Collaterals issued by these longitudinally running fiber bundles detach at right angles, entering each segmental domain in a ventrodorsal direction (Fig. 17B). The tractus limitans is a conspicuous sheet of such fibers that innervates the rostral pretectum where it borders the dorsal thalamus. More diffuse systems of collateral fibers penetrate the ventral and dorsal thalamic domains. Third order collaterals do not seem to cross intersegmental boundaries and ramify exclusively within the diverse optic or non-optic neuropiles described above within each segmental domain.

Comparison with previous studies in reptiles

Cell bodies. The ChATi cell bodies in the telencephalon of the lizard *Gallotia* are largely distributed like those in the lizard *Gekko* (Hoogland and Vermeulen-VanderZee, '90). In the telencephalon of both lizards, immunoreactive neurons are present in the nucleus olfactorius anterior, olfactory tubercule, nucleus accumbens, striatum, ventral pallidum, nucleus of the diagonal band of Broca, anterior dorsal ventricular ridge, and around the anterior commissure. The presence of ChATi neurons in all these telencephalic structures, with the exception of the olfactory structures and ADVR, seems to be a primitive character in reptiles, since they have been described also in crocodiles (Brauth et al., '85) and turtles (Mufson et al., '84). The present study has revealed additional ChATi cell bodies within the medial and lateral olfactory tracts, the nucleus of the lateral olfactory tract, the globus pallidus, and some cortical areas in the lizard *Gallotia,* which have not been described in other reptiles. Particularly, the present finding of ChATi cell bodies in the cortex of lizards deserves some comment. Although acetylcholine esterase studies always revealed differential staining of cortical neurons, by means of the rat anti-ChAT antibodies cholinergic, immunoreactive neurons could not be demonstrated in previous studies of reptiles (Mufson et al., '84; Brauth et al., '85; Hoogland and Vermeulen-VanderZee, '90). With the latter antiserum we were also unable to detect putative cholinergic cell bodies in cortical regions of lizards. However, with a rabbit anti-chicken ChAT antiserum such cells were easily recog-

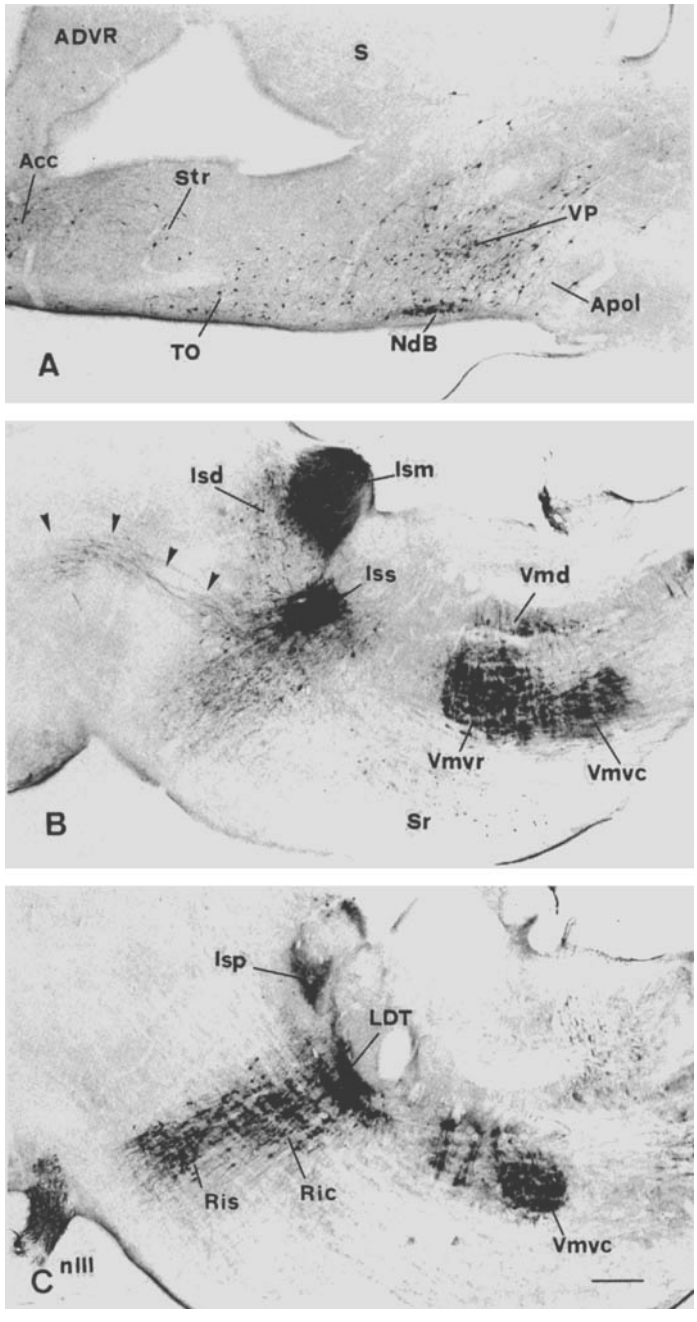

Fig. 16. High magnification photographs illustrating details of the sections in Figure 15. **A:** ChAT immunoreactivity in the basal telencephalon. **B,C:** ChATi cell bodies and fibers in the isthmus and rostral rhombencephalon. Note the bundle of ChATi fibers coursing rostralwards (arrowheads, B), and the three subnuclei of the motor trigeminal complex. Scale bar = 200 μm.

nized in lizards (present results), but not in turtles (Reiner, '91). Although additional studies using this anti-chicken ChAT antibody in the brain of other reptilian species are needed, it seems that the presence of cholinergic neurons in the cortex is not a feature shared by all reptiles.

As in lizards, ChATi neurons have been described in the area preoptica lateralis in other reptiles (Mufson et al., '84; Brauth et al., '85; Hoogland and Vermeulen-VanderZee, '90). The present account presents the first description of ChATi neurons in the periventricular hypothalamic nucleus, lateral hypothalamic area, and infundibular region of a reptile.

The lack of detailed studies makes it difficult to compare our results in the brainstem and spinal cord in lizards with those in other reptiles. Cholinergic neurons were observed in the isthmic nucleus and the lateral reticular formation in crocodiles (Brauth et al., '85), and several species of lizards (Medina and Smeets, '92). These neurons probably correspond to the magnocellular and semilunar isthmic nuclei, and to the isthmic reticular and laterodorsal tegmental nuclei described in the present study. Our results provide evidence of ChATi fiber bundles leaving this large isthmic complex and coursing rostralwards into the diencephalon, and dorsalwards into the tectum and the cerebellum (Figs. 1p, 6, 11C, 12B, 13A). In the lizard *Gallotia,* some ChATi fibers arising in this complex could be traced into the optic nerve and may represent a retinopetal system (Medina and Smeets, '92). This is supported by the finding of retro-

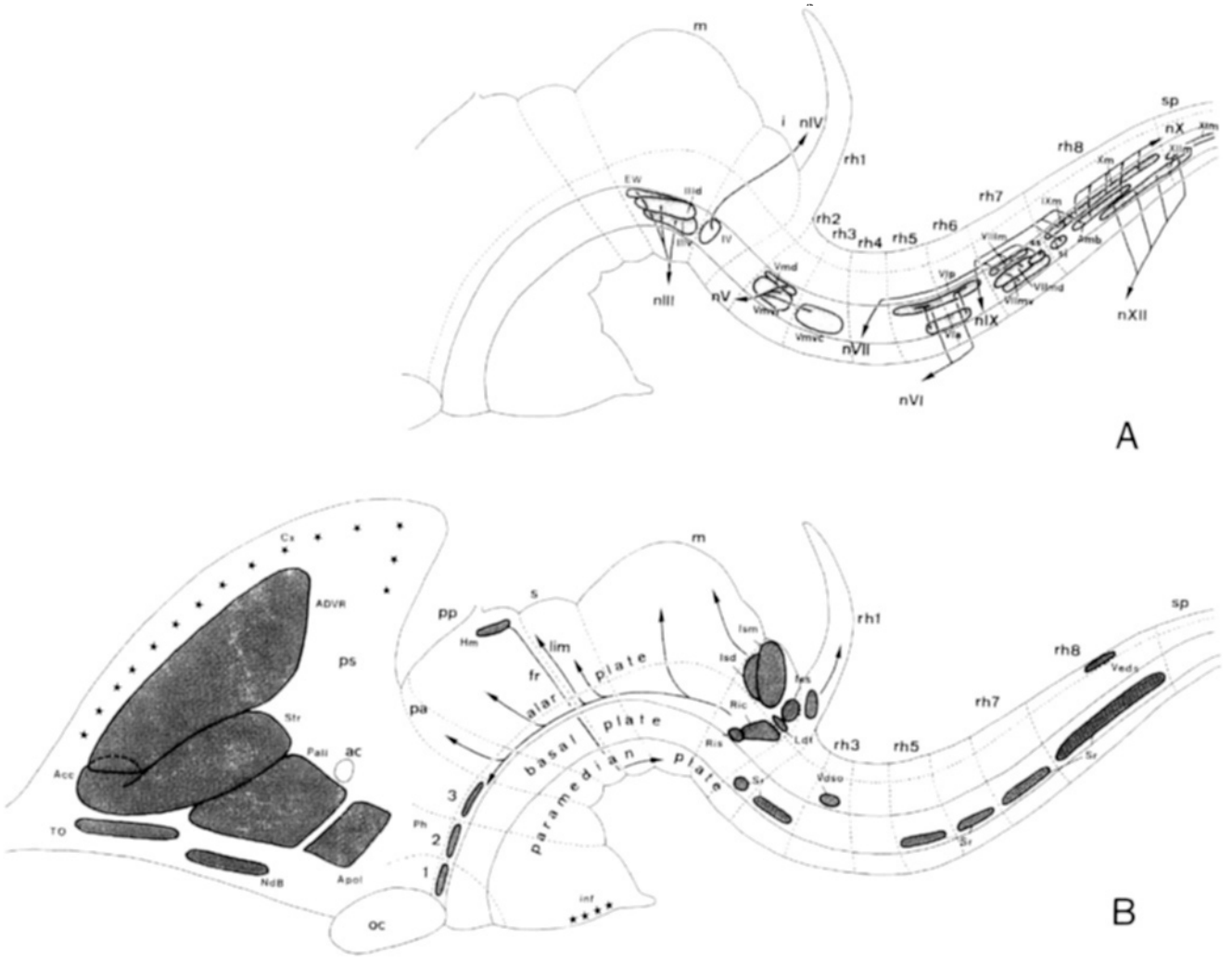

Fig. 17. Sagittal schematic drawing of the brain of the lizard *Gallotia galloti,* showing the topography of ChATi cell bodies and main fiber tracts in relation to the segmental domains, and to the alar, basal, and paramedian longitudinal plates. **A:** ChATi motor nuclei of the cranial nerves with respect to brainstem segments (mesencephalon, isthmus, and eight rhombomeres), which can be easily identified in all vertebrates by using the cranial nerves as a reference (see text for more details). **B:** All non-motor cell groups with respect to brainstem segments (identified as described above), as well as with respect to forebrain segments (secondary prosencephalon, anterior parencephalon, posterior parencephalon, and synencephalon), which were identified following Puelles et al. ('87)'s criteria. Asterisks represent cell bodies in the cortex (cx) and infundibular region (inf) that were only immunoreactive with the rabbit anti-chicken ChAT antibody.

gradely labeled neurons in this area after intraocular injections of HRP (Ferguson et al., '78; Medina and Smeets, '92).

In addition, cholinergic neurons have also been found in "the motor nuclei in the brainstem and spinal cord" of turtles (Mufson et al., '84), as we have seen in lizards, together with some immunoreactive neurons of the reticular formation.

Fibers. The plexiform layers of the medial and dorsal cortices are innervated by ChATi fibers in lizards (present results; Hoogland and Vermeulen-VanderZee, '90). Comparison of the distribution of ChATi terminals in the medial cortex of *Gallotia galloti* with that of acetylcholinesterase (AChE) in *Gallotia stehlini* (Regidor and Poch, '88), which is another endemic lizard from the Canary Islands and taxonomically very close to *G. galloti,* shows an apparent overlap of both enzymes, as in other lizards (Hoogland and Vermeulen-VanderZee, '90).

The cholinergic innervation observed in the medial and dorsal cortices of lizards (present results; Hoogland and Vermeulen-VanderZee, '90) may partially originate in the ventral pallidum/diagonal band, since projections from this zone to those cortices have been shown in several reptiles (Bruce and Butler, '84; Ouimet et al., '85; Russchen and Jonker, '88; Ten Donkelaar and de Boer-van Huizen, '88). Other cholinergic terminals in the lizard cortex probably originate in intrinsic ChATi neurons (present study).

The distribution of ChATi fibers in the subcortical telencephalic areas of the lizard *Gallotia* resembles in many respects that described in the lizard *Gekko* (Hoogland and Vermeulen-VanderZee, '90). In *Gallotia,* however, the ChATi neurons of the rostral and intermediate striatal levels lie within plexuses of immunoreactive varicose fibers, like those of the nucleus accumbens. At caudal striatal levels, most of the ChATi neurons lie around dense immunoreactive plexuses of varicose fibers, and show processes

directed into them. In *Gekko*, mainly the second type of organization has been observed (Hoogland and Vermeulen-VanderZee, '90), whereas the condition in other reptiles is still unknown. Most of the ChATi fibers observed in the striatum of *Gallotia* seem to originate in the ChATi intrinsic neurons, as suggested in other reptiles (Brauth et al., '85; Hoogland and Vermeulen-VanderZee, '90). The nucleus accumbens of *Gallotia* also contains dispersed ChATi fibers that seem to arise in the intrinsic cholinergic cell bodies, whereas distinct plexuses were not observed within the nucleus accumbens of *Gekko* (Hoogland and Vermeulen-VanderZee, '90). Moderate plexuses are seen in the nucleus septalis impar and caudolateral to the ADVR in both lizards.

The present study reveals that all the optic neuropiles of the diencephalon and the mesencephalon are densely innervated by ChATi fibers, as was already described by us in the other lizards (Medina and Smeets, '92). A dark staining of AChE has been found in the optic neuropiles of several reptiles, including lizards, snakes, and turtles (Martínez-de-la-Torre, '85), showing a pattern that largely resembles that of the ChAT immunoreactivity.

The lack of detailed studies in other reptiles makes it difficult to compare our results in the brainstem of lizards. The laminated pattern of ChATi innervation observed in the midbrain tectum is similar to that of other lizards (Medina and Smeets, '92). Numerous ChATi fibers have also been described in the intermediate and deep layers of the midbrain tectum of crocodiles (Brauth et al., '85).

Comparison with other vertebrates and evolutionary considerations

Cell bodies. The distribution of ChAT immunoreactivity in reptiles resembles in many respects the pattern observed in the brain of mammals and other non-mammalian vertebrates. The main groups of ChATi neurons observed in reptiles have also been described in other vertebrates. In addition to them, cholinergic cell bodies have been found in the midbrain tectum/superior colliculus and in the pretectum of some non-reptilian vertebrates that were not observed in the reptilian species studied so far.

Cortical areas. As in lizards, intrinsic cholinergic cell bodies are present in the cortex of mammals (Parnavelas et al., '86; Hendry et al., '87; Blaker et al., '88; Reiner, '91). In contrast, no ChATi neurons have been found in the cortical areas of other reptiles and birds (Mufson et al., '84; Brauth et al., '85; Hoogland and Vermeulen-VanderZee, '90; Shimizu and Karten, '90; Reiner, '91), or in the pallium of amphibians and fishes (Ekström, '87; Brantley and Bass, '88; Ciani et al., '88). From these data, it seems that the presence of cholinergic cell bodies in the cortical areas is a feature acquired rather late during evolution of vertebrate brain. Supporting this suggestion, it has been hypothesised that the neurons of the superficial layers of the mammalian isocortex (layers II–IV, where cholinergic neurons are located) were not present in the telencephalic cortex of reptiles ancestral to modern reptiles and mammals, and these neurons were added later in evolution (Reiner, '91). If this is so, the presence of cholinergic neurons in the cortex of lizards and mammals might constitute a case of convergent homoplasy (Striedter and Northcutt, '91).

Striatum, accumbens and olfactory structures. As in reptiles, the olfactory tubercle, the striatum, and the nucleus accumbens of mammals contain cholinergic neurons (Houser et al., '83; Mesulam et al., '84; Satoh and Fibiger, '85a; Kása, '86; Vincent et al., '86; Vincent and Reiner, '87; Maley et al., '88), which are thought to be intrinsic cells (Kása, '86; Alheid and Heimer, '88; Woolf, '91). The rostrobasal telencephalon of teleost fishes also contains ChATi neurons (Ekström, '87; Brantley and Bass, '88), whereas some AChE positive cells were found in the striatum and the accumbens of amphibians (Ciani et al., '88, nucleus accumbens identified as septum). Observations of ChAT immunoreactivity in the brain of chicken and pigeon indicate that the lobus paraolfactorius, paleostriatum augmentatum (comparable to accumbens-striatum), and olfactory tubercule contain cholinergic neurons (Puelles, Medina, and Reiner, unpublished observations). This means that, in all vertebrates studied so far, the striatal areas of the forebrain contain cholinergic neurons, suggesting that these neurons could have originated very early in the evolution of vertebrates and that they represent a primitive condition.

The rostral cholinergic column. The ChATi neurons observed in the caudal part of the basal forebrain of reptiles largely resembles that described in mammals within the substantia innominata and associated areas (Houser et al., '83; Mesulam et al., '84; Satoh and Fibiger, '85a; Kása, '86; Vincent et al., '86; Vincent and Reiner, '87; Maley et al., '88; Woolf, '91). These cells also form a continuum that includes the septal-diagonal band complex, the pallidal-peripallidal areas, and the nucleus basalis of Meynert. The large group of ChATi neurons observed at caudal levels in the ventral pallidum of reptiles seems analogous to the mammalian nucleus basalis of Meynert, on the basis of its position and its large cholinergic neurons. As was proposed for the analogous areas of reptiles (see discussion above), the cholinergic neurons of the rostral cholinergic column of mammals form a corticopetal complex which provides a direct input to the cortex (Kása, '86; Alheid and Heimer, '88; Woolf, '91). In addition, the rostral cholinergic column of mammals project to the thalamus and the cholinergic cell groups of the ponto-mesencephalic reticular formation (tegmenti pedunculo-pontine and laterodorsal tegmental nuclei) (Kása, '86; Hallager et al., '87; Alheid and Heimer, '88; Parent et al., '88). This may also be true in reptiles, since efferent projections from the ventral pallidum and the striato-amygdaloid transition area have been reported to reach several ventral thalamic nuclei, the isthmic reticular formation and the griseum centrale (Russchen and Jonker, '88), where cholinergic neurons are present (present results).

Cholinergic cells in the caudobasal telencephalon of fishes and amphibians resemble the cholinergic column of the caudobasal forebrain described in reptiles and mammals. In fishes, these cholinergic neurons may project to the pallial areas, where ChATi terminals have been described (Ekström, '87; Brantley and Bass, '88). The observation of ChATi fibers ascending from the cholinergic groups of the basal forebrain to the pallium supports this hypothesis, although we are well aware that some of the ascending fibers may represent an input from the brainstem (Brantley and Bass, '88). Although further studies are needed, the cholinergic neurons of the basal forebrain may represent a primitive feature of the brain of vertebrates and thus constitute a case of static homology (Striedter and Northcutt, '91).

Cholinergic cell groups of the preoptic region and hypothalamus. As in reptiles, cholinergic neurons have been described in the preoptic and hypothalamic regions of

mammals (Tago et al., '87; Woolf, '91). The hypothalamic ChATi neurons of mammals are located in either periventricular or lateral regions, like those of lizards. In addition, ChATi neurons are present in the infundibular region of mammals (Tago et al., '87) and reptiles (present results), so that the distribution of cholinergic neurons in the reptilian and mammalian hypothalamus seems to be very similar. ChATi neurons were observed in the hypothalamus of teleost fishes (Ekstrom, '87), but data on the distribution of ChAT immunoreactivity in the hypothalamus of amphibians and birds are lacking.

Epithalamus. As in reptiles, cholinergic cell bodies are present in the habenula of mammals (Houser et al., '83; Mesulam et al., '84; Satoh and Fibiger, '85a; Kása, '86; Vincent et al., '86; Vincent and Reiner, '87; Maley et al., '88; Woolf, '91), birds (Sorenson et al., '89), amphibians (Ciani et al., '88), and teleost fishes (Ekström, '87; Brantley and Bass, '88). These epithalamic cholinergic neurons were probably present in the habenula of primitive vertebrates, and they were highly conserved during evolution, representing another case of static homology (Striedter and Northcutt, '91).

Cholinergic cell bodies of pretectum and midbrain tectum. In contrast to reptiles and mammals studied so far, in the chicken and teleost fishes a cholinergic cell group has been described in the pretectum (chicken: nucleus spiriformis medialis, Sorenson et al., '89; teleost fishes: nucleus pretectalis superficialis pars magnocellularis, Ekstrom, '87). AChE positive neurons are also present in the pretectum of amphibians, although it is not known whether they are indeed cholinergic (Ciani et al., '88).

In contrast to reptiles (crocodiles: Brauth et al., '85; lizards: present results in *Gallotia* and unpublished observations in *Gekko* and *Podarcis*) and some mammals (baboon: Satoh and Fibiger, '85a; macaque: Mesulam et al., '84; guinea pig: Maley et al., '88), intrinsic cholinergic cell bodies have been described in the midbrain tectum of other mammals (rat: Tago et al., '89; cat: Vincent and Reiner, '87), birds (Sorenson et al., '89), amphibians (Ciani et al., '88), and teleost fishes (Ekström, '87; Brantley and Bass, '88; Zottoli et al., '88), which contribute to the innervation of this structure. The presence of intrinsic cholinergic neurons in the midbrain tectum of a wide variety of vertebrates, and their absence in reptiles studied so far makes it difficult to know what was the primitive condition in the brain of vertebrates. On the one hand, the absence of cholinergic cell bodies in the tectum of reptiles makes it is likely that they were absent in reptiles ancestral to modern ones. On the other hand, the presence of cholinergic perikarya in the midbrain tectum of fishes, amphibians, birds, and some mammals indicates that this may be a primitive feature in the brain of vertebrates; if this is so, at least primitive reptiles, if not modern reptiles, should possess such intrinsic tectal cholinergic cell bodies. One hypothesis that includes both possibilities is that the first primitive reptiles possessed cholinergic tectal neurons, and later in the reptilian evolution there was a bifurcation, so that some reptiles kept those cholinergic neurons, whereas others lost them. If this is true, we should expect that at least reptilian species evolutionarily closer to birds, such as crocodiles, might possess tectal cholinergic cell bodies. However, Brauth et al. ('85), using a rat anti-ChAT antibody, did not find such neurons in the tectum of crocodiles. Considering that the present study has revealed cholinergic perikarya in the brain that were only immunoreactive with a rabbit anti-chicken ChAT antibody, a reinvestigation of the ChAT immunoreactivity in the midbrain tectum of crocodiles and other reptiles with this antibody may be of interest.

The caudal cholinergic column. The column of cholinergic cell bodies observed in the isthmic region of reptiles generally resembles the parabigeminal-parabrachial cholinergic complex of mammals, which includes the parabigeminal nucleus, the nucleus tegmenti pedunculo-pontinus (TPP), the laterodorsal tegmental nucleus (LDT), and the parabrachial nucleus, amongst others (Houser et al., '83; Mesulam et al., '84; Satoh and Fibiger, '85a; Vincent and Reiner, '87; Maley et al., '88; Tago et al., '89; Woolf, '91). In reptiles and mammals, the cholinergic neurons of the caudal column extend longitudinally between the catecholaminergic neurons of the substantia nigra, rostrally, and the locus coeruleus, caudally (Satoh and Fibiger, '85b). The neurons of the caudal part of this column are located close to the superior cerebellar peduncle. The pattern of projections of the caudal cholinergic column of reptiles (see discussion above) resembles strongly that described for the cholinergic neurons of the parabigemino-parabrachial complex of mammals, where they project to the cerebellum, midbrain tectum, thalamus, and the basal forebrain (Sofroniew et al., '85; Beninato and Spencer, '86; Kása, '86; Woolf and Butcher, '86; Dolabela De Lima and Singer, '87; Hallanger et al., '87; Fitzpatrick et al., '88; Woolf, '91). Considering the respective positions within the caudal cholinergic column, the reptilian magnocellular isthmic nucleus is probably homologous to the mammalian parabigeminal nucleus, and the large cholinergic neurons observed in the isthmic reticular formation are comparable to the mammalian TPP, whereas those cholinergic neurons observed in the laterodorsal tegmental nucleus of reptiles are comparable to the mammalian LDT.

The cholinergic neurons observed in the isthmic-parabigeminal nuclei of reptiles and mammals, as well as those of the TPP-LDT-pontine complex, largely resemble the ChATi neurons of the isthmic nucleus and those of the nucleus reticularis superior, nucleus a, and nucleus lateralis valvulae, respectively, of teleost fishes (Ekström, '87; Brantley and Bass, '88; Zottoli et al., '88). Supporting this hypothesis, the pattern of connections of these cholinergic centers is similar to that of reptiles and mammals. On the one hand, the isthmic nucleus of fishes projects to the midbrain tectum, as do the isthmic-parabigeminal nuclei of reptiles and mammals. On the other hand, the axons of the nucleus reticularis superior of fishes course rostralwards and decussate in the postoptic commissure, whereas those of the nucleus lateralis valvulae apparently reach the molecular layer of the valvula cerebelli, thus resembling the connections of Ri/Iss-TPP and LDT of reptiles and mammals.

As in other vertebrates, cholinergic neurons projecting to the tectum have been described in the isthmic nuclei of amphibians (Ricciuti and Gruberg, '85; Ciani et al., '88) and the chicken (Sorenson et al., '89). Although cholinergic cell groups are present in the tegmentum of amphibians and birds, more data about their connections are needed in order to establish homologies.

Motor neurons (cranial nerve nuclei and spinal cord). The cholinergic character of most of the motor neurons of the brainstem and spinal cord seems to be a primitive and highly conserved condition. As in reptiles, cholinergic motor neurons are present in the nuclei of nerves III, IV, V, VI, VII, VIII, IX, X, and XII of fishes (Ekström, '87; Brantley

and Bass, '88), amphibians (Ciani et al., '88), and mammals (Houser et al., '83; Satoh and Fibiger, '85a; Vincent and Reiner, '87; Maley et al., '88; Tago et al., '89).

Medullary cholinergic cell groups. Cholinergic neurons are present in the reticular formation of reptiles, mammals, and fishes (present results; Brauth et al., '85; Ekström, '87; Brantley and Bass, '88; Tago et al., '89; Woolf, '91), and this is probably a primitive condition. As in reptiles, other cholinergic cell groups have been described in the nucleus descendens nervi trigemini and/or some vestibular nuclei of mammals, amphibians, and teleost fishes (Ekstrom, '87; Brantley and Bass, '88; Ciani et al., '88; Tago et al., '89).

Fibers. The distribution of cholinergic fibers in the brain of reptiles resembles in many respects that observed in mammals. Remarkable similarities appear in the cholinergic innervation of the cortical areas, nucleus accumbens, striatum, and basal forebrain (Woolf, '91). As suggested in reptiles, the ChATi innervation observed in the striatum and nucleus accumbens of mammals arises in the intrinsic cholinergic neurons. On the other hand, the cholinergic innervation of the mammalian cortex mostly originates in the basal forebrain (Woolf, '91), although part of it must have an intrinsic origin (Parnavelas et al., '86).

The optic neuropiles of the diencephalon and midbrain show a dense cholinergic innervation in mammals (Kimura et al., '81; Houser et al., '83; Levey et al., '83; Mesulam et al., '84; Maley et al., '88), birds (Sorenson et al., '89), reptiles (present results; Medina and Smeets, '92), amphibians (Ciani et al., '88), and teleost fishes (Ekström, '87; Brantley and Bass, '88). The idea of a retinal origin for this innervation has been rejected, since the retinal ganglion cells of vertebrates studied so far do not contain ChAT (see Medina and Smeets, '92 for discussion). Instead, this innervation originates in cholinergic cell bodies of the brain. As suggested in reptiles (present results; Medina and Smeets, '92), most of the cholinergic innervation of the primary visual centers of mammals originates in the caudal cholinergic column (Sofroniew et al., '85; Beninato and Spencer, '86; Kása, '86; Woolf and Butcher, '86; Dolabela De Lima and Singer, '87; Hallanger et al., '87; Fitzpatrick et al., '88; Woolf, '91). The cholinergic plexuses observed in the neuropile of the Ncp and in the nucleus laminaris of the torus semicircularis of reptiles are obviously comparable to those described in the neuropile of the nucleus spiriformis lateralis and in the nucleus intercollicularis of birds (Sorenson et al., '89; Martínez and Puelles, unpublished observations).

In reptiles and all the mammals studied so far (Houser et al., '83; Mesulam et al., '84; Satoh and Fibiger, '85a; Kása, '86; Vincent et al., '86; Vincent and Reiner, '87; Maley et al., '88; Woolf, '91), the fasciculus retroflexus contains a highly positive component, coursing from the medial habenula to the interpeduncular nucleus. The cholinergic character of the habenulo-interpeduncular projection through the fasciculus retroflexus seems to be also a primitive condition, since it is present in birds (Sorenson et al., '89), amphibians (Ciani et al., '88), and teleost fishes (Ekström, '87; Brantley and Bass, '88), as well as in reptiles and mammals.

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LITERATURE CITED

Alheid, G.F., and L. Heimer (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. Neuroscience *27:*1–39.

Barbas-Henry, H.A., and A.H.M. Lohman (1988) Primary projections and efferent cells of the VIIIth cranial nerve in the Monitor lizard, *Varanus exanthematicus*. J. Comp. Neurol. *277:*234–249.

Beninato, M., and R.F. Spencer (1986) A cholinergic projection to the rat superior colliculus demonstrated by retrograde transport of horseradish peroxidase and choline acetyltransferase immunohistochemistry. J. Comp. Neurol. *253:*525–538.

Bergquist, H., and B. Källén (1954) Notes on the early histogenesis and morphogenesis of the central nervous system in vertebrates. J. Comp. Neurol. *100:*627–660.

Blaker, S.N., D.M. Armstrong, and F.H. Gage (1988) Cholinergic neurons within the rat hippocampus: Response to fimbria-fornix transection. J. Comp. Neurol. *272:*127–138.

Brantley, R.K., and A.H. Bass (1988) Cholinergic neurons in the brain of a teleost fish (*Porichthys notatus*) located with a monoclonal antibody to choline acetyltransferase. J. Comp. Neurol. *275:*87–105.

Brauth, S.E., C.A. Kitt, D.L. Price, and B.H. Wainer (1985) Cholinergic neurons in the telencephalon of the reptile *Caiman crocodilus*. Neurosci. Lett. *58:*235–240.

Bruce, L.L., and A.B. Butler (1984) Telencephalic connections in lizards. I. Projections to cortex. J. Comp. Neurol. *229:*585–601.

Ciani, F., V. Franceschini, and P. Del Grande (1988) Histochemical and biochemical study on the acetylcholinesterase and choline acetyltransferase in the brain and spinal cord of frog, *Rana esculenta*. J. Hirnforsch. *29:*157–163.

Dolabela De Lima, A., and W. Singer (1987) The brainstem projections to the lateral geniculate nucleus in the cat: Identification of cholinergic and monoaminergic elements. J. Comp. Neurol. *259:*92–121.

Ekström, P. (1987) Distribution of choline acetyltransferase-immunoreactive neurons in the brain of a cyprinid teleost (*Phoxinus phoxinus* L.). J. Comp. Neurol. *256:*494–515.

Ferguson, J.L., P.L. Mulvanny, and S.E. Brauth (1978) Distribution of neurons projecting to the retina of *Caiman crocodilus*. Brain Behav. Evol. *15:*294–306.

Fitzpatrick, D., M. Conley, G. Luppino, M. Matelli, and I.T. Diamond (1988) Cholinergic projections from the midbrain reticular formation and the parabigeminal nucleus to the lateral geniculate nucleus in the tree shrew. J. Comp. Neurol. *272:*43–67.

Hallanger, A.E., A.I. Levey, H.J. Lee, D.B. Rye, and B.H. Wainer (1987) The origins of cholinergic and other subcortical afferents to the thalamus in the rat. J. Comp. Neurol. *262:*105–124.

Hendry, S.H.C., E.G. Jones, H.P. Killackey, and L.M. Chalupa (1987) Choline acetyltransferase-immunoreactive neurons in fetal monkey cerebral cortex. Dev. Brain Res. *37:*313–317.

Hoogland, P.V., and E. Vermeulen-VanderZee (1990) Distribution of choline acetyltransferase immunoreactivity in the telencephalon of the lizard *Gekko gecko*. Brain Behav. Evol. *36:*378–390.

Houser, C.R., G.D. Crawford, R.P. Barber, P.M. Salvaterra, and J.E. Vaughn (1983) Organization and morphological characteristic of cholinergic neurons: An immunocytochemical study with a monoclonal antibody to choline acetyltransferase. Brain Res. *266:*97–119.

Johnson, C.D., and M.L. Epstein (1986) Monoclonal antibodies and polyvalent antiserum to chicken choline acetyltransferase. J. Neurochem. *46:*968–976.

Kása, P. (1986) The cholinergic systems in brain and spinal cord. Prog. Neurobiol. *26:*211–272.

Keynes, R., and A. Lumsden (1990) Segmentation and the origin of regional diversity in the vertebrate central nervous system. Neuron, *2:*1–9.

Keyser, A. (1972) The development of the diencephalon of the Chinese hamster. An investigation of the validity of the criteria of subdivision of the brain. Acta. Anat. (Basel) *83 (Suppl. 59):*1–178.

Kimmel, D.L. (1940) Differentiation of the bulbar motor nuclei and the coincident development of associated root fibers in the rabbit. J. Comp. Neurol. *72:*83–148.

Kimura, H., P.L. McGeer, J.H. Peng, and E.G. McGeer (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. J. Comp. Neurol. *200:*151–201.

Levey, A.I., D.M. Armstrong, S.F. Atweh, R.D. Terry, and B.H. Wainer (1983) Monoclonal antibodies to choline acetyltransferase: Production, specificity and immunohistochemistry. J. Neurosci. *3:*1–9.

Lumsden, A., and R. Keynes (1989) Segmental patterns of neuronal development in the chick hindbrain. Nature *337:*424–428.

Maley, B.E., M.L. Frick, A.I. Levey, B.H. Wainer, and R.P. Elde (1988) Immunohistochemistry of choline acetyltransferase in the guinea pig brain. Neurosci. Lett. *84:*137–142.

Martínez-de-la-Torre (1985) Estructura del mesencéfalo y diencéfalo en aves y reptiles: Aportaciones a una síntesis en la búsqueda de homologías. Thesis, University of Murcia, Spain.

Medina, L., and W.J.A.J. Smeets (1992) Cholinergic, monoaminergic and peptidergic innervation of the primary visual centers in the brain of the lizards *Gekko gecko* and *Gallotia galloti*. Brain Behav. Evol. *40:*157–181.

Mesulam, M.M., E.J. Mufson, A.I. Levey, and B.H. Wainer (1984) Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. Neuroscience *12:*669–686.

Mufson, E.J., P.H. Desan, M.M. Mesulam, B.H. Wainer, and A.I. Levey (1984) Choline acetyltransferase-like immunoreactivity in the forebrain of the red-eared pond turtle (*Pseudemys scripta elegans*). Brain Res. *323:*103–108.

Noden, D.M. (1991) Vertebrate craniofacial development: The relation between ontogenetic process and morphological outcome. Brain Behav. Evol. *38:*190–225.

Ouimet, C.C., R.L. Patrick, and F.F. Ebner (1985) The projection of three extrathalamic cell groups to the cerebral cortex of the turtle *Pseudemys*. J. Comp. Neurol. *237:*77–84.

Parent, A., D. Paré, Y. Smith, and M. Steriade (1988) Basal forebrain cholinergic and noncholinergic projections to the thalamus and brainstem in cats and monkeys. J. Comp. Neurol. *277:*281–301.

Parnavelas, J.G., W. Kelly, E. Franke, and F. Eckenstein (1986) Cholinergic neurons and fibres in the rat visual cortex. J. Neurocytol. *15:*329–336.

Price, M., M. Lemaistre, M. Pischetola, R. Di Lauro, and D. Duboule (1991) A mouse gene related to Distal-less shows a restricted expression in the developing forebrain. Nature *351:*748–751.

Puelles, L., J.A. Amat, and M. Martínez-de-la-Torre (1987) Segment-related, mosaic neurogenetic pattern in the forebrain and mesencephalon of early chick embryos: I. Topography of AChE-positive neuroblast up to stage HH18. J. Comp. Neurol. *266:*247–268.

Puelles, L., M. Guillén, and M. Martínez-de-la-Torre (1991) Observations on the fate of the nucleus superficialis magnocellularis of Rendahl in the avian diencephalon, bearing on the organization and nomenclature of neighboring retinorecipient nuclei. Anat. Embryol. *183:*221–233.

Reiner, A. (1991) A comparison of neurotransmitter-specific and neuropeptide-specific neuronal cell types present in the dorsal cortex in turtles with those present in the isocortex in mammals: Implications for the evolution of isocortex. Brain Behav. Evol. *38:*53–91.

Regidor, J., and L. Poch (1988) Histochemical analysis of the lizard cortex: An acetylcholinesterase, cytochrome oxidase and NADPH-diaphorase study. In W.K. Schwerdtfeger and W.J.A.J. Smeets (eds): The Forebrain of Reptiles. Current Concepts of Structure and Function. Basel: Karger, pp. 77–84.

Ricciuti, A.J., and E.R. Gruberg (1985) Nucleus isthmi provides most tectal choline acetyltransferase in the frog *Rana pipiens*. Brain Res. *341:*399–402.

Russchen, F.T., and A.J. Jonker (1988) Efferent connections of the striatum and the nucleus accumbens in the lizard *Gekko gecko*. J. Comp. Neurol. *276:*61–80.

Russchen, F.T., W.J.A.J. Smeets, and P. Hoogland (1987) Histochemical identification of pallidal and striatal structures in the lizard *Gekko gecko:* Evidence for compartmentalization. J. Comp. Neurol. *256:*329–341.

Satoh, K., and H.C. Fibiger (1985a) Distribution of central cholinergic neurons in the baboon (*Papio papio*). I. General morphology. J. Comp. Neurol. *236:*197–214.

Satoh, K., and H.C. Fibiger (1985b) Distribution of central cholinergic neurons in the baboon (*Papio papio*). II. A topographic atlas correlated with catecholamine neurons. J. Comp. Neurol. *236:*215–233.

Shimizu, T. and H.J. Karten (1990) The origins of neocortex: Connections and lamination as distinct events in evolution. J. Cognitive Sci. *1:*291–301.

Smeets, W.J.A.J., P.V. Hoogland, and A.H.M. Lohman (1986a) A forebrain atlas of the lizard *Gekko gecko*. J. Comp. Neurol. *254:*1–19.

Smeets, W.J.A.J., P.V. Hoogland, and P. Voorn (1986b) The distribution of dopamine immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko:* An immunohistochemical study with antibodies against dopamine. J. Comp. Neurol. *253:*46–60.

Sofroniew, M.V., J.V. Priestley, A. Consolazione, F. Eckenstein, and A.C. Cuello (1985) Cholinergic projections from the midbrain and pons to the thalamus in the rat, identified by combined retrograde tracing and choline acetyltransferase immunohistochemistry. Brain Res. *329:*213–223.

Sorenson, E.M., D. Parkinson, J.L. Dahl, and V.A. Chiappinelli (1989) Immunohistochemical localization of choline acetyltransferase in the chicken mesencephalon. J. Comp. Neurol. *281:*641–657.

Striedter, G.F., and R.G. Northcutt (1991) Biological hierarchies and the concept of homology. Brain Behav. Evol. *38:*177–189.

Tago, H., P.L. McGeer, G. Bruce, and L.B. Hersh (1987) Distribution of choline acetyltransferase-containing neurons of the hypothalamus. Brain Res. *415:*49–62.

Tago, H., P.L. McGeer, E.G. McGeer, H. Akiyama, and L.B. Hersh (1989) Distribution of choline acetyltransferase immunopositive structures in the rat brainstem. Brain Res. *495:*271–297.

Ten Donkelaar, H.J., and de Boer-van Huizen (1988) Brain stem afferents to the anterior dorsal ventricular ridge in a lizard (*Varanus exanthematicus*). Anat. Embryol. *177:*465–475.

Ten Donkelaar, H.J., G.C. Bangma, H.A. Barbas-Henry, R. de Boer-van Huizen, and J.G. Wolters (1987) The brain stem in a lizard, *Varanus exanthematicus*. Adv. Anat. Embryol. Cell Biol. 107.

Vaage, S. (1969) The segmentation of the primitive neural tube in chick embryos (*Gallus domesticus*). A morphological, histochemical and autoradiographical investigation. Adv. Anat. Embryol. Cell Biol. *41:*1–88.

Vaage, S. (1973) The histogenesis of the isthmic nuclei in chick embryos (*Gallus domesticus*). I. A morphological study. Z. Anat. Entwickl.-Gesch. *142:*283–314.

Vincent, S.R., and P.B. Reiner (1987) The immunohistochemical localization of choline acetyltransferase in the cat brain. Brain Res. Bull. *18:*371–415.

Vincent, S.R., K. Satoh, and H.C. Fibiger (1986) The localization of central cholinergic neurons. Prog. Neuropsychopharmacol. Biol. Psychiatry *10:*637–656.

Wächtler, K. (1983) The acetylcholine-system in the brain of cyclostomes with special references to the telencephalon. J. Hirnforsch. *24:*63–70.

Windle, W.F. (1933) Neurofibrillar development in the central nervous system of cat embryos between 8 and 12 mm. long. J. Comp. Neurol. *58:*643–723.

Woolf, N.J. (1991) Cholinergic systems in mammalian brain and spinal cord. Prog. Neurobiol. *37:*475–524.

Woolf, N.J., and L.L. Butcher (1986) Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. Brain Res. Bull. *16:*603–637.

Zottoli, S.J., K.J. Rhodes, J.G. Corrodi, and E.J. Mufson (1988) Putative cholinergic projections from the nucleus isthmi and the nucleus reticularis mesencephali to the optic tectum in the goldfish (*Carassius auratus*). J. Comp. Neurol. *273:*385–398.