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# Distribution of Choline Acetyltransferase and Acetylcholinesterase in Vocal Control Nuclei of the Budgerigar (*Melopsittacus undulatus*)

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ABSTRACT

The present study used histochemical methods to map the distributions of choline acetyl transferase (ChAT) and acetylcholinesterase (AChE) in the vocal control nuclei of a psittacine, the budgerigar (*Melopsittacus undulatus*). The distributions of ChAT and AChE in budgerigars appeared similar to that in oscine songbirds despite evidence that these systems have evolved independently. The magnicellular nucleus of the lobus parolfactorius in budgerigars, like the area X in songbirds, contained many ChAT labeled somata, fibers, and varicosities and stained densely for AChE. In contrast, the robust nucleus of the archistriatum (RA) and the supralaminar area of the frontal neostriatum in budgerigars, like the RA and the magnicellular nucleus of the neostriatum (MAN) in songbirds, respectively, contained few or no ChAT labeled somata, fibers, and varicosities and stained lightly for AChE. The central nucleus of the lateral neostriatum in budgerigars, like the higher vocal center (HVC) in songbirds, contained no ChAT labeled somata, moderate densities of ChAT labeled fibers and varicosities, and moderate levels of AChE staining. Two nuclei, the oval nucleus of the hyperstriatum ventrale (HVo) and the oval nucleus of the anterior neostriatum (NAo), contained no ChAT labeled somata, dense ChAT labeled fibers and varicosities, and moderate to high levels of AChE staining. The HVo and the NAo have no counterparts in songbirds but may be important vocal control nuclei in the budgerigar. Cholinergic enzymes are also described in other regions which may be involved in budgerigar vocal behavior, including the basal forebrain, the torus semicircularis, and the hypoglossal nuclei (nXII). 

**Indexing terms: immunohistochemistry, acetylcholine, vocal learning, vocalization**

Vocal learning is believed to have evolved independently in at least three avian groups—the apodiformes, the oscine songbirds, and the psittacines. Two of these groups, the apodiformes and oscines, are derived from the higher landbird division of neognathe birds. Psittacines, on the other hand, evolved from the basal landbird division of neognathe birds (Carr, 1992). The independent evolution of vocal learning in each of these lineages raises an intriguing question. Are the neural mechanisms underlying vocal learning similar, or did the evolution of brain mechanisms for vocal learning follow a different course in each?

A great deal of evidence has now accumulated indicating that vocal learning in oscines depends on a set of anatomically and functionally distinct telencephalic nuclei located in the neostriatum, archistriatum, and paleostriatum (Nottebohm et al., 1976). Specialized circuits interconnect these telencephalic nuclei with brainstem motor and premotor nuclei of the respiratory and tracheosyringeal systems (Wild, 1994). Functional specialization within the oscine vocal control circuit is reflected not only by the anatomical connections of telencephalic vocal control nuclei, but also by the fact that these nuclei can be distinguished from surrounding neural fields chemoarchitectonically using receptor binding and histochemical stains (Ball et al., 1994). One prominent histochemical feature of these nuclei is conspicuous staining for cholinergic markers including both acetylcholinesterase (AChE) and choline acetyltransferase (ChAT).

Discrete vocal control nuclei within the neostriatum, archistriatum, and paleostriatum have also been identified in a psittacine species, the budgerigar (*Melpsittacus undulatus*) (Brauth et al., 1994; Paton et al., 1981; Striedter, 1994). As in oscines, telencephalic vocal control nuclei in

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budgerigars are cytoarchitectonically discrete and project to respiratory and syringeal motor and premotor nuclei in the brainstem (Brauth et al., 1994; Paton et al., 1981; Striedter, 1994). In the present study, we mapped the distributions of cells and fibers staining for AChE and ChAT in the budgerigar brain with special attention given to the vocal control nuclei. Our goal was to compare these distributions to those in oscines in order to identify similarities and differences. Recent studies (Brauth et al., 1994) have also shown that neurons in the basal forebrain project to telencephalic vocal control nuclei in the budgerigar. Another aim of the present study was to determine if the ventral paleostriatum (VP) neurons in the budgerigar also contain AChE or ChAT as is the case in other avian species (Medina and Reiner, 1994).

## METHODS

Ten adult budgerigars of both sexes were used. Five were used for AChE histochemistry and five for ChAT immunohistochemistry. All procedures were conducted under the auspices of protocols approved by the campus Animal Care and Use Committee.

All subjects were deeply anesthetized with pentobarbitol (8 mg/bird, i.m. in saline vehicle); the chest was opened and the circulatory systems perfused via cannulae inserted either into the left ventricle or via the carotid arteries with 50 ml heparinized sodium phosphate buffer, followed by 200 ml of a 4% paraformaldehyde fixative. The brain was then steroetaxically blocked, removed from the skull, and placed in a phosphate buffer containing 30% sucrose overnight. The following day the brain was sectioned on a freezing microtome at 40-μm thickness and placed in tissue wells containing potassium phosphate buffered saline (KPBS, pH = 7.2; 0.1M).

### AChE histology

The Tsuji (1974) and Hedreen et al. (1985) modifications of the Roots-Karnovsky method were used for histochemical identification of AChE in the budgerigar brain (cf. Brauth, 1990). The Hedreen et al. (1985) method stains both cells and fibers intensely, whereas the Tsuji (1974) method preferentially stains somata.

For the Hedreen et al. (1985) method, sections were rinsed in sodium acetate buffer (pH = 6.0; 0.1M) for 15 minutes, after which they were incubated in a reaction solution containing 65 ml of 0.1M sodium acetate buffer, 50 mg of acetylthiocholine iodide, 10 ml of 0.03M aqueous copper sulphate, 5 ml of 0.1M aqueous sodium citrate, 16 ml distilled water, and 4 ml 0.005M potassium ferricyanide

*Abbreviations*

| | |
|---|---|
| A | archistriatum |
| Aid | archistriatum intermedium, pars dorsalis |
| Aiv | archistriatum intermedium, pars ventralis |
| AL | ansa lenticularis |
| An | nucleus angularis |
| APH | area parahippocampalis |
| AVT | area ventralis (Tsai) |
| Bas | nucleus basalis |
| BC | brachium conjunctivum |
| BCD | brachium conjunctivum descendens |
| CA | commissura anterior |
| CbL | nucleus cerebellaris lateralis |
| CbM | nucleus cerebellaris intermedius |
| Cc | central toral nucleus |
| DB | diagonal band |
| DMm | magnicellular nucleus of the dorsomedial thalamus |
| DMP | nucleus dorsomedialis posterior |
| DMv | ventrolateral nucleus of the dorsomedial thalamus |
| E | ectostriatum |
| EM | nucleus ectomammillaris |
| FPL | fasciculus prosencephali lateralis |
| FLM | fasciculus longitudinalis medialis |
| FRL | formatio reticularis lateralis mesencephali |
| HA | hyperstriatum accessorium |
| HD | hyperstriatum dorsale |
| HIS | hyperstriatum intercalatus superior |
| Hp | hippocampus |
| HV | hyperstriatum ventrale |
| HVC | high vocal center |
| HVo | oval nucleus of the ventral hyperstriatum |
| Ic | core portion of the intercollicular area |
| Imc | nucleus isthmi, pars magnocellularis |
| Ipc | nucleus isthmi, pars parvocellularis |
| L | field 'L' |
| L2a | lamina 2a of Field 'L' (ovoidalis projection field) |
| La | nucleus laminaris |
| LHy | nucleus lateralis hypothalami |
| LLd | nucleus lemnisci lateralis, pars dorsalis |
| LLv | nucleus lemnisci lateralis, pars ventralis |
| LMD | lamina medullaris dorsalis |
| LMmc | nucleus lentiformis mesencephali, pars magnocellularis |
| LPO | lobus parolfactorius |
| LPOm | magnicellular nucleus of the lobus parolfactorius |
| MC | nucleus magnocellularis |
| NAo | oval nucleus of the anterior neostriatum |
| NAs | supralaminar area of the frontal neostriatum |
| NC | neostriatum caudale |
| NI | neostriatum intermedium |
| NIDL | neostriatum intermedium, pars dorsolateralis |
| NIL | neostriatum intermedium, pars lateralis |
| NIVL | neostriatum intermedium, pars ventrolateralis |
| NLc | central nucleus of the lateral neostriatum |
| NLs | supracentral nucleus of the lateral neostriatum |
| NLv | ventral nucleus of the lateral neostriatum |
| NV | nervus trigeminus |
| nX | nucleus motorius dorsalis nervi vagi |
| nXII | nucleus nervi hypoglossi |
| nXIIts | tracheosyringeal portion of the hypoglossal nucleus |
| OM | tractus occipitomesencephalicus |
| Ov | nucleus ovoidalis |
| PA | paleostriatum augmentatum |
| PC | paracentral toral nucleus |
| PM | nucleus pontis medialis |
| POA | nucleus preopticus anterior |
| PPC | nucleus principalis precommissuralis |
| PT | nucleus pretectalis |
| QF | tractus quintofrontalis |
| RA | nucleus robustus archistriatalis |
| Rb | nucleus retroambigualis |
| RP | nucleus reticularis pontis caudalis |
| RPgc | nucleus reticularis parvocellularis |
| RT | nucleus rotundus |
| S | septum |
| SAC | stratum album centrale |
| SCd | nucleus subceruleus dorsalis |
| SGC | stratum griseum centrale |
| SGF | stratum griseum et fibrosum superficiale |
| SGP | substantia grisea et fibrosa periventricularis |
| Sl | nucleus septalis lateralis |
| SLu | nucleus semilunaris |
| Sm | nucleus septalis medialis |
| SMT | septomesoencephalic tract |
| SOp | stratum opticum |
| SPC | nucleus superficialis parvocellularis |
| SpL | nucleus spiriformis lateralis |
| TPc | nucleus tegmenti pedunculo-pontinus, pars compacta |
| TSM | tractus septomesencephalicus |
| V | ventricle |
| VP | ventral paleostriatum |

for 30 minutes with gentle agitation. Tissue sections were rinsed five times in acetate buffer for 5 minutes each and placed in 4% aqueous sodium sulphide for 1 minute to produce a brown precipitate. Sections were washed three times for 5 minutes each in 0.1M sodium nitrate, then placed in 0.1% silver nitrate for 1 minute to intensify the brown reaction product. Tissue sections were rinsed three times in 0.1M sodium nitrate for 5 minutes each rinse, mounted onto subbed slides, air-dried overnight, dehydrated through a series of graded alchohols, cleared in xylenes, and coverslipped.

For the Tsuji (1974) method, sections were rinsed in acetate buffer for 15 minutes, preincubated for 10 minutes in 90 ml acetate buffer containing 10 ml copper glycine solution, then incubated for 2 hours in the same solution containing 300 mg acetylthiocholine iodide. Sections were rinsed 5 times for 3 minutes each in acetate buffer then developed in 3% aqueous potassium ferricyanide solution for 15 minutes. Sections were rinsed 5–6 times in acetate buffer, mounted onto subbed slides, air-dried overnight, cleared, and coverslipped. Selected sections were charted and/or photographed through the microscope.

The density of AChE was quantified using the Bioquant system. Optical density was measured in various nuclei, with ten sample measurements taken from each nucleus. Average log density values were then calculated using the following formula:

$$D_n = \frac{\Sigma[\mathrm{Log}_{10}(255/d)]}{10}$$

where d is the optical density value, 255 is the maximum brightness value on the grayscale used to digitize the image, and 10 is the number of measures taken for each nucleus in each bird. Average log density values were also calculated for white matter ($D_w$), with measurements taken in both the anterior commissure (CA) and the fasciculus prosencephali lateralis (FPL). The above procedures were repeated using tissues from five different cases. The log density values for each nucleus ($D_n$) and for white matter ($D_w$) were averaged across the five cases to give overall mean log density values.

The density of AChE staining may vary between cases due to variations in histochemical processing. Variations between cases were observed in white matter staining. The highest mean log density value for white matter was twice as large as the lowest mean log density value for white matter. Because no differences were expected in the content of cholinergic markers in white matter, it was assumed that differences in the log density values for white matter indicated variations in histochemical processing. Therefore, average ratio density (ARD) values were calculated by dividing the mean log density value for each nucleus ($D_n$) by the mean log density value for white matter ($D_w$). The ARD values were used to determine whether AChE staining in a given nucleus was low (ARD = 1.0–1.5), medium (ARD = 1.5–1.8), or high (ARD = 1.8–2.2).

## ChAT immunohistochemistry

ChAT immunohistochemistry was performed using a rabbit polyclonal anti-ChAT antibody kindly supplied by Dr. Miles Epstein and characterized by Johnson and Epstein (1986). Tissue sections were rinsed three times in KPBS for 15 minutes each and then placed in blocking solution consisting of 4% normal goat serum (NGS) in KPBS with 0.4% Triton X-100 (TX) for 20 minutes at room temperature under gentle agitation before being transferred to a solution containing the anti-ChAT antibody diluted 1:5,000 in KPBS containing 0.4% TX and 1% NGS. Sections were incubated in the ChAT primary antibody solution under gentle agitation at 4°C for 70 hours and at room temperature for 1–2 hours. Sections were then rinsed ten times at 10 minutes each in KPBS containing 0.02% TX, transferred to KPBS containing biotinylated goat anti-rabbit serum diluted 1:1500 with 0.02% TX at room temperature for 60 minutes, and washed in fresh KPBS. The sections were incubated in avidin-biotinylated HRP complex (Vectastain) and the label developed in 0.04% diaminobenzidine (DAB) in Tris buffer (0.1M; pH = 7.2) with 0.003% $H_2O_2$ for 5–25 minutes. After development in the chromagen solution, tissue sections were rinsed twice in fresh Tris buffer for 15 minutes each, mounted onto subbed slides, and air-dried overnight. The DAB reaction product was then silver-gold intensified using previously reported procedures (Kitt et al., 1994) and coverslipped. Sections were examined under a Zeiss Axioplan microscope in both brightfield and darkfield. The distribution of labeled cells and fibers were charted onto large drawings of the sections.

The specificity of the ChAT antibody used in the present study has already been demonstrated in other avian species (Medina and Reiner, 1994; Sorenson et al., 1989). A limited number of controls were therefore employed in the present study. Tissues processed without the primary ChAT antibody serum showed no labeling of either somata or fibers. Different series of tissues from one case were processed using different concentrations of the primary ChAT antibody serum (1:5000, 1:8000, 1:10,000). It was assumed that nonspecific binding of the ChAT antibody would occur with lower affinity and, therefore, that nonspecific binding patterns would either not be detected with lower concentrations of the antibody serum or would "dilute out" more quickly than specific binding patterns. The lower concentrations resulted in lighter labeling, making ChAT labeled somata and fibers more difficult to detect, but no differences were observed in the pattern of ChAT distribution. Finally, two cases were processed using a secondary antibody conjugated with fluorescein. No differences were observed in the distribution of ChAT fibers and somata using biotinylated or fluorescein-conjugated secondary antibodies.

## Nomenclature for vocal control nuclei

Paton et al. (1981) identified a discrete archistriatal nucleus projecting to the tracheosyringeal portion of the hypoglossal nucleus (nXIIts) called robustus archistriatalis (RA). Paton et al. (1981) also showed that RA receives input from an adjacent neostriatal higher vocal center (HVC). The RA in budgerigars contains a dorsal subregion and a ventral subregion (Durand, Heaton, Amateau, and Brauth, personal communication). The dorsal subregion was previously identified by Paton et al. (1981) as the RA in budgerigars, and in the present report we likewise refer to the dorsal subregion as RA. The RA in budgerigars receives projections from a nucleus in the adjacent neostriatum. Striedter (1994) has designated this neostriatal nucleus the central nucleus of the lateral neostriatum (NLc) and showed it receives input from the immediately dorsal supracentral nucleus of the lateral neostriatum (NLs). The NLc is considered comparable to the HVC in songbirds.

Striedter (1994) also described the oval nuclei of the hyperstriatum ventrale (HVo) and anterior neostriatum

(NAo). Brauth et al. (1994) showed that these nuclei receive input from the RA and project to the NLc and, on the basis of these projections, should be considered part of the vocal control system. Both the HVo and the NAo project to the magnicellular nucleus of the lobus parolfactorius (LPOm), which in turn projects to the magnicellular nucleus of the dorsomedial thalamus (DMm) (Brauth et al., 1994; Hall et al., 1994; Striedter, 1994). The DMm projects to the NLc and the RA (Brauth et al., 1994). For these reasons, the LPOm and the DMm are considered to be vocal control nuclei in the present paper.

The midbrain dorsomedial nucleus of the intercollicular area, the hypoglossal nucleus (nXII), and the nucleus ambigualis are each part of vocal or respiratory control circuits (Wild, 1994). Puelles et al. (1994) have recently described the torus semicularis in detail and have renamed the dorsomedial nucleus of the intercollicular area as the core portion of the intercollicular area (Ic). The present study adopts the nomenclature proposed by Puelles et al. (1994) for nuclei in the torus semicircularis. Specific attention was given to mapping cholinergic markers in these areas as well.

## RESULTS

### ChAT immunohistochemistry

The distribution of ChAT labeled cells and fibers is plotted onto a rostrocaudal series of standard drawings of the budgerigar brain in the transverse plane (Brauth et al., 1987, Hall et al., 1993) with accompanying photomicrographs (Figs. 1–4). The standard drawings include only the transverse sections containing vocal control nuclei described in the methods section. No sex differences were noted in the distribution of ChAT labeled cells and fibers.

ChAT labeled somata, fibers, and varicosities in the rostral telencephalon are depicted in Figure 1. All portions of the LPO and the PA contain ChAT labeled somata. Many ChAT labeled somata in LPO and PA have stellate profiles that generally vary from 20 to 25 μm in diameter. Some ChAT labeled somata in LPO and PA have pyramidal or bipolar-shaped profiles with diameter sizes comparable to the stellate neurons. The LPOm seems to contain approximately the same density of ChAT labeled somata as in the surrounding LPO and PA, although the LPOm could be distinguished from surrounding portions of the LPO by more substantial background labeling (see Fig. 1B). The dense background labeling in LPOm could not be clearly identified as either perikaryal or fibrous. No ChAT labeled somata were observed in pallial areas of the telencephalon, although coarse and fine fibers and many varicosities were observed in both the HVo and the NAo, as shown in Figure 1C,D.

ChAT labeled fibers were observed throughout the hyperstriatum and neostriatum. In the hyperstriatum, accumulations of ChAT labeled fibers and varicosities were conspicuously dense in a continuous field extending across lamina from the dorsomedial accessory hyperstriatum (HA) to the intermediate region of the ventral hyperstriatum (HV) including the HVo. The rostral portion of the densely labeled field was confined to HA, whereas the caudal portion of the field was confined to the HVo and to the region immediately medial to the HVo. Figure 1 shows only the caudal portion of the field in the HV and the HVo. In the neostriatum, dense accumulations of ChAT labeled fibers and varicosities were observed in the NAo and in regions immediately medial and lateral to the NAo. The ChAT labeled fibers observed in the lateral neostriatum were most dense along the dorsal boundary with the HV. A few labeled fibers were observed in the nucleus basalis (Bas), although labeling in the Bas was less dense than in the HVo and the NAo.

Figure 2 depicts ChAT labeled somata, fibers, and varicosities more caudally at the level of the NLc and the RA. Within the paleostriatum, the LPO and the PA contained substantial numbers of labeled fibers and many labeled somata. ChAT labeled fibers and somata were also observed in the area ventral to the LPO, corresponding to the ventral paleostriatum (VP) (see Fig. 2B). Most ChAT labeled somata in the VP had either pyramidal or bipolar-shaped profiles and were generally 15–20 μm in diameter. A smaller number of ChAT labeled somata in VP had stellate-shaped profiles and varied from 10 to 25 μm in diameter. The lateral VP was perforated by the fasciculus prosencephali lateralis (FPL). ChAT labeled neurons were observed in the VP both medial and lateral to the FPL. Some fibers from ChAT labeled somata in the VP extended across the FPL.

A sparse plexus of ChAT labeled fibers and occasional varicosities was present in the NLc. Labeled fibers in the NLc were present primarily in the ventral portion of the central nucleus. Fewer labeled fibers or varicosities were observed in the supracentral nucleus of the lateral neostriatum (NLs), and these were primarily concentrated in the lateral regions (see Fig. 2A,B). ChAT labeled fibers were consistently as observed in the lateral neostriatum dorsal to NLc as well as in the ventral nucleus of the lateral neostriatum (NLv) immediately ventral to the NLc.

Labeled fibers were more abundant in the ventral archistriatum than in the dorsal archistriatum or the lateral neostriatum. Coarse ChAT labeled fibers passed through the region medial to the rostral RA toward the lateral neostriatum and toward the lateral portion of the dorsal medullary lamina (LMD) (see Fig. 2A,C). It was not possible to follow fibers from the LMD farther. A small number of labeled fibers appeared to pass through the most medial portion of RA, and a few labeled fibers were observed extending through the RA toward the NLc. It was not possible to determine whether these fibers terminated in RA or passed through RA without terminating.

The neostriatal field located immediately dorsal to the LMD has been identified as the supralaminar area of the frontal neostriatum (NAs) by Striedter (1994). In contrast to the NLc, few ChAT labeled fibers were observed in the NAs. We were unable to determine if these fibers terminated in the NAs or passed through without terminating.

Labeled somata and fibers in the telencephalon and diencephalon immediately caudal to the anterior commissure at the main level of the DMm are depicted in Figure 3. As shown in Figure 3A, accumulations of ChAT labeled fibers and varicosities were observed in the ventral archistriatum (A) and the dorsomedial hyperstriatum accessorium (HA). Many ChAT labeled somata and fibers were observed in the septum, the diagonal band (DB), the ventral paleostriatum (VP), and the anterior preoptic nucleus (POA). ChAT labeled somata in the medial septum clustered around the septomesencephalic tract (TSM). These somata generally had bipolar-shaped profiles, 15–25 μm in diameter, with processes extending dorsally toward the TSM and ventrolaterally toward the DB. Most ChAT labeled somata in the DB had bipolar-shaped profiles, 15–25 μm in diameter, with processes extending medially toward the medial septum and laterally toward the VP. A

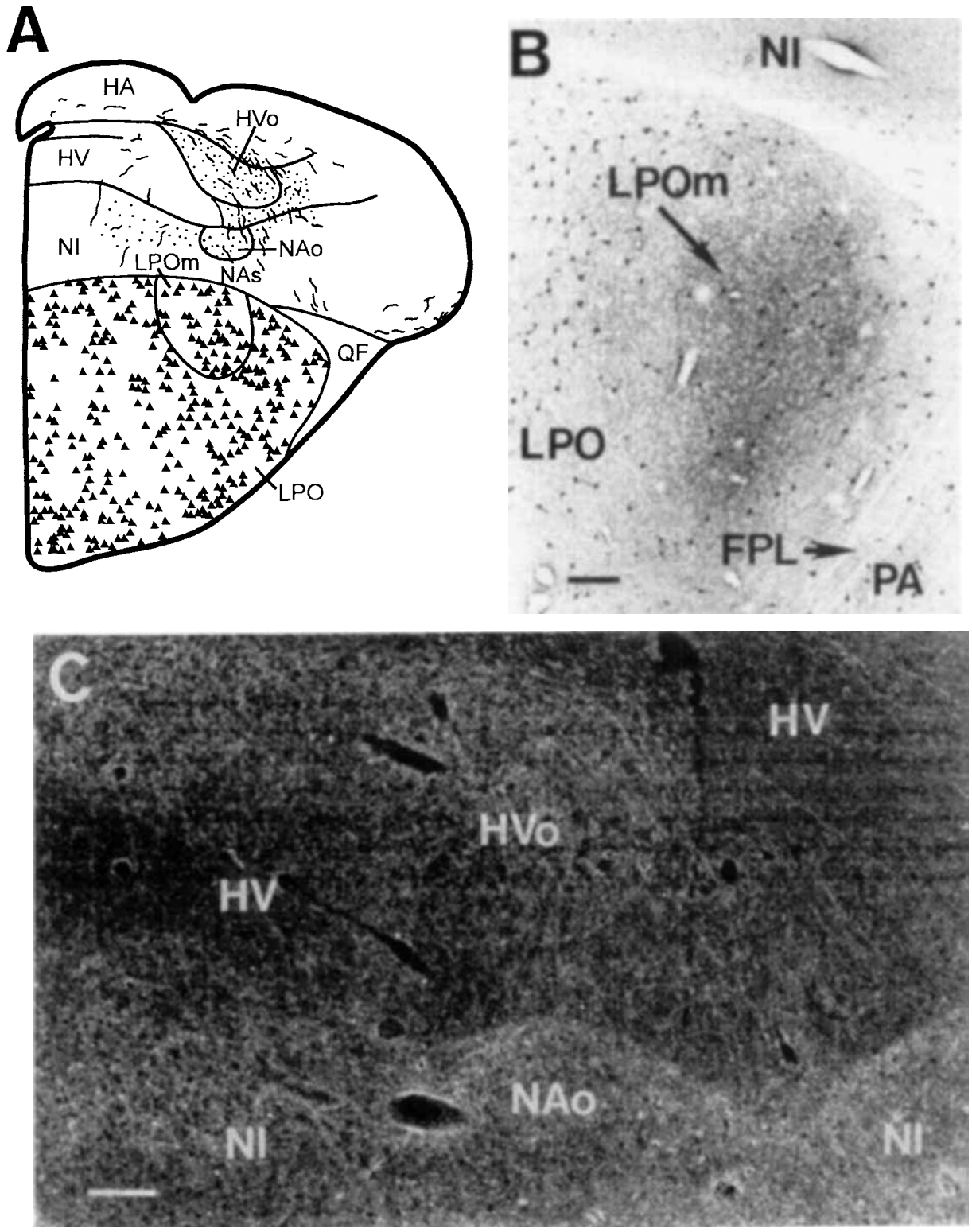



Fig. 1. Transverse section showing the distribution of ChAT labeled somata, fibers, and varicosities in the rostral telencephalon at the level of the HVo, the NAo, and the LPOm. **A:** Drawing of the rostral telencephalon showing ChAT labeled somata, fibers, and varicosities. Labeled somata are indicated as filled triangles, labeled profiles are shown as broken lines and varicosities are shown as fine dots. The drawn fibers indicate regions of relatively dense fiber accumulations. **B:** Brightfield photomicrograph showing the distribution of ChAT labeled somata in the LPOm. **C:** Darkfield photomicrograph showing the distribution of ChAT labeled fibers and varicosities in the HVo and the NAo.

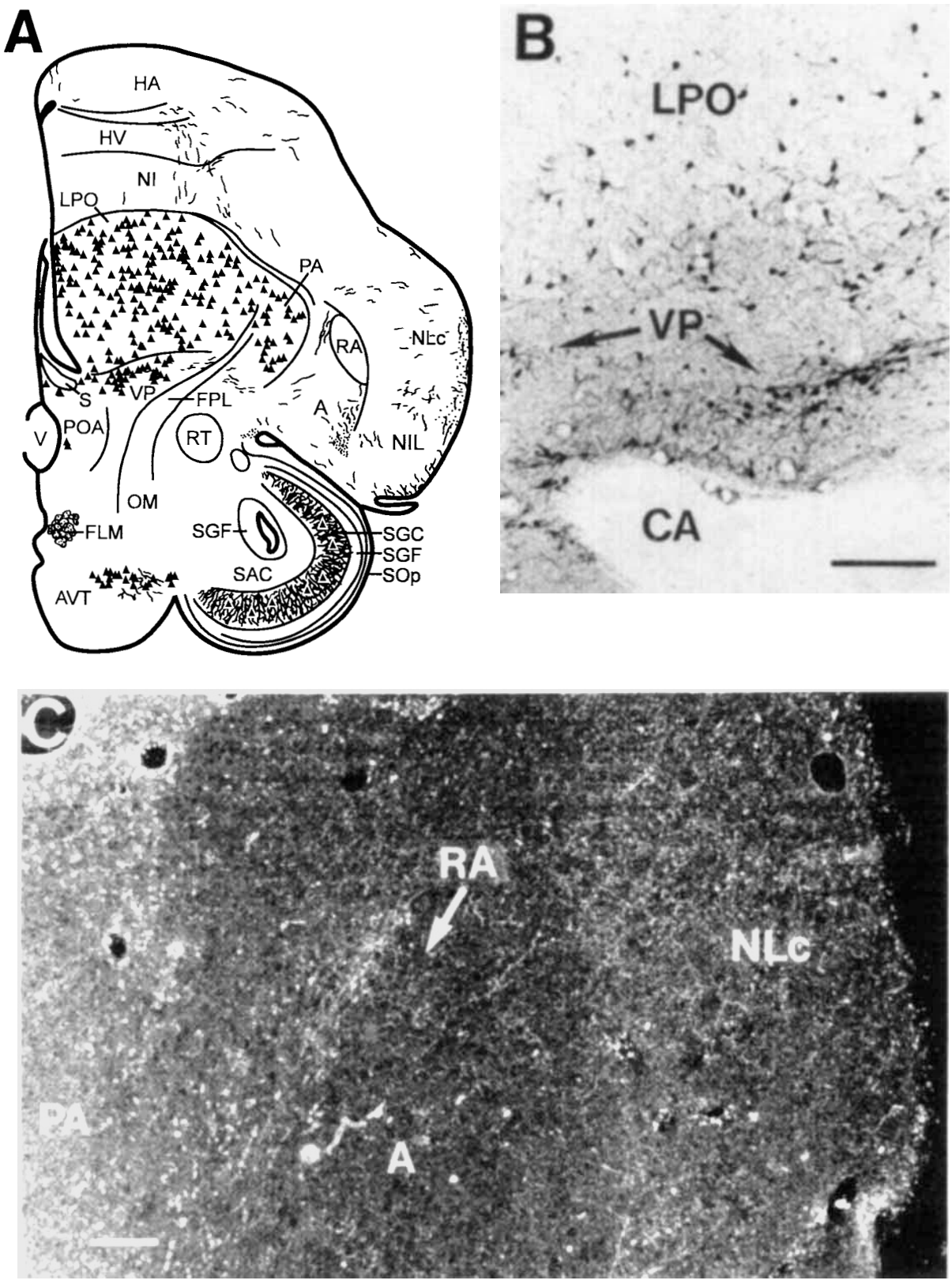
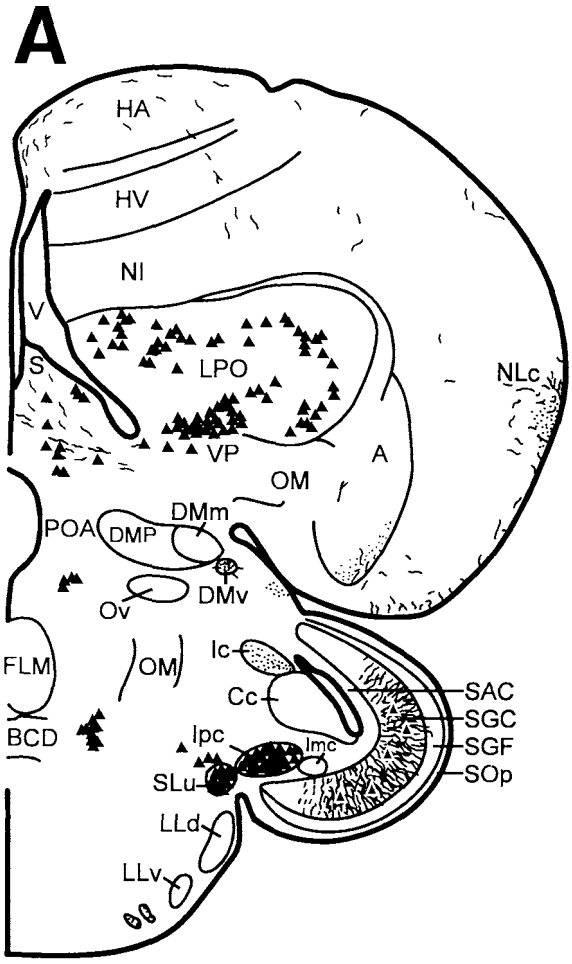
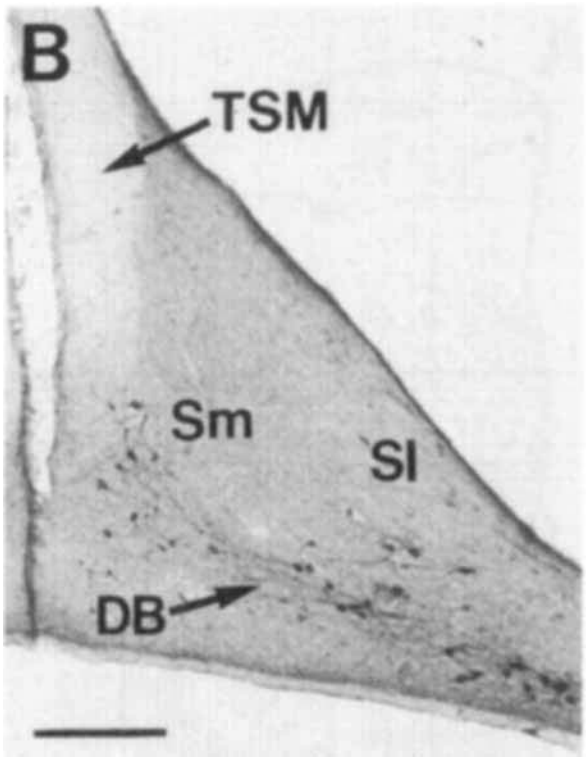



Fig. 2. Transverse section showing the distribution of ChAT labeled somata, fibers, and varicosities in the telencephalon and diencephalon at the level of the RA, the NLc, and the VP. **A:** Drawing of the telencephalon and diencephalon showing ChAT labeled somata, fibers, and varicosities. Labeled somata are indicated as filled triangles, labeled profiles are shown as broken lines, and varicosities are shown as fine dots. The drawn fibers indicate regions of relatively dense fiber accumulations. **B:** Brightfield photomicrograph showing the distribution of ChAT labeled somata and fibers in the VP. **C:** Darkfield photomicrograph showing the distribution of ChAT labeled fibers and varicosities in the RA and the NLc.



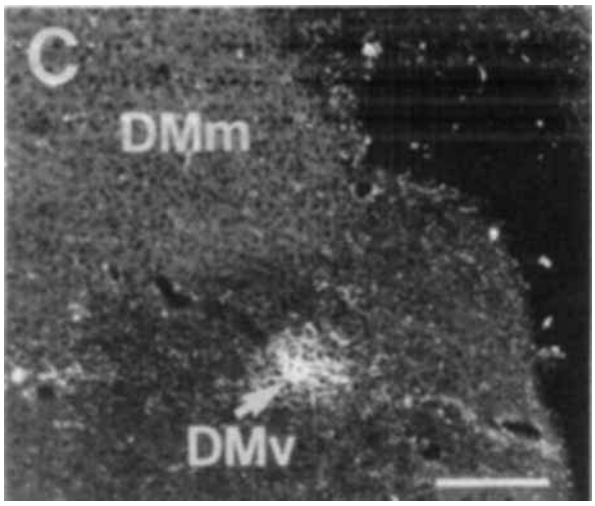




Fig. 3. Transverse section showing the distribution of ChAT labeled somata, fibers, and varicosities in the telencephalon and diencephalon at the level of DMP and the intercollicular area. **A:** Drawing of the telencephalon and diencephalon showing ChAT labeled somata, fibers, and varicosities. Labeled somata are indicated as filled triangles, labeled profiles are shown as broken lines and varicosities are shown as fine dots. The drawn fibers indicate regions of relatively dense fiber accumulations. **B:** Brightfield photomicrograph showing the distribution of ChAT labeled somata and fibers in the medial septum (Sm), lateral septum (Sl), and diagonal band (DB). **C:** Darkfield photomicrograph showing the distribution of ChAT labeled fibers and varicosities in the DMv.

few ChAT labeled somata were observed in the lateral septum. In rostral regions of the lateral septum, ChAT labeled somata were located near the horn of the lateral ventricle. These somata generally had bipolar-shaped profiles and contained processes that extended toward the ventrolateral septum, the dorsomedial septum, and the diagonal band. In more caudal regions of the lateral septum, a few lightly labeled somata were also observed along the lateral ventricle.

Two groups of ChAT labeled somata were observed in the POA (see Fig. 3A). The majority of these were observed in an inverted L-shaped region along the medial wall of the POA, apparently forming a distinct nuclear region. Most of these ChAT labeled somata had stellate or bipolar shaped

profiles with diameters ranging from 10 to 20 μm. Dorsal to the L-shaped nuclear region along the medial wall of the POA, a second group of lightly labeled somata were observed immediately ventral to the anterior commissure. The ChAT labeled somata immediately ventral to the anterior commissure did not form a distinct nuclear region.

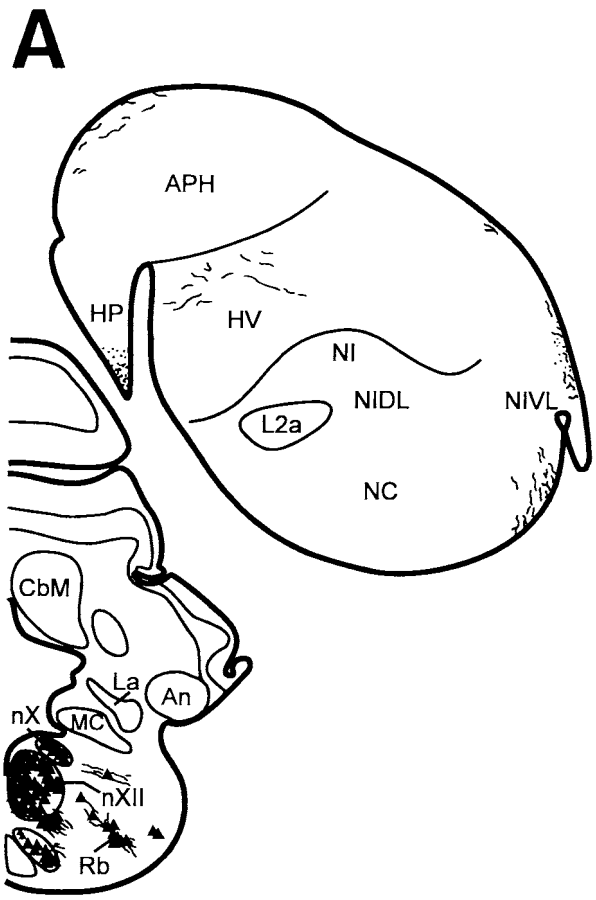
Within the midbrain, a small plexus of labeled fibers and varicosities was observed immediately ventral and lateral to the magnicellular subregion of the nucleus dorsomedialis posterior (DMP) (see Fig. 3A,D). The cell group corresponding to plexus of fibers and varicosities is here referred to as ventrolateral nucleus of the dorsomedial thalamus (DMv). The DMP and other cell groups immediately surrounding the DMP contained few or no labeled somata, fibers, or varicosities.

Coarse and fine ChAT labeled fibers and varicosities were observed in the core portion of the intercollicular area (Ic) (see Fig. 3A). This dorsomedial subregion corresponds to a region which receives afferent projections from the RA (Paton et al., 1981; Striedter, 1994). No ChAT labeled somata and few or no ChAT labeled fibers or varicosities were observed in the core portion of the central toral nucleus (Cc) (see Fig. 3A). Many ChAT labeled fibers and several ChAT labeled somata were present in the optic tectum. The stratum griseum centrale (SGC) and the stratum griseum et fribrosum superficiale (SGF) contain the most substantial number of fibers in the optic tectum. The ChAT labeled somata were located primarily in the SGC. These somata generally had bipolar-shaped profiles, 10–15 μm in diameter, with long processes that extended into the SGF.

Sparse ChAT labeled fibers were present in the caudal telencephalon within the parahippocampus and within the ventral and lateral neostriatum (see Fig. 4A). Sensory telencephalic areas such as Field L contain few ChAT labeled fibers and no ChAT labeled somata. Only a portion of Field L, Field L2a, is shown in Figure 4A. Brainstem motoneuron pools, including the hypoglossal nucleus (nXII) and the reticulated area lateral and ventral to the nXII, contained conspicuously labeled somata (see Fig. 4A,B). The reticulated area lateral and ventral to the nXII includes to the nucleus retroambigualis (Rb).

## AChE Histochemistry

AChE stained fibers and somata were observed throughout much of the forebrain and brainstem. The distribution of AChE stained fibers and somata are plotted onto standard drawings in Figures 5–8. Although material stained with both the Tsuji (1974) and Hedreen et al. (1985) methods were examined, only material stained with the Hedreen et al. (1985) method was used to quantify the density of staining as shown in the drawings. No sex differences were observed in the distribution of AChE. AChE staining in the rostral telencephalon is shown in Figure 5. Dense accumulations of AChE stained fibers and varicosities were observed in a continuous field extending across lamina from the dorsomedial HA to the intermediate region of the HV including the HVo. This field of dense AChE staining corresponded to the previously described field of ChAT fibers and varicosities. Figure 5B shows only the caudal portion of the field in the HVo. In the neostriatum, AChE stained fibers and varicosities were observed in the NAo and in regions immediately medial and lateral to the NAo. Staining in adjacent portions of the hyperstriatum



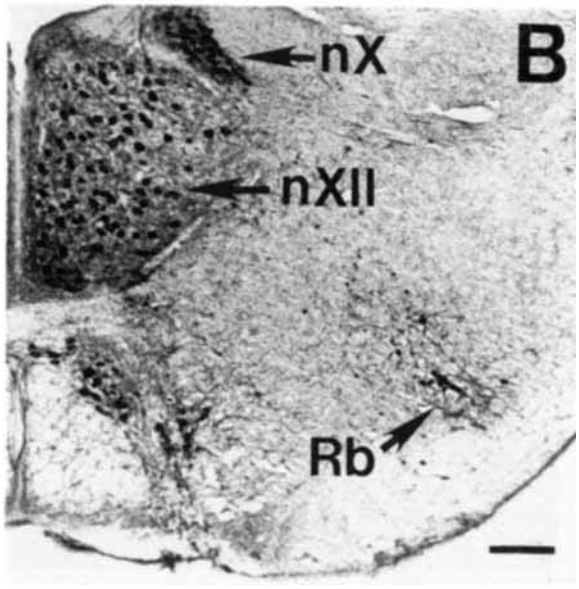




Fig. 4. Transverse section showing the distribution of ChAT labeled somata, fibers, and varicosities in the caudal telencephalon, medulla, and cerebellum. **A:** Drawing of the caudal telencephalon, medulla, and cerebellum showing ChAT labeled somata, fibers, and varicosities. Labeled somata are indicated as filled triangles, labeled profiles are shown as broken lines and varicosities are shown as fine dots. The drawn fibers indicate regions of relatively dense fiber accumulations. **B:** Brightfield photomicrograph showing the distribution of ChAT labeled somata and fibers in the nXII and the Rb.

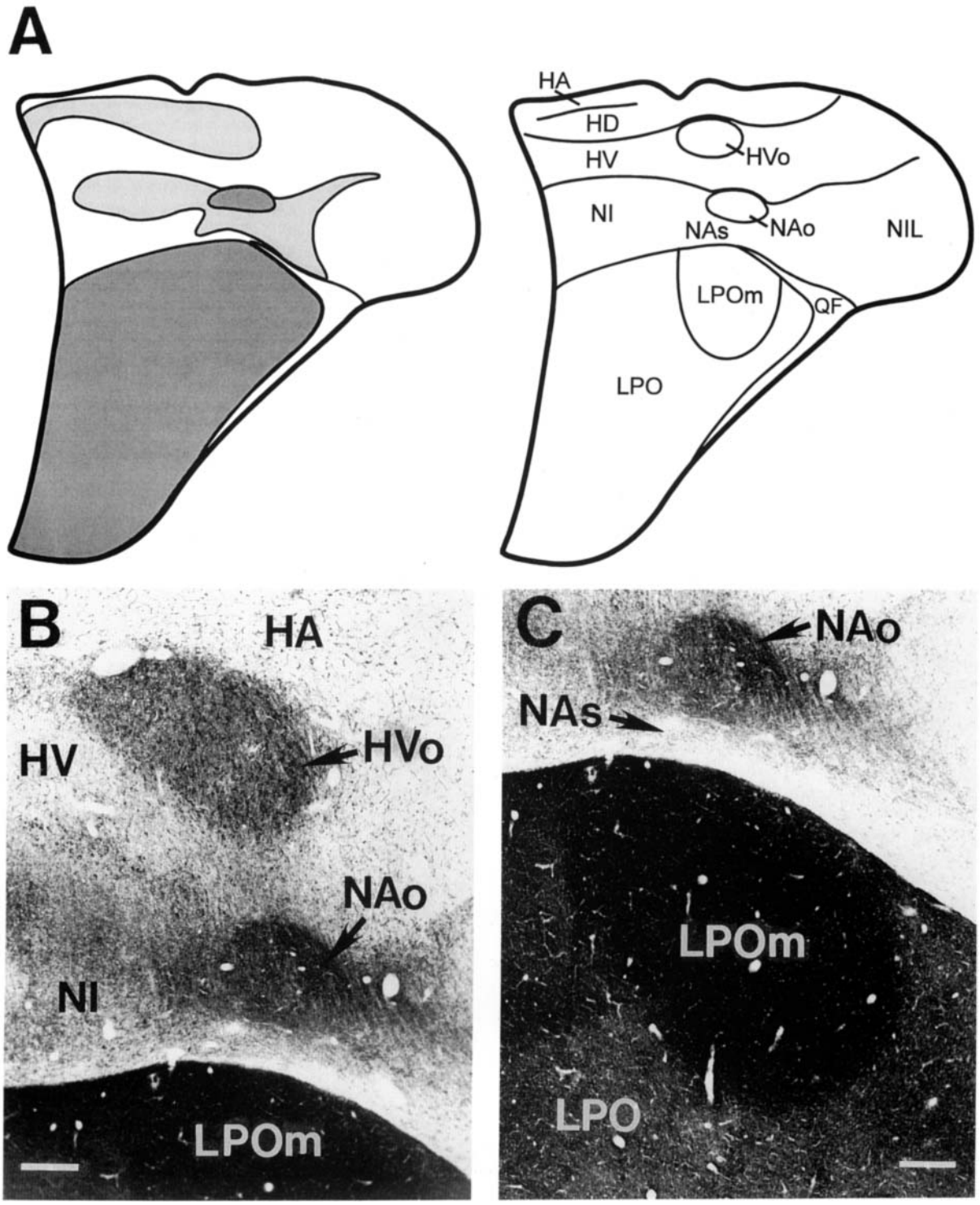



Fig. 5. Transverse section showing the distribution of AChE staining in the rostral telencephalon at the level of the HVo, the NAo, and the LPOm. **A:** Drawings showing the relative density of AChE in the rostral telencephalon. The drawing on the left side schematically indicates the density of AChE staining. Low AChE staining density (ARD = 1.0–1.5) is shown as white, medium AChE staining density (ARD = 1.5–1.8) is shown as light gray, and high AChE staining density (ARD = 1.8–2.2) is shown as dark gray. The drawing on the right side shows anatomical nomenclature at the same level as the AChE staining in the drawing on the left side. **B:** Brightfield photomicrograph showing AChE staining in the HVo and the NAo. **C:** Brightfield photomicrograph showing AChE staining in the NAo and the LPOm.

and neostriatum, including the NAs, was less intense (see Fig. 5A,B).

The LPOm and the LPO were both heavily stained. Although the LPOm and the LPO both had "high" overall ARD values (see Fig. 5A), the LPOm was distinguished by a greater density of AChE staining than surrounding areas of the LPO using the Tsuji (1974) method (see Fig. 5C). The high density of staining made it difficult to clearly identify somata and fibers in the LPOm.

Figure 6 depicts AChE staining at the level of the RA and the NLc. Many AChE stained fibers and somata were observed in the RA and the NLc. AChE stained fibers were less abundant in the RA, although the RA contained many AChE stained somata (see Fig. 6B). Note that Figure 6A illustrates AChE staining densities at more rostral levels of the RA, whereas Figure 6B,C shows AChE staining at more caudal levels of the RA. The most dense AChE staining in the archistriatum occurred in an oval shaped field ventral and caudal to the RA (see Fig. 6B). The NLc contained relatively fewer AChE stained somata, but contained a greater accumulation of AChE stained fibers, than the RA.

AChE staining at the level of the thalamic DMm is depicted in Figure 7A. As shown, substantial AChE staining was present in the telencephalon at this level, including dense labeling of somata and fibers in LPO. Portions of the hyperstriatum ventrale, lateral neostriatum, and dorsal archistriatum (see Fig. 7A) also contained labeled varicosities and fibers and some somata. Within the thalamus, DMm contained conspicuously lower levels of AChE staining than surrounding the DMP (see Fig. 7B). The DMv also contained densely stained fibers and varicosities. Within the midbrain, the Ic and the Cc contained higher levels of AChE in fibers than surrounding fields (see Fig. 7C).

Figure 8 portrays AChE staining in the caudal telencephalon, medulla, and cerebellum. Motoneuron somata in the nXII are intensely stained (see Fig. 8A,B). Some less intensely stained somata are also observed in the reticulated area ventrolateral to nXII.

## DISCUSSION

As in songbirds, vocal control nuclei in the budgerigar exhibit staining patterns for both ChAT and AChE which distinguish these nuclei from surrounding neuronal fields (see Figs. 1–8). The HVo and the NAo are heavily labeled with AChE and contain substantially more ChAT labeled fibers than surrounding fields in the hyperstriatum and neostriatum. The LPOm is readily visualized and distinguished from surrounding fields in the LPO using both ChAT and AChE material. The NLc and the RA both stain more intensely for AChE than surrounding fields, although neither nucleus contains many ChAT fibers. In the thalamus, the DMm contains conspicuously less AChE staining than surrounding portions of the DMP, whereas the DMv shows dense AChE staining and many ChAT labeled fibers and varicosities. In the midbrain, the Ic contains many ChAT labeled fibers and varicosities and moderate AChE staining, whereas the Cc contains very few ChAT labeled fibers and varicosities and moderate levels of AChE staining. Motoneurons of the nXII are heavily stained with both ChAT and AChE, and the reticular area ventrolateral to the nXII contains some ChAT and AChE labeled neurons as well. No sex differences were noted in the distributions of ChAT and AChE.

### ChAT vs. AChE in vocal control nuclei

Although both ChAT and AChE were generally present in the same areas of the budgerigar brain, there were significant differences in the distributions of these markers in vocal control nuclei. AChE stained somata were observed in the HVo, the NAo, the NAs, the NLc, and the RA; but no ChAT labeled somata were observed in these nuclei. This may mean that while neurons in these nuclei are not cholinergic, they are cholinoceptive. Alternatively, the discrepancy between AChE and ChAT labeling may indicate that at least some somata in the HVo, the NAo, the NAs, the NLc, and the RA process other neurochemicals, such as neuropeptides, which also involve the enzymatic activity of AChE.

At least some of the AChE stained somata in vocal control nuclei may reflect noncholinergic inputs. Only a few ChAT labeled fibers were observed in the NAs, yet AChE staining in the somata of the NAs was almost as intense as that of the NAo and the HVo—areas in which many ChAT labeled fibers were observed. Similarly, few coarse ChAT labeled fibers appeared to pass through the RA, yet AChE staining was similar to that of the NLc in which ChAT labeled fibers were more numerous (see Figs. 2 and 6). These results suggest that the NAs and the RA receive at most sparse cholinergic input, in contrast to the fact that AChE stained somata are observed throughout these regions.

The distributions of ChAT and AChE in the LPOm were essentially concordant (see Figs. 1A, 2A, 4A, and 5A). The LPOm contains many ChAT labeled somata, although dense background or varicosities staining made it difficult to clearly identify AChE stained somata. The presence of ChAT labeled fibers and somata in the LPOm is consistent with the idea that axons of ChAT positive LPOm neurons ramify within the LPOm. It is also possible that the LPOm receives input from other nuclei containing ChAT positive neurons, such as the surrounding LPO and the VP. Although the possibility of cholinergic afferents to the LPOm cannot be ruled out, previous studies mapping the connections of the LPOm have failed to reveal inputs from the surrounding LPO, from the VP, or from other nuclei observed in the present report to contain ChAT labeled somata (Brauth et al., 1994; Striedter, 1994).

### Sources of cholinergic inputs to vocal control nuclei

No major discrepancies were observed between the distribution of ChAT labeled somata and AChE labeled somata in basal forebrain nuclei, leaving little doubt that the VP, the septum, the diagonal band, and the POA contain many cholinergic neurons. Moreover, the distribution of these putatively cholinergic neurons is highly similar to that of pigeons (Krebs et al., 1991; Medina and Reiner, 1994). ChAT labeled neurons have been described in the VP of reptiles (Brauth et al., 1985; Mufson et al., 1984; Powers and Reiner, 1993) and are thought to be a primitive feature of amniote brain organization (Brauth, 1990).

The distribution of ChAT and AChE stained fibers and varicosities in the HVo, the NAo, and the NLc is consistent with the hypothesis that vocal control nuclei in budgerigars receive cholinergic input. The absence of ChAT labeled somata suggests these nuclei do not project cholinergic efferents to other nuclei. Notably fewer ChAT labeled fibers were observed in the RA and the NAs. The RA and the NAs may therefore receive sparse cholinergic afferents, or, alter-

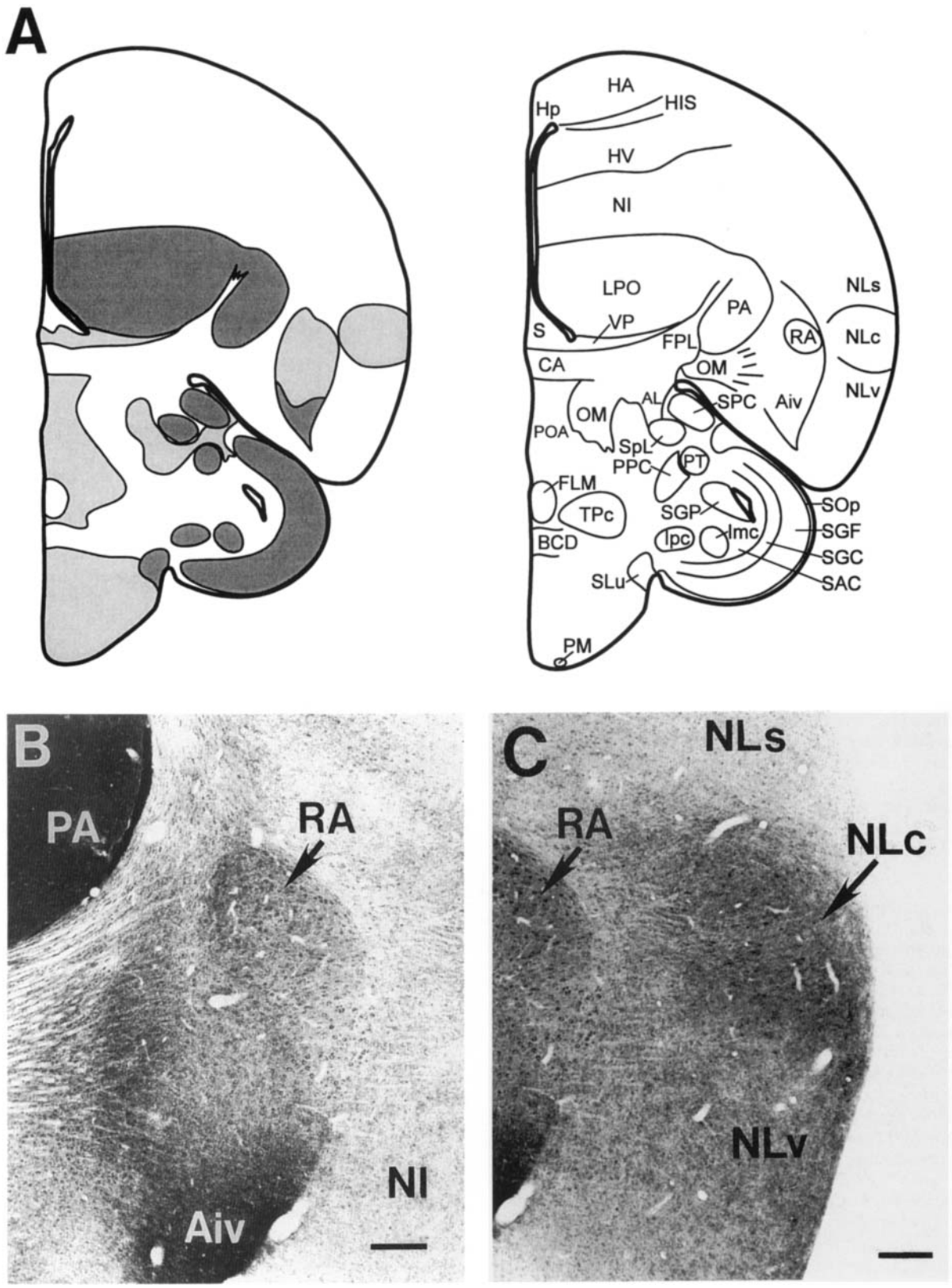



Fig. 6. Transverse section showing the distribution of AChE staining in the telencephalon and diencephalon at the level of the RA and the NLc. **A:** Drawings showing the relative density of AChE in the telencephalon and diencephalon. The drawing on the left side schematically indicates the density of AChE staining. Low AChE staining density (ARD = 1.0–1.5) is shown as white, medium AChE staining density (ARD = 1.5–1.8) is shown as light gray, and high AChE staining density (ARD = 1.8–2.2) is shown as dark gray. The drawing on the right side shows anatomical nomenclature at the same level as the AChE staining in the drawing on the left side. **B:** Brightfield photomicrograph showing AChE staining in the RA and the Av. Note that this photomicrograph shows AChE staining at a level of RA more caudal to that illustrated in Figure 6A. **C:** Brightfield photomicrograph showing AChE staining in the NLc at the same level as shown in Figure 6B.

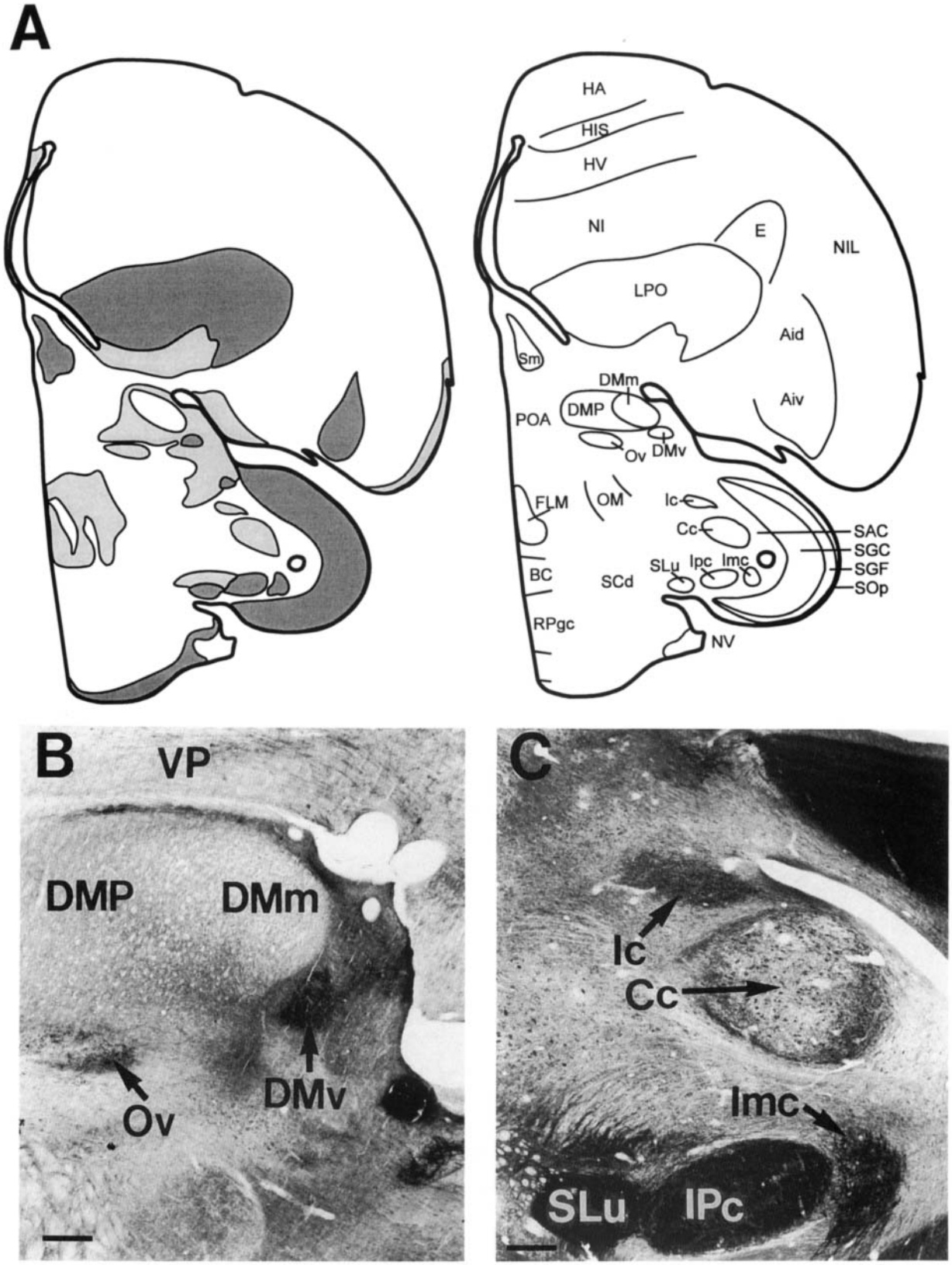



Fig. 7. Transverse section showing the distribution of AChE staining in the telencephalon and diencephalon at the level of DMP and the intercollicular area. **A:** Drawings showing the relative density of AChE in the telencephalon and diencephalon. The drawing on the left side schematically indicates the density of AChE staining. Low AChE staining density (ARD = 1.0–1.5) is shown as white, medium AChE staining density (ARD = 1.5–1.8) is shown as light gray, and high AChE staining density (ARD = 1.8–2.2) is shown as dark gray. The drawing on the right side shows anatomical nomenclature at the same level as the AChE staining in the drawing on the left side. The drawing on the right side shows anatomical nomenclature at the same level. **B:** Brightfield photomicrograph showing AChE staining in the DMm, the DMP, the DMv, the OV. **C:** Brightfield photomicrograph showing AChE staining in Ic and Cc.

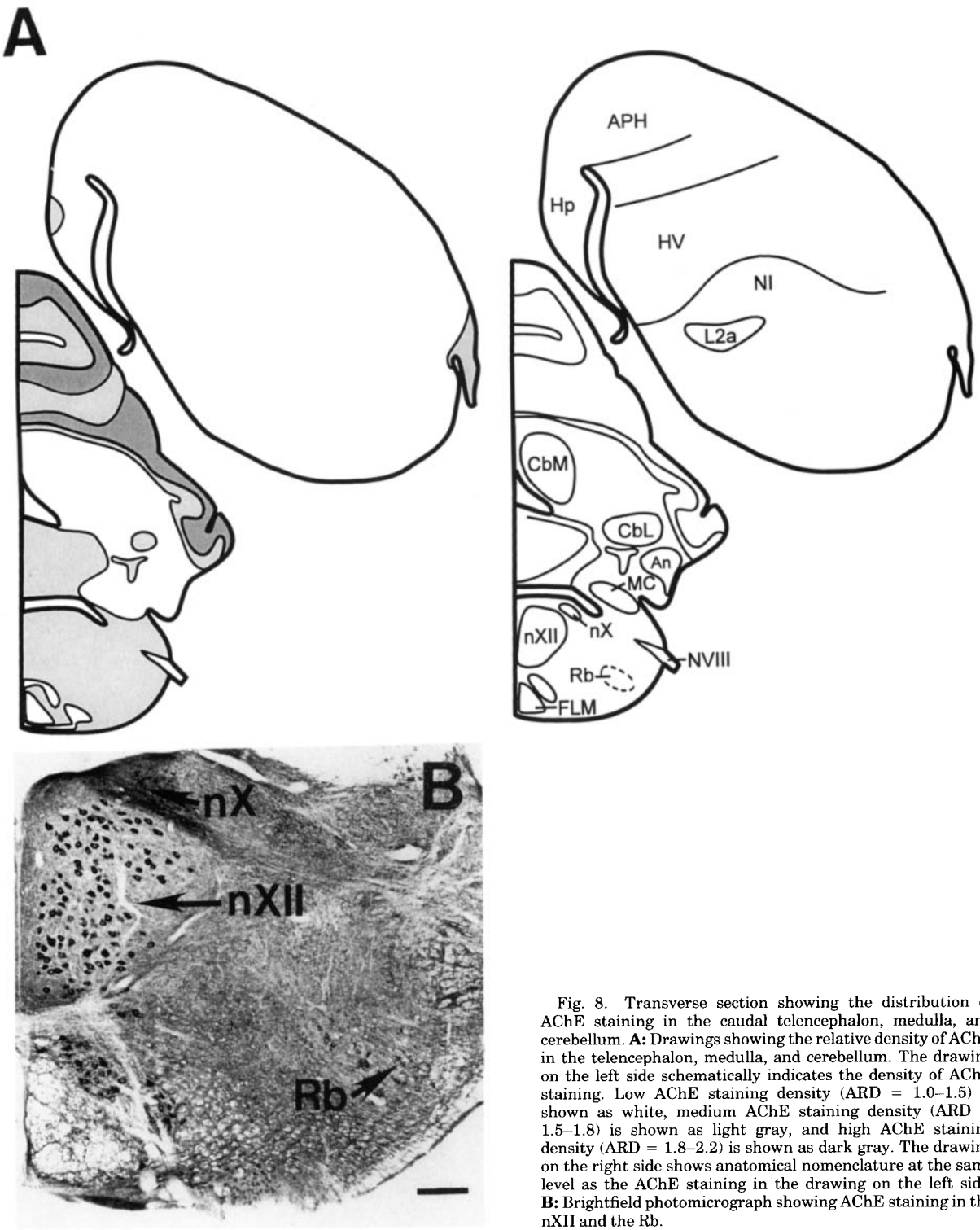



Fig. 8. Transverse section showing the distribution of AChE staining in the caudal telencephalon, medulla, and cerebellum. **A:** Drawings showing the relative density of AChE in the telencephalon, medulla, and cerebellum. The drawing on the left side schematically indicates the density of AChE staining. Low AChE staining density (ARD = 1.0–1.5) is shown as white, medium AChE staining density (ARD = 1.5–1.8) is shown as light gray, and high AChE staining density (ARD = 1.8–2.2) is shown as dark gray. The drawing on the right side shows anatomical nomenclature at the same level as the AChE staining in the drawing on the left side. **B:** Brightfield photomicrograph showing AChE staining in the nXII and the Rb.

natively, the ChAT labeled fibers may pass through the RA and the NAs without terminating. The absence of ChAT labeled somata in the RA and the NAs indicates that, like the HVo, the NAo, and the NLc, these nuclei do not project cholinergic efferents to other nuclei. Finally, the LPOm contains both ChAT labeled fibers and somata and may, as discussed above, possess an intrinsic cholinergic fiber system, receive extrinsic cholinergic afferents or project cholinergic efferents to other vocal nuclei. This latter possibility seems remote, however, insofar as the only known projection target of the LPOm within the vocal system is the DMm (Brauth et al., 1994; Hall et al., 1993, 1994; Striedter,

TABLE 1. Vocal Control Nuclei

| Species | HVo | NAo | MAN/NAs | LPOm/X | HVC/NLc | RA |
|---|---|---|---|---|---|---|
| Zebra Finches | | | | | | |
| AChE | * | * | S, F | S, F, V | S, V | S, V |
| ChAT | * | * | F' | F, S | F, V' | F', V' |
| Budgerigars | | | | | | |
| AChE | S, F, V | S, F, V | S, F | V | S, F, V | S, V |
| ChAT | F, V | F, V | F' | S, F, V | F, V | F' |

S = Labeled Somata.
F = Labeled Fibers (F' = very few fibers).
V = Labeled Varicosities (V' = very few varicosities).
* = Not considered a Vocal Control Nucleus in Zebra Finches.

1994) and the DMm contains virtually no ChAT labeled fibers and varicosities (see Fig. 3C).

The distribution of ChAT and AChE is consistent with the hypothesis that the basal forebrain and perhaps the septal and preoptic regions are sources of cholinergic projections to the HVo, the NAo, the NLc, and possibly the NAs and the RA in budgerigars. Pathway tracing studies using amino acid autoradiography (Brauth et al., 1994) report projections to the ventral portions of the NLc and the RA as well as many areas in the hyperstriatum ventrale and medial neostriatum from the VP. Double-label experiments are needed to evaluate the neurotransmitter content of these pathways. Nevertheless, it seems highly plausible that at least some of the projections of the VP to vocal control nuclei, especially the HVo and the NAo, are cholinergic in nature.

## Comparison with oscine songbirds

The distribution of cholinergic enzymes in vocal control nuclei in zebra finches and budgerigars is compared in Table 1. Data on the distribution of cholinergic enzymes in the zebra finch were compiled from previously published reports (Ryan and Arnold, 1981; Sakaguchi and Saito, 1991; Zuschratter and Scheich, 1990). AChE stained fibers and somata are observed in vocal control nuclei for both species and, as tabulated, are similar in many ways.

The distribution of ChAT and AChE in vocal control nuclei appears greatest in the LPO regions both in zebra finches and in budgerigars. The area X of oscine songbirds exhibits greater levels of ChAT labeling and AChE staining than the surrounding regions of the LPO (Zuschratter and Scheich, 1990). The present study reveals that the LPOm in budgerigars also shows greater levels of ChAT labeling and AChE staining than the surrounding regions of the LPO. The area X of zebra finches and the LPOm of budgerigars thus share similar distributions of cholinergic enzymes. Interestingly, Ball et al. (1990) have reported a higher density of muscarinic receptors in the area X compared to surrounding regions of the LPO of songbirds. Acetylcholine may be an important neurotransmitter both in the area X of oscine songbirds and in the LPOm of budgerigars.

In contrast to the dense localization of cholinergic enzymes in the area X and the LPOm, the RA of zebra finches and budgerigars contains very few ChAT labeled fibers. Zuschratter and Scheich (1990) found AChE stained somata and varicosities in the RA of zebra finches, but found no ChAT labeled somata and very few ChAT labeled fibers and varicosities. The present study likewise found AChE stained somata in the RA of budgerigars, but no ChAT labeled somata and few ChAT labeled fibers. These findings suggest the RA does not receive a substantial cholinergic input in either zebra finches or budgerigars. Consistent with the view that the RA receives little or no cholinergic input, the RA in oscine songbirds and budgerigars contains low densities of muscarinic receptors. Ball et al. (1990) have reported a low density of muscarinic receptors in the RA of oscine songbirds. Although Ball (1994) has suggested a high density of muscarinic receptors in the RA of budgerigars, the data reported actually shows a high density of receptor binding in the archistriatum both ventral and caudal to the RA (see Ball, 1994: his Fig. 4). This ventral region of the archistriatum containing a high density of muscarinic receptors corresponds to a region showing dense ChAT labeling and AChE staining in the present study (see Figs. 2A, 3A, 6B, and 7A in this article). The data reported by Ball (1994: his Fig. 4) reveals a low density of muscarinic receptors in the budgerigar RA. Thus, the RA apparently receives little or no cholinergic input in either oscine songbirds or budgerigars.

The HVC of zebra finches contains many AChE stained somata, but no ChAT labeled somata (Zuschratter and Scheich, 1990). The density of ChAT labeled fibers and varicosities in the zebra finch HVC is less than that observed in area X and greater than that observed in RA. Similarly, the NLc of budgerigars contains many AChE stained somata and fibers, but no ChAT labeled somata. The density of ChAT labeled fibers and varicosities in the NLc of budgerigars is less than that observed in LPOm and greater than that observed in RA. The HVC of zebra finches and the NLc of budgerigars both contain intermediate densities of cholinergic enzymes compared to area X/LPOm and RA.

Paton et al. (1981) described projections to the HVC from a cell group situated immediately dorsal to the LMD considered comparable to the magnocellular nucleus of the anterior neostriatum (MAN) of oscine songbirds and which Striedter (1994) renamed the NAs. As shown in Table 1, the NAs of budgerigars is similar to the MAN of songbirds in that both contain only a few ChAT labeled fibers. The ChAT labeled fibers observed in the budgerigar NAs were relatively large and may be fibers of passage. Consistent with these findings, previous studies have shown that MAN in oscine songbirds contains a low density of muscarinic cholinergic receptors (Ball, 1994; Ball et al., 1990). Thus, the MAN in oscine songbirds and the NAs in budgerigars apparently receive only sparse cholinergic input.

There are no direct counterparts in oscine songbirds to either the HVo or the NAo. The patterns of AChE and ChAT staining in the budgerigar HVo seem similar to those of the oscine HVC. Interestingly, Ball (1994) found a low density of muscarinic receptors in the hyperstriatum ventrale (HV) in budgerigars, although no data are reported for the HVo per se. The low density of muscarinic receptors stands in contrast to the dense ChAT labeled fibers and varicosities and the dense AChE staining observed in HVo in the present study (see Figs. 1A and 5A). The discrepancy between the low density of muscarinic receptors in HV and the high density of cholinergic enzymes in the HVo raises the possibility that cholinergic activity in the budgerigar HVo may be mediated by nicotinic receptors rather than muscarinic receptors. The present investigation reveals a similar pattern of cholinergic enzyme distribution in the vocal control nuclei of oscine songbirds and psittacines. Similarity in the distribution of cholinergic enzymes may be interpreted in two ways. On the one hand, similarity in the distribution of cholinergic enzymes may result from evolutionary homology of vocal control nuclei in oscine songbirds and budgerigars. On the other hand, similarity in the

distribution of cholinergic enzymes may reflect homoplasy of neurotransmitter functions in vocal control nuclei rather than evolutionary homology. The latter interpretation is more consistent with Striedter's (1994) theory, based on a phyletic analysis of pathway connections, that telencephalic vocal control nuclei have evolved independently in oscines and psittacines. Further studies are needed to clarify whether similarities in cholinergic enzyme distribution in oscines and budgerigars result from homology of vocal control nuclei or from homoplasy of cholinergic functions in vocal control nuclei.

### Comparison with nonvocal learning species

Budgerigars are the only species of basal landbirds to exhibit flexible vocal learning. It is therefore of interest to compare the distribution of cholinergic enzymes in budgerigars with the distribution of enzymes in other species of basal landbirds which do not exhibit flexible vocal learning. Two species of basal landbirds which do not exhibit flexible vocal learning are pigeons (Columbiformes) and domestic chickens (Galliformes).

Pigeons do not have specialized vocal control nuclei, although they do possess basal forebrain nuclei comparable to those found in budgerigars. The distribution of ChAT in the basal forebrain appears similar in pigeons and budgerigars. Krebs et al. (1991) and Medina and Reiner (1994) found ChAT labeled somata and fibers in the diagonal band and the medial septum of pigeons. They also found ChAT labeled fibers in the TSM and in the anterior-dorsal region of the hippocampus. No ChAT labeled somata were observed in the pigeon hippocampus. A similar pattern of cholinergic distribution was observed in budgerigars. ChAT labeled somata and fibers were observed in the diagonal band and medial septum in budgerigars. Some fibers in the medial septum extended toward the TSM, and ChAT labeled fibers were observed both in the hippocampus and in the area parahippocampalis. No ChAT labeled somata were observed in the hippocampus in budgerigars. Thus, pigeons and budgerigars share a similar pattern of cholinergic enzyme distribution in the basal forebrain.

The distribution of ChAT labeled fibers in the striatal regions of the telencephalon are also similar in pigeons and budgerigars. Slight differences in ChAT distribution are observed in the HV. In budgerigars, a relatively dense distribution of ChAT labeled fibers is located along the dorsal boundary of the HV with a greater concentration of labeled fibers and varicosities observed in the HVo. In pigeons, a relatively sparse and homogenous distribution of ChAT labeled fibers is located along the dorsal boundary of the HV (Medina and Reiner, 1994). Medina and Reiner (1994) have suggested that the distribution of ChAT in the HV may differ in pigeons and in chickens due to specialized imprinting nuclei in the HV in chickens. Similarly, differences in the distribution of ChAT in the HV in pigeons and budgerigars may be due to specialized call learning nuclei in the HV in budgerigars.

The greatest discrepancy in ChAT distribution between pigeons and budgerigars occurred in the thalamus. Medina and Reiner (1994) reported many ChAT labeled somata in several thalamic nuclei in pigeons. In contrast, ChAT labeled fibers were quite sparse and no ChAT labeled somata were observed in the thalamus in budgerigars. These differences may be due either to species differences in the thalamic distribution of ChAT or to nonspecific labeling of a ChAT-like antigen in the thalamus in pigeons. Nonspecific labeling in the thalamus in pigeons seems the more likely explanation. Medina and Reiner (1994) noted that ChAT labeled somata have not been reported in the thalamus of chickens, and that some of the ChAT labeled somata in the thalamus in pigeons did not label when human ChAT antibodies were used. They suggested somata in the thalamus in pigeons may contain an antigen similar to ChAT.

Domestic chickens, like pigeons, do not possess specialized vocal control nuclei, although chickens do possess nuclei specialized for visual and auditory imprinting. Nuclei specialized for visual and auditory imprinting include the hyperstriatal accessorium and hyperstriatum dorsale (HAD), the medial hyperstriatum and neostriatum (MNH), and the lateral hyperstriatum and neostriatum (LNH) (Horn, 1991, 1993; Scheich et al., 1991). In domestic chickens, the MNH receives input from the hippocampus (Bradley et al., 1985; Davies and Horn, 1983; Horn, 1991). Davies and Horn (1983) suggested projections from the hippocampus to the MNH may be cholinergic on the basis of AChE staining in the MNH somata.

The data presented here and in previous studies on pigeons do not support the hypothesis that the hippocampus sends cholinergic efferents to the medial hyperstriatal and neostriatal region. No ChAT labeled somata were observed in the present study within the hippocampus in budgerigars. The absence of ChAT labeled somata in the hippocampus in budgerigars is consistent with the absence of ChAT labeled somata in the hippocampus in pigeons (Krebs et al., 1991; Medina and Reiner, 1994). The lack of ChAT labeled somata in the hippocampus of both budgerigars and pigeons suggests that the hippocampus in basal landbirds does not send cholinergic projections to the medial hyperstriatal and neostriatal region. Cholinergic projections to the MNH in domestic chicks more likely originate from basal forebrain nuclei such as the septum or ventral paleostriatum, a possibility which Davies and Horn (1983) themselves suggested.

## CONCLUSIONS

The distribution of ChAT and AChE in budgerigars indicate that the HVo, the NAo, the NLc, and some regions of the tourus semicircularis receive substantial cholinergic input, whereas the RA, the NAs, and the DMm receive little or no cholinergic input. None of these nuclei contain ChAT labeled somata. The presence of both ChAT labeled fibers and somata in the LPOm is consistent with the view that axons of the LPOm neurons ramify within the LPOm. The motoneurons of the nXII and neurons of the Rb also show ChAT immunoreactivity and stain intensely for AChE.

Comparisons of the distribution of AChE and ChAT in the vocal control nuclei of budgerigars and oscine songbirds reveal many similarities. Similarities in the distribution of cholinergic enzymes may be interpreted as either homology or homoplasy. Interpreting similarities in the distribution of cholinergic enzymes as homoplasy is more consistent with Striedter's (1994) theory, based on pathway tracing studies, that vocal control nuclei in budgerigars evolved independently and are homoplastic with vocal control nuclei in oscine songbirds. Homoplastic distributions in cholinergic enzymes may reflect similar functions of the neurotransmitter acetylcholine in the vocal control systems of psittacines and oscine songbirds.

Comparisons of the distribution of AChE and ChAT in budgerigars and other basal landbirds, including Columbi-

formes and Galliformes, reveal strong similarities in basal forebrain nuclei. Budgerigars, however, are the only basal landbird species to have evolved specialized vocal control nuclei. Similarities and differences between vocal control nuclei in budgerigars and nuclei in other basal landbird species need to be clarified by further research.

Although the present study did not definitively determine the sources of cholinergic inputs to vocal control nuclei, the greatest number of ChAT labeled somata are present in the LPO and in basal forebrain nuclei including the septum, the POA, and the VP. The basal forebrain may be an important source of cholinergic inputs to vocal control nuclei in budgerigars. Pathway tracing studies have shown projections from the VP to several vocal control nuclei (Brauth et al., 1994), but double label experiments are needed to evaluate the neurotransmitter content of these pathways.

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