The Immunohistochemical Localization of Choline Acetyltransferase in the Cat Brain

STEVEN R. VINCENT AND PETER B. REINER

Division of Neurological Sciences, Department of Psychiatry
The University of British Columbia, Vancouver, B.C., V6T 1W5 Canada

Received 19 August 1986

VINCENT, S. R. AND P. B. REINER. The immunohistochemical localization of choline acetyltransferase in the cat brain. BRAIN RES BULL 18(3) 371-415, 1987.—The distribution of neurons displaying choline acetyltransferase (ChAT) immunoreactivity was examined in the feline brain using a monoclonal antibody. Groups of ChAT-immunoreactive neurons were detected that have not been identified previously in the cat or in any other species. These included small, weakly stained cells found in the lateral hypothalamus, distinct from the magnocellular rostral column cholinergic neurons. Other small, lightly stained cells were also detected in the parabrachial nuclei, distinct from the caudal cholinergic column. Many small ChAT-positive cells were also found in the superficial layers of the superior colliculus. Other ChAT-immunoreactive neurons previously detected in rodent and primate, but not in cat, were observed in the present study. These included a dense cluster of cells in the medial habenula, together with outlying cells in the lateral habenula. Essentially all of the cells in the parabigeminal nucleus were found to be ChAT-positive. Additional ChAT-positive neurons were detected in the periolivary portion of the superior olivary complex, and scattered in the medullary reticular formation. In addition to these new observations, many of the cholinergic cell groups that have been previously identified in the cat as well as in rodent and primate brain such as motoneurons, striatal interneurons, the magnocellular rostral cholinergic column in the basal forebrain and the caudal cholinergic column in the midbrain and pontine tegmentum were confirmed. Together, these observations suggest that the feline central cholinergic system may be much more extensive than previous studies have indicated.

Choline acetyltransferase Cat Acetylcholine Immunohistochemistry Central nervous system

THE localization of central cholinergic neurons was a mystery for many years, due to the lack of a specific method with which to identify unambiguously such cells. Although acetylcholinesterase (AChE) histochemistry has provided much valuable information, the cholinergic nature of many esterase-positive neurons has always been in doubt [26, 45, 80, 81]. The last few years have witnessed a revolution in our understanding of central cholinergic anatomy, brought about primarily by the introduction of antibodies to the cholinergic marker, choline acetyltransferase (ChAT).

The first major description of immunohistochemically-identified central cholinergic neurons was the mapping of the cat brain by Kimura et al. [43], who used an antiserum raised in rabbit against human ChAT. Since that time various groups have produced specific, monoclonal antibodies to ChAT [15, 19, 22, 47]. These have been used to map the distribution of central cholinergic neurons in the rat [3, 4, 16, 22, 23, 32, 36, 48, 54, 72, 82, 94] and primate [55, 60, 73, 74] brains. However, the cat brain has not yet been mapped with these new reagents. Therefore, in the present study a well characterized monoclonal antibody to ChAT [22] was used to examine the central cholinergic system of the cat. The data indicate that this system in the feline brain displays many features distinct from what has previously been found in other species. A preliminary report of these observations has been presented [92].

METHOD

Four adult male cats weighing three to four kg were studied. The animals were anesthetized with sodium pentobarbital (50 mg/kg, IP), heparinized (10,000 units, intracardiac) and perfused transcardially with 200 ml of phosphate buffered saline (0.1 M, pH 7.4) at room temperature, followed by 1500 ml of ice-cold fixative. The fixatives used were 4% paraformaldehyde in 0.1 M phosphak buffer, pH 7.4 (3 cats) or the buffered picric acid paraformaldehyde solution of Zamboni and DeMartino [99] (1 cat). The brain and spinal cord were removed and post-fixed for 2 hr in the same fixative, blocked and placed in cryoprotectant (2% sucrose, 10% glycerol in 0.05 M phosphate buffer) for 48 hr at 4°C. Three of the brains were sectioned coronally and one sagittally at 30 μm thickness on a freezing microtome, and collected serially in nine wells containing Tris-buffered saline (TBS, 0.05 M, pH 7.4) with 0.02% sodium azide.

Adjacent sets of sections were stained for Nissl with cresyl violet and for choline acetyltransferase immunoreactivity using a well characterized rat monoclonal antibody to porcine ChAT [22]. Sections were incubated in 0.3% H₂O₂ in TBS for 1 hr at room temperature to inhibit endogenous peroxidase activity, and then rinsed in TBS. The free-floating sections were then incubated with the monoclonal antibody, diluted 1:200 in TBS containing 2% normal rabbit
CA). The sections were rinsed 3x20 min in TBS, and then washed once again 3 x20 min in TBS and then reacted for containing normal rabbit sera and BSA. The sections were X-100, for 48 hr at 4°C. The sections were then processed for immunoperoxidase using the avidin-biotin complex method peroxidase activity.

anti-rat IgG diluted I:200 in TBS containing normal rabbit incubated for I hr at room temperature in biotinylated rabbit horseradish peroxidase complex, diluted 1: 100 in TBS con-

sections from the other three animals were reacted using a rat ABC Kit (Vector Laboratories, Burlingame, CA). The sections were rinsed 3x20 min in TBS, and then incubated for 1 hr at room temperature in biotinylated rabbit anti-rat IgG diluted 1:200 in TBS containing normal rabbit sera, BSA and Triton. Next, the sections were rinsed again 3x20 min in TBS and then incubated in avidin-biotinylated horseradish peroxidase complex, diluted 1:100 in TBS containing normal rabbit sera and BSA. The sections were washed once again 3x20 min in TBS and then reacted for peroxidase activity. The sections from one of the coronally sectioned, aldehyde-fixed cats were incubated in 50 mM Tris-Cl, pH 7.4 containing 0.025% 3,3′-diaminobenzidine, 0.01 M imidazole and 0.0075% H₂O₂ for 8 min. The sections from the other three animals were reacted using a nickel intensification technique in 50 mM Tris-Cl containing 0.02% diaminobenzidine, 0.0015% H₂O₂, and 0.6% nickel ammonium sulfate. Following rinsing in TBS, the sections were mounted on chrom-alum coated slides, dehydrated and coverslips were applied with Permount. The sections were examined under bright and dark field illumination, and with Normarski differential interference contrast optics. Selected sections stained for ChAT were mounted in a photographic enlarger and projected directly onto 8 x 10" Ilfospeed grade 4 glossy photographic paper to illustrate the distribution of ChAT-immunoreactive cells and neuropil in the forebrain (Figs. 1-8) and caudal brainstem (Figs. 17-40). The anatomical nomenclature used follows that of Rerman [6,7] and Bleier [8] in most instances, while in the brainstem, Taber [87] was also consulted.

For comparison, young adult male Wistar rats were perfused and processed in parallel with the cats, using identical procedures throughout.

RESULTS

Cells exhibiting ChAT immunoreactivity had a widespread distribution throughout the central nervous system of the cat. Although cell bodies were the most obvious stained feature, numerous regions showed dense terminal fields which were best appreciated in the prints made directly from

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>nucleus ambiguus</td>
</tr>
<tr>
<td>aa</td>
<td>anterior amygdaloid nucleus</td>
</tr>
<tr>
<td>ab</td>
<td>basolateral nucleus of the amygdala</td>
</tr>
<tr>
<td>ac</td>
<td>central nucleus of the amygdala</td>
</tr>
<tr>
<td>AC</td>
<td>anterior commissure</td>
</tr>
<tr>
<td>ap</td>
<td>area postrema</td>
</tr>
<tr>
<td>aq</td>
<td>cerebral aqueduct</td>
</tr>
<tr>
<td>BC</td>
<td>brachium conjunctivum</td>
</tr>
<tr>
<td>BCX</td>
<td>decussation of the brachium conjunctivum</td>
</tr>
<tr>
<td>c</td>
<td>caudate nucleus</td>
</tr>
<tr>
<td>CC</td>
<td>crus cerebri</td>
</tr>
<tr>
<td>ci</td>
<td>inferior colliculus</td>
</tr>
<tr>
<td>cm</td>
<td>central medial nucleus of the thalamus</td>
</tr>
<tr>
<td>cu</td>
<td>cuneiform nucleus</td>
</tr>
<tr>
<td>dbh</td>
<td>horizontal limb of the nucleus of the diagonal band of Broca</td>
</tr>
<tr>
<td>dbv</td>
<td>vertical limb of the nucleus of the diagonal band of Broca</td>
</tr>
<tr>
<td>dh</td>
<td>dorsal horn of the spinal cord</td>
</tr>
<tr>
<td>dmx</td>
<td>dorsal motor nucleus of the vagus nerve</td>
</tr>
<tr>
<td>ep</td>
<td>entopeduncular nucleus</td>
</tr>
<tr>
<td>fc</td>
<td>central tegmental field</td>
</tr>
<tr>
<td>ftc</td>
<td>magnocellular tegmental field</td>
</tr>
<tr>
<td>g</td>
<td>nucleus gracilis</td>
</tr>
<tr>
<td>gp</td>
<td>globus pallidus</td>
</tr>
<tr>
<td>h</td>
<td>hilus of the lateral superior olive</td>
</tr>
<tr>
<td>ha</td>
<td>anterior hypothalamic area</td>
</tr>
<tr>
<td>hl</td>
<td>lateral hypothalamic area</td>
</tr>
<tr>
<td>hm</td>
<td>medial habenula</td>
</tr>
<tr>
<td>ic</td>
<td>islands of Calleja</td>
</tr>
<tr>
<td>icm</td>
<td>internal capsulate</td>
</tr>
<tr>
<td>icm</td>
<td>medial island of Calleja</td>
</tr>
<tr>
<td>ip</td>
<td>interpeduncular nucleus</td>
</tr>
<tr>
<td>ipi</td>
<td>inner division of the posterior interpeduncular nucleus</td>
</tr>
<tr>
<td>ipo</td>
<td>outer division of the posterior interpeduncular nucleus</td>
</tr>
<tr>
<td>ipp</td>
<td>paramedian division of the posterior interpeduncular nucleus</td>
</tr>
<tr>
<td>lc</td>
<td>locus ceruleus</td>
</tr>
<tr>
<td>LL</td>
<td>lateral lemniscus</td>
</tr>
<tr>
<td>lld</td>
<td>dorsal nucleus of the lateral lemniscus</td>
</tr>
<tr>
<td>lv</td>
<td>ventral nucleus of the lateral lemniscus</td>
</tr>
<tr>
<td>lot</td>
<td>nucleus of the lateral olfactory tract</td>
</tr>
<tr>
<td>lso</td>
<td>lateral superior olive</td>
</tr>
<tr>
<td>MLF</td>
<td>medial longitudinal fasciculus</td>
</tr>
<tr>
<td>ms</td>
<td>medial septal nucleus</td>
</tr>
<tr>
<td>mso</td>
<td>medial superior olive</td>
</tr>
<tr>
<td>na</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>OT</td>
<td>optic tract</td>
</tr>
<tr>
<td>P</td>
<td>putamen</td>
</tr>
<tr>
<td>P</td>
<td>pyramidal tract</td>
</tr>
<tr>
<td>pc</td>
<td>paracentral nucleus of the thalamus</td>
</tr>
<tr>
<td>pg</td>
<td>periaqueductal gray</td>
</tr>
<tr>
<td>pb</td>
<td>parabrachial nuclei</td>
</tr>
<tr>
<td>pbg</td>
<td>paragigeminal nucleus</td>
</tr>
<tr>
<td>pc</td>
<td>paracentral nucleus of the thalamus</td>
</tr>
<tr>
<td>pg</td>
<td>pontine gray</td>
</tr>
<tr>
<td>sc</td>
<td>spinal cord</td>
</tr>
<tr>
<td>si</td>
<td>substantia innominata</td>
</tr>
<tr>
<td>sn</td>
<td>substantia nigra</td>
</tr>
<tr>
<td>ST</td>
<td>stria terminalis</td>
</tr>
<tr>
<td>TB</td>
<td>trapezoid body</td>
</tr>
<tr>
<td>TD</td>
<td>dorsal tegmental nucleus of Gudden</td>
</tr>
<tr>
<td>TS</td>
<td>solitary tract</td>
</tr>
<tr>
<td>tld</td>
<td>laterodorsal tegmental nucleus</td>
</tr>
<tr>
<td>tv</td>
<td>ventral tegmental nucleus of Gudden</td>
</tr>
<tr>
<td>vh</td>
<td>ventral horn of the spinal cord</td>
</tr>
<tr>
<td>III</td>
<td>third ventricle</td>
</tr>
<tr>
<td>IV</td>
<td>fourth ventricle</td>
</tr>
<tr>
<td>3</td>
<td>oculomotor nucleus</td>
</tr>
<tr>
<td>4</td>
<td>trochlear nucleus</td>
</tr>
<tr>
<td>4n</td>
<td>trochlear nerve</td>
</tr>
<tr>
<td>5</td>
<td>motor nucleus of the trigeminal nerve</td>
</tr>
<tr>
<td>5r</td>
<td>retrotrigeminal nucleus</td>
</tr>
<tr>
<td>6</td>
<td>abducens nucleus</td>
</tr>
<tr>
<td>6n</td>
<td>abducens nerve</td>
</tr>
<tr>
<td>7</td>
<td>facial nucleus</td>
</tr>
<tr>
<td>7a</td>
<td>accessory facial nucleus</td>
</tr>
<tr>
<td>7g</td>
<td>genu of the facial nerve</td>
</tr>
<tr>
<td>7n</td>
<td>facial nerve</td>
</tr>
<tr>
<td>7r</td>
<td>retrofacial nucleus</td>
</tr>
<tr>
<td>12</td>
<td>hypoglossal nucleus</td>
</tr>
</tbody>
</table>
the microscope slides in the photographic enlarger (Figs. 1-8, 17-40). Also, the dendritic fields of many of the cell groups were well delineated, and numerous fiber bundles, such as the cranial nerves, the fasciculus retroflexus and the dorsal tegmental pathway were well stained. No glial elements were immunoreactive.

Only normal adult male cats were examined. It is possible that colchicine pretreatment might have allowed the visualization of additional cell groups that were not detected in the present study. However, it has been found in previous studies in rat [21] that colchicine treatment does not improve ChAT immunostaining.

Telencephalon

No ChAT-immunoreactive cell bodies were detected in the olfactory bulb, hippocampus, or cerebral cortex. The caudate nucleus and putamen contained a population of large, mostly multipolar ChAT-positive neurons. These were sparsely scattered throughout the entire striatum, including the nucleus accumbens (Figs. 1-8). In addition, a heterogeneous terminal field was also clearly evident throughout the striatum, with scattered, lightly-stained regions embedded in the more intensely stained neuropil. The striatal terminal field was most dense within the nucleus accumbens (Figs. 1, 2).

Between the nucleus accumbens and the septum, in the medial island of Calleja, a group of much smaller ChAT-positive cells was present (Fig. 1). These were larger (10-15 μm diameter) than the islet granule cells among which they were scattered, but still much smaller than the ChAT-positive cells in the nucleus accumbens (25-35 μm diameter) (Fig. 9). The islet cells displayed only one or two poorly stained processes. In each of the other islands of Calleja similar clusters of small ChAT-immunoreactive cells were also found (Fig. 1). These often occurred within the cup-like depression formed by the granule cells, and extended processes into the islets, which exhibited dense terminal fields (Fig. 10). The size of the ChAT-immunoreactive cells associated with the islets appeared to increase in the more laterally placed islets, with the most medial islet having the smallest ChAT-positive cells. Indeed, the cells in the most lateral islets were similar in size and morphology to those in the nucleus accumbens, caudate nucleus and putamen, and in fact appeared to be a ventral extension of the striatum, via striatal bridges across the polymorphic layer of the olfactory tubercle.

The major ChAT-immunoreactive cell group of the forebrain was the rostral cholinergic column of the basal forebrain (Figs. 1-8). This extensive collection of magnocellular, intensely-immunoreactive neurons began rostrally in the medial septum. Scattered single ChAT-positive cells were found within the lateral septum, which also contained a dense terminal field. Similarly, a few of these large, ChAT-intense cells could be found within and surrounding the fibers of the columns of the fornix, and caudally within and about the commissure of the fornix (Figs. 2-4). Ven-

trally, the cells of the medial septum were continuous with similar large multipolar cells in the vertical limb of the nucleus of the diagonal band of Broca (Fig. 2).

The cells of both the vertical and horizontal limbs of the diagonal band of Broca were large, intensely stained, and mostly multipolar (Fig. 11c). The ChAT-positive cells of the horizontal limb spread laterally in clumps across the polymorphic layer of the caudal olfactory tubercle and the rostral preoptic area to the medial edge of the lateral olfactory tract. Here a dorsal branch rose through the substantia innominata, penetrated the putamen, and surrounded the globus pallidus from its ventral and lateral surfaces (Figs. 3-5). A dense band of such cells coursed dorsally in the medullary lamina between the globus pallidus and putamen to reach the internal capsule. Another, sparser ascending branch arose medial to the globus pallidus beneath the anterior commissure and surrounded the medial and dorsal surfaces of the globus pallidus (Figs. 4-6).

Both these branches of the basolateral cholinergic column invaded the internal capsule extensively (Figs. 2-5). Many, very large, and very heavily stained cells were intercalated among the fiber bundles dorsal to the globus pallidus, between the caudate and putamen. These cells were larger (up to 50 μm diameter) than the striatal ChAT-positive cells (25-35 μm diameter) seen at these levels (Fig. 11). It is interesting to note that although the globus pallidus was surrounded on all sides by magnocellular ChAT-immunoreactive neurons, such cells only penetrated within the pallidum at its caudal pole (Figs. 6-8). A similar situation was seen in the ventral pallidum, beneath the posterior limb of the anterior commissure, which contained very few ChAT-positive cells and very little fiber staining, but was surrounded by the many ChAT-positive cells of the substantia innominata (Fig. 2).

Moving caudally, the ChAT-positive cells of the substantia innominata extended laterally above the nucleus of the lateral olfactory tract into the anterior amygdaloid area (Figs. 5-8). Cells continued to extend dorsally from here around the pallidum in the medullary lamina next to the putamen and within the external capsule. Magnocellular ChAT-positive cells also appeared to invade the ventral third of the putamen, where they mixed with the smaller striatal ChAT cells. Both the nucleus of the lateral olfactory tract and the anterior cortical nucleus of the amygdala contained dense terminal fields (Figs. 5-8).

ChAT-positive cells extended beneath the pallidum above the central nucleus of the amygdala, and within the putamen. A few small ChAT-positive cells were found within the central nucleus of the amygdala (Figs. 8, 11f). Some cells also occurred ventromedially along the surface of the globus pallidus beneath the rostral entopeduncular nucleus in the ansa lenticularis (Figs. 6-8). No ChAT-positive cells were found within the entopeduncular nucleus. The caudal extension of the rostral cholinergic column continued above the dorsal tip of the optic tract to the caudal end of the entopeduncular nucleus (Fig. 8).
FIG. 1. The rostral pole of the rostral column is found in the medial septal nucleus (ms). The striatal ChAT-positive cells are found in the caudate nucleus (c), putamen (p) and the nucleus accumbens (na). In addition, very small ChAT-immunoreactive cells are found in association with the medial island of Calleja (icm) and the other small islands of Calleja (ic, arrowheads) which also show a dense terminal field.
FIG. 2. The cells of the septal area are continuous caudally with those in the vertical limb of the diagonal band of Broca (dbv) which are themselves continuous with the cells found in the horizontal limb of the diagonal band (dbh).
FIG. 3. At the rostral pole of the globus pallidus (gp), the ChAT-positive neurons of the rostral column extend out from the horizontal limb of the diagonal band (dbh) into the anterior hypothalamic area (ha), substantia innominata (si) and dorsally up into the internal capsule (IC), between the caudate nucleus (c) and the putamen (p). Note the absence of ChAT-immunoreactive neurons within the globus pallidus.
FIG 4. At the level of the anterior commissure (AC), many of the magnocellular ChAT-positive neurons of the rostral column are found within the internal capsule (IC), dorsal to the globus pallidus (gp). Other cells are present in the bed nuclei of the anterior commissure, and the stria terminalis (ST), while many more are present in the horizontal limb of the diagonal band and the substantia innominata (si). Scattered smaller ChAT-immunoreactive cells are present in the anterior hypothalamic area (ha).
FIG. 5. A dense terminal field is present in the nucleus of the lateral olfactory tract (lot), which lies beneath the rostral cholinergic column neurons in the substantia innominata (si). The magnocellular ChAT-positive neurons can be seen surrounding the globus pallidus (gp) in the internal capsule (IC), and even invading the ventral third of the putamen (p). Medially the cells invade the lateral portion of the anterior hypothalamic area (ha), where smaller weakly ChAT-positive cells are also found.
FIG. 6. At the rostral pole of the entopeduncular nucleus (ep), some of the magnocellular neurons invade the globus pallidus (gp) and the anterior amygdaloid area (aa), which also contains a rather dense terminal field. A few magnocellular neurons together with many small faint cells are present in the lateral hypothalamus (hl).
FIG. 7. Within the amygdala, dense terminal fields are present in the central nucleus (ac) and the basolateral nucleus (lb). ChAT-positive neurons of the rostral column are found mostly along the medial edge of the globus pallidus (gp), but they extend into the globus pallidus, and within the amygdala. Striatal ChAT-positive neurons continue in the caudate nucleus (c) and putamen (p).
FIG. 8. The caudal end of the rostral cholinergic column coincides with the caudal end of the globus pallidus. Striatal cells are found in the putamen (p), and some cells are found in and around the central nucleus of the amygdala (ac) which together with the basolateral nucleus (ab) contains a dense terminal field. Other terminal fields are present in the intralaminar central medial (cm) and paracentral (pc) thalamic nuclei. The small ChAT-positive cells of the medial habenula (hm) can also be seen.
FIG. 9. This set of micrographs illustrates the ChAT-positive neurons present in the nucleus accumbens (na), the medial island of Calleja (icm) and the medial septal nucleus (ms). (a) is a cresyl violet section showing the granule cells of the medial island. The ChAT-immunoreactive cells are shown in (b). (c,d,e) are higher power micrographs taken from (b) to illustrate the relative sizes of the ChAT-positive neurons in the na, icm and ms, respectively. Scale bars indicate 500 μm for (a,b) and 50 μm for (c,d,e).
FIG. 10. In (a), the small ChAT-immunoreactive neurons seen capping the islands of Calleja are illustrated. The very small granule cells of the islet are apparent in a nearby section stained for cresyl violet (b). Scale bar indicates 100 μm.
FIG. 11. These micrographs illustrate the relative sizes of the ChAT-immunoreactive neurons present in the caudate nucleus (a) and the putamen (b), in the nucleus of the diagonal band of Broca (c) and within the internal capsule (d). An example of the larger cells surrounding the central nucleus of the amygdala is shown (e) as are some of the small cells found within this nucleus (f). Scale bar indicates 50 μm for all figures.
FIG. 12. Many small, weakly ChAT-positive cells are scattered in the lateral hypothalamus (arrows) above some of the magnocellular basal forebrain ChAT-positive cells which lie dorsal to the supraoptic nucleus (a). The relative sizes and staining patterns of the two hypothalamic ChAT cell populations are illustrated in (b). Some of the magnocellular rostral column cells are found embedded in the fibers of the stria terminalis (c) and positive cells are found within the bed nucleus of the stria terminalis (d). Scale bars indicate 200 μm in (a) and 50 μm for all other figures.
Diencephalon

Beneath the anterior commissure in the anterior and lateral hypothalamic areas, two types of ChAT-positive cells were noted (Fig. 12a,b). The magnocellular cells of the lateral hypothalamus were continuous with those in the adjacent horizontal limb of the diagonal band and substantia innominata. These were scattered in a dorsolateral direction in the lateral hypothalamus medial to the globus pallidus. A cluster of these magnocellular ChAT-positive neurons was found just overlying the supraoptic nucleus (Figs. 6, 12a).

In addition, a population of much smaller (15 μm diameter), less intensely stained ChAT-immunoreactive cells was present, which extended into the bed nuclei of the stria terminalis, anterior commissure and stria medullaris. This type of cell was most common medial to the internal capsule in the lateral hypothalamic area. Some similar cells also extended medially into the anterior hypothalamic area. This cell group continued caudally, rising over the fornix to reach the dorsal aspect of the third ventricle. A few similar cells could also be found ventral and medial to the fornix in the anterior hypothalamic area, adjacent to the periventricular and arcuate nuclei. These small cells continued caudally in the lateral hypothalamus to the level of the zona incerta.

No ChAT-immunoreactive cell bodies were observed in the thalamus. However, a well-organized pattern of terminal fields was observed. The densest terminal fields were associated with the rostral intralaminar nuclei, in particular the central medial and paracentral nuclei (Fig. 8). The anteroventral, reticular and dorsal lateral geniculate nuclei also contained noteworthy terminal fields.

Within the epithalamus, a moderately dense terminal field was present in the lateral habenula. In the medial habenula, a dense cluster of small ChAT-positive cells was found in the ventral half of this nucleus (Fig. 8). These were so densely clustered together that cell processes could not be distinguished. In addition, scattered cells with clearly stained processes were observed in the medial portion of the lateral habenula (Fig. 13a,b). These extended caudally among the fibers of the habenular commissure and descended down the fasciculus retroflexus to the level of the parafascicular nucleus.

Mesencephalon

The fibers of the fasciculus retroflexus were ChAT-positive. These ended in the interpeduncular nucleus in a distinct pattern. Rostrally, the cholinergic fibers began as two compact pear-shaped bodies (Fig. 13c). These expanded caudally to fill the central interpeduncular nucleus with a unique pattern of horizontally organized fibers. This pattern extended caudally into the inner division of the posterior nucleus (Fig. 13d,e). The apical nucleus and the outer division of the posterior nucleus were only poorly innervated.

The oculomotor complex began rostrally just above the caudal oculomotor nucleus, where occasional ChAT-positive cell bodies were observed (Fig. 12a). The caudal cholinergic column was first detected at the level of the caudal oculomotor nucleus, where occasional ChAT-positive cells were seen, but these came together caudally in a compact cluster lying between the bundles of the fasciculus retroflexus, beneath the rostral pole of the aqueduct. These cells were smaller and less intensely stained than those found further caudally. These cells increased in number and moved dorsally between the medial longitudinal fasciculi at the level of the interfascicular nucleus. Scattered, larger and more intensely stained cells were also found at this level, scattered among the moderately stained cells. At the level of the exit of the third nerve, the darker stained cells formed two distinct clusters lateral to the moderately immunostained cells which lay dorsally in the midline. The moderately immunoreactive cells decreased in number further caudally, while the larger, intensely labelled cells increased and formed a single large cluster lying dorsal to the medial longitudinal fasciculus (Fig. 14). Some of these cells were also found scattered within this fiber bundle ventrolateral to the main cell group. The cells of the oculomotor nucleus extended long dendritic processes laterally into the central gray and among the fibers of the medial longitudinal fasciculus. In addition, the axons of the third nerve showed strong immunoreactivity (Fig. 14).

At its most caudal level, the cells of the oculomotor complex above and within the medial longitudinal fasciculus appeared continuous with those of the trochlear nucleus. The cells of the trochlear nucleus formed bilateral clusters above the dorsomedial surface of the fasciculus (Fig. 18). There appeared to be two cell populations, small intense cells capping the dorsomedial surface of the nucleus, with larger cells ventrally and extending within the medial longitudinal fasciculus. The trochlear nucleus ended with the exit of the stained axons of the fourth nerve dorsolaterally (Fig. 22).

The superior colliculus displayed a striking pattern of ChAT immunoreactivity (Fig. 15). A fiber network was present in lamina one of the superficial gray layer. In addition, small intensely stained patches of ChAT-positive fibers were observed in the intermediate gray layer (Fig. 17). Weakly stained ChAT-positive cell bodies were also observed in the superior colliculus. They were scattered sparsely in the superficial gray layers, predominantly in laminae two and three. These cells were small (15–20 μm diameter) and round, and had poorly stained processes (Fig. 15b). Often only a single process oriented dorsally was apparent, although some cells had horizontally oriented processes as well.

Another group of ChAT-positive cells was detected in the parabigeminal nucleus. Essentially all of the densely packed, medium-sized (25 μm diameter) cells of this nucleus were immunoreactive (Figs. 16–18). The ChAT-positive neurons were mostly multipolar and fusiform in shape, and a dense network of immunoreactive processes was also present throughout the parabigeminal nucleus.

The major ChAT-positive cell group of the midbrain and pontine tegmentum comprised the caudal cholinergic column. This extended from the mesencephalic reticular formation caudally into the pontine tegmentum (Figs. 17–25). The caudal cholinergic column was first detected at the level of the caudal oculomotor nucleus, where occasional ChAT-positive cell bodies were observed. These were less intensely stained than those found in the rostral oculomotor nuclei.
FIG. 14. The ChAT-positive motoneurons of the oculomotor complex, and the positive axons in the exit of the third nerve are illustrated (a). The inset (b) shows some of the large multipolar cells extending their processes out into the medial longitudinal fasciculus. Scale bars indicate 500 μm in (a) and 50 μm in (b).
FIG. 15. ChAT-immunoreactive elements in the superior colliculus include positive cell bodies in the superficial gray layers (arrows) and dense terminal patches in the intermediate gray layer. The cells and patches are shown at higher magnification in (b) and (c) respectively. Scale bars indicate 200 μm in (a) and 50 μm in (b,c).
A cluster of ChAT-positive cells is present in the parabigeminal nucleus (pbg) which lies dorsolateral to the substantia nigra (sn) at the level of the trochlear nucleus (4) and the interpeduncular nucleus (ip). The rostral tip of the caudal cholinergic column (arrows) also reaches up to this level. (b) is a higher magnification of some of the parabigeminal ChAT-positive neurons. Scale bars indicate 500 μm for (a) and 25 μm for (b).
FIGS. 17-40. Projection prints illustrating the distribution of ChAT-immunoreactive neurons in the caudal midbrain, pons and medulla of the cat.

FIG. 17. The caudal cholinergic column (small arrowhead) begins the central tegmental field of the midbrain just dorsal to the caudal lateral substantia nigra (sn) and just medial to the small ChAT-immunoreactive cells of the parabigeminal nucleus (pbg). This section is at the level of the oculomotor nucleus (3). Terminal fields are present in the interpeduncular nucleus (ip) and in patches in the intermediate gray layers of the superior colliculus (large arrowheads).
found in the rostral pole of the laterodorsal tegmental nucleus (arrowhead) in the periaqueductal gray. The small ChAT-positive cells of the parabigeminal nucleus (pbg) continue caudally to this level.
FIG. 19. At the level of the decussation of the brachium conjunctivum (BCX) the caudal cholinergic column occupies the entire dorsal portion of the central tegmental field (ftc) ventral to the cuneiform nucleus (cu). ChAT-positive cells extend from this main group dorsomedially into the central gray. The ChAT-immunoreactive terminal field in the inner division of the posterior interpeduncular nucleus (ipi) is very distinct at this level.

FIG. 20. The dorsal portion of the central tegmental field is full of the large, intensely ChAT-positive cells of the caudal cholinergic column, which extend dorsomedially to form a distinct group in the laterodorsal tegmental nucleus (tld) in the floor of the fourth ventricle.
FIG. 21. The laterodorsal tegmental nucleus (td) contains numerous ChAT-positive cells at this level. Neurons extend ventrolaterally out of the central gray beneath and within the brachium conjunctivum.

FIG. 22. The ChAT-positive cells are found in the laterodorsal tegmental nucleus between the dorsal tegmental nucleus (Gudden) (td) and the locus ceruleus (lc). A few cells extend out of the central gray beneath the brachium conjunctivum. Some similar cells are found dorsolateral to the brachium conjunctivum, wedged between the dorsal nucleus of the lateral lemniscus and the caudal pole of the inferior colliculus (arrowhead).
FIG. 23. The level of the caudal end of the caudal cholinergic column, in the laterodorsal tegmental nucleus (tlD). At this level a separate and distinct group of small, weakly ChAT-positive cells is found in the parabrachial nucleus, especially in its dorsolateral aspect (arrowhead).

FIG. 24. The parabrachial cell group is quite extensive at this level surrounding the brachium conjunctivum ventrally and laterally (arrowheads). These small cells are quite distinct from the remaining cells of the caudal cholinergic column in the laterodorsal tegmental nucleus. At this level a few of the pcroillary cells (arrows) are found lateral to the trapezoid body (TB) at the rostral pole of the superior olivary complex.
FIG. 25. The rostral pole of the motor nucleus of the trigeminal nerve (5) lies at the caudal extreme of the caudal cholinergic column, which ends in the laterodorsal tegmental nucleus (tltd) in the floor of the fourth ventricle. Some of the smaller and fainter ChAT-positive cells of the parabrachial nucleus (pb) can also be seen at this level, beneath the brachium conjunctivum (BC).

FIG. 26. The main body of the motor nucleus of the trigeminal nerve (5) can be seen at this level. Also present are some of the periolivary neurons (arrowheads) surrounding the lateral superior olivary nucleus (lsn).
FIG. 27. Small cells (arrow) can be seen extending between the retrotrigeminal nucleus (r5), next to the stained fibers of the descending seventh nerve (7n), and the rostral end of the abducens nucleus (6). Many periolivary cells (arrowheads) are present at this level between the exits of the sixth (6n) and seventh nerves.

FIG. 28. The main body of the abducens nucleus (6) lies beneath the genu of the seventh nerve (7g). Small periolivary cells are present within the hilus (h), while ChAT-positive cells are present in the magnocellular tegmental field (arrow).
FIG. 29. A few small ChAT-positive cells (arrows) extend from the caudal pole of the abducens nucleus (6) ventrolaterally toward the accessory facial nucleus (7a) and the cluster of small cells lying lateral to it (arrowheads), dorsal to the caudal end of the lateral superior olivary nucleus (5o).

FIG. 30. Scattered cells (arrows) are found in the magnocellular tegmental field (ftm) medial to the facial nucleus (7). Another group of cells (arrowheads) is found just lateral to the accessory facial nucleus (7a).
Fig. 31. Towards the caudal pole of the facial nucleus (7) many small ChAT-positive cells continue in the lateral tegmental field (small arrowheads). Another cluster of similar cells (large arrowhead) is found dorsomedially in the nucleus prepositus hypoglossi. Scattered larger cells (arrows) are found medial to the facial nucleus in the magnocellular tegmental field (ftm).

Fig. 32. The retrofacial nucleus (7r) arises dorsolateral to the caudal pole of the facial nucleus. Small ChAT-positive cells (small arrowheads) extend dorsal to the retrofacial nucleus to the rostral pole of the dorsal motor nucleus of the vagus (dmn). A cluster of similar cells (large arrowheads) continues in the nucleus prepositus hypoglossi, and the adjacent medial longitudinal fasciculus (MLF), while scattered larger cells (arrows) are found ventral to this in the magnocellular tegmental field above the inferior olive (io).
FIG. 33. The nucleus ambiguus (a) appears as a caudal extension of the retrofacial nucleus, and scattered small cells (arrowheads) continue to extend dorsally from it to the dorsal motor nucleus of the vagus (dmx).

FIG. 34. The hypoglossal nucleus (12) makes its appearance medial to the dorsal motor nucleus of the vagus (dmx) in the floor of the fourth ventricle. A few scattered cells (arrowheads) are found in the inferior vestibular nucleus at this level.
FIG. 35. Scattered small cells (arrowheads) continue to extend between the dorsal motor nucleus of the vagus (dmx) and the nucleus ambiguus (a). The hypoglossal nucleus (12) is quite extensive at this level.

FIG. 36. A few scattered cells (arrowheads) lie dorsal to the nucleus ambiguus (a) towards the dorsal motor nucleus of the vagus (dmx) which has moved medially to lie dorsal to the hypoglossal nucleus (12) at the level of the area postrema (ap). Other scattered cells (arrow) lie along the exit of the twelfth nerve.
immunoreactive neurons were seen in the ventrolateral mesencephalic reticular formation, just dorsal to the pars lateralis of the substantia nigra (Figs. 17, 18). At the level of the trochlear nucleus and nerve, ChAT-positive neurons were seen extending in a band across the tegmentum, within the pedunculopontine nucleus in the central tegmental field of Berman [6] (Figs. 18, 19). Still further caudal, the location of this column of ChAT-positive neurons shifted dorsomedially; scattered ChAT-immunoreactive perikarya were seen embedded within the fiber bundles of the brachium conjunctivum which bisected the pedunculopontine nucleus at this level (Fig. 20). At the level of the dorsal and ventral tegmental nuclei of Gudden, most ChAT-positive neurons were located medial to the brachium conjunctivum within the laterodorsal tegmental nucleus and the subjacent pedunculopontine nucleus (Fig. 21).

Further caudal, as the pedunculopontine nucleus merged with the medial parabrachial nucleus, neurons staining deeply for ChAT were seen within the laterodorsal tegmental nucleus and extended out into the medial parabrachial nucleus (Fig. 22). Additionally, at this level, a sheet of large spindle-shaped, horizontally oriented ChAT-positive neurons was seen capping the dorsal nucleus of the lateral lemniscus, near the lateral edge of the brachium of the inferior colliculus (Fig. 22). Further caudal, large ChAT-positive neurons were restricted to the laterodorsal tegmental nucleus in the periventricular gray (Figs. 23, 24). At the most caudal level of this column, occasional ChAT-immunoreactive neurons were seen in the lateral periventricular gray, apparently within the medial vestibular nucleus.

A group of small (12-15 μm diameter) spindle-shaped neurons which stained lightly for ChAT was seen within both the medial and lateral parabrachial nuclei (Figs. 21-25). In the ventral portion of the lateral parabrachial nucleus, these cells formed a distinct cluster (Fig. 24). These neurons could be distinguished clearly from the neurons of the caudal cholinergic column by both size and staining intensity (Fig. 41), even at rostral levels where the two cell populations intermingled (Fig. 21, 22).

**Pons and Medulla**

The motor nucleus of the trigeminal nerve began rostrally at the caudal pole of the laterodorsal tegmental nucleus, beneath the small ChAT-positive cells in the parabrachial nucleus (Fig. 25). These motoneurons formed a dense ball of large multipolar cells similar to those of the oculomotor nucleus. In addition, a cluster of much smaller, but strongly staining cells was found at the ventrolateral pole of the trigeminal motor nucleus beneath the exit of the fifth nerve, the axons of which were stained. At the level of the peduncle pole of the nucleus, another group of small (25-25 μm diameter) ChAT-positive cells extended up from the dorsomedial aspect beneath the descending limb of the seventh nerve. A few such cells were also found along the dorsal surface of the seventh nerve. These continued caudally and formed a cluster beneath the descending limb of the seventh nerve caudal to the motor nucleus of the trigeminal. At slightly more caudal levels, these small cells seemed to form bridges between the larger motoneurons of the abducens nucleus lying just beneath the genu of the seventh nerve and the retrotrigeminal nucleus embedded in the descending limb of the seventh nerve laterally and the accessory facial nucleus lying among the ascending fibers of the seventh nerve medially (Figs. 27-29).

As the caudal end of the abducens nucleus, other small ChAT-positive cells were found scattered ventrally in the lateral tegmental field between the descending sixth and seventh nerves, dorsal to the superior olivary complex and lateral to the accessory facial nucleus (Figs. 28, 42).

A separate group of ChAT-positive cells was found within the superior olivary complex. These first appeared rostrally as multipolar cells (20-25 μm diameter) in the preolivary nucleus beneath the lateral superior olivary nucleus (Fig. 26). Further caudal, these neurons spread out medially beneath both the medial and lateral superior olivary nuclei (Fig. 27). Smaller (10 μm diameter), mostly bipolar cells were also found sweeping over the dorsolateral surface of the lateral superior olive and into the hilus (Figs. 28, 43). Scattered periolivary ChAT-positive cells continued to surround the principal olivary nuclei, some being present medial to the nucleus of the trapezoid body, and some just dorsal to the fibers of the trapezoid body beneath the rostral pole of the facial nucleus.

At the level of the facial nucleus proper, scattered medium-size cells continued to extend dorsally from the nucleus almost to the genu (Fig. 30). A few large, weakly-stained, multipolar cells appeared at this level in the magnocellular tegmental field (Figs. 30, 42c). Near the caudal pole of the facial nucleus the retrofacial nucleus made its appearance dorsally, just medial to the ascending limb of the seventh nerve (Fig. 32). Lateral to the retrofacial nucleus, the scattered medium-sized cells continued in the lateral tegmental field, while a few larger multipolar cells were scattered in the magnocellular tegmental field medially.

The retrofacial nucleus extended caudally and appeared to merge with the nucleus ambiguus dorsolaterally at the level of the inferior olivary complex and the exit of the twelfth nerve (Fig. 33). The nucleus ambiguus was made up of large, well stained cells similar to those in the facial and retrofacial nuclei. It appeared quite compact in coronal sections, but had a considerable rostro-caudal extent. Medium-sized, weakly staining cells lay dorsal to the nucleus ambiguus, and extended up towards the dorsal motor nucleus of the vagus, which first appeared as a small dense ball of cells lying just ventromedial to the nucleus of the solitary tract (Figs. 32, 33).

Another group of ChAT-positive cells was found scattered in the paramedian reticular nucleus in the dorsal portion of the medial longitudinal fasciculus and the adjacent prepositus hypoglossal nucleus. This appeared to correspond to the accessory hypoglossal nucleus (Fig. 32). Some similar cells extended ventrally and were found just dorsal to the inferior olivary complex and along the exit of the twelfth nerve (Fig. 32).

The dorsal motor nucleus moved dorsomedially further caudal to enter the central gray as a dense ball ventromedial to the nucleus of the solitary tract (Figs. 32-34). A diffuse group of lighter stained cells still bridged it and the nucleus ambiguus (Fig. 33), and scattered cells of this accessory nucleus spread into the nucleus of the solitary tract near the tract. Some similar cells were even found dorsal to the nucleus of the solitary tract among the fibers of the descending root of the vestibular nerve in the inferior vestibular nucleus (Figs. 34, 44).

The hypoglossal nucleus formed a compact group just dorsolateral to the medial longitudinal fasciculus, although scattered accessory cells were still present ventrally and along the twelfth nerve (Figs. 34-38, 44). The nucleus ambiguus became quite diffuse caudally, ending at the level of the commissural nucleus of the solitary
FIG. 37. The hypoglossal nucleus (12), dorsal motor nucleus of the vagus (DMV), and the nucleus ambiguus (a) continue caudally past the commissural nucleus of the solitary tract.

FIG. 38. The caudal extensions of the hypoglossal nucleus (12) and the dorsal motor nucleus of the vagus (DMV) extend into the spinal cord, lateral to the spinal canal (SC).
FIG. 39. Motoneurons are found first at the medial edge of the ventral horn, while the caudal extension of the hypoglossal nucleus (12) continues lateral to the spinal canal.

FIG. 40. Within the spinal cord, ChAT-positive neurons are found within the ventral horn (VH), just lateral to the spinal canal and scattered (arrowheads) within the dorsal horn (DH).
FIG. 41. These micrographs illustrate the ChAT-immunoreactive neurons of the caudal cholinergic column in the pedunculopontine nucleus (a), the laterodorsal tegmental nucleus (b) and the outlying cells found dorsal to the dorsal nucleus of the lateral lemniscus (ltd), beneath the caudal inferior colliculus (c). Cells in the separate cell group in the parabrachial nuclei surrounding the caudal brachium conjunctivum (BC) are shown in (d) at the same magnification for comparison. Scale bar indicates 100 μm for all figures.
FIG. 42. (a) This micrograph illustrates some of the small ChAT-positive cells associated with the motor nuclei of the caudal pons and medulla. These small cells are scattered just lateral to the abducens nucleus which lies beneath the genu of the facial nerve (7g). The stained axons of the sixth nerve (6n) can also be seen. (b) This is a higher magnification of some of these smaller ChAT-positive cells next to the abducens nucleus. (c) This is an example of one of the large multipolar ChAT-immunoreactive cells found scattered in the magnocellular tegmental field at the level of the facial nucleus (see Fig. 30). Scale bars indicate 100 μm for (a), and 50 μm for (b,c).

FACING PAGE

FIG. 43. The ChAT-immunoreactive neurons (arrows) associated with the superior olivary complex beneath the accessory facial nucleus (7a) are illustrated (a). The large periolivary cells lying lateral to the exit of the sixth nerve (6n) and medial to the medial superior olive (mso) are shown in (b), while the small cells in the hilus of the lateral superior olive (lsb) are shown in (c). Scale bars indicate 250 μm in (a) and 50 μm in (b,c).
FIG. 44. At the level of the hypoglossal nucleus (12) positive cells appear to be scattered out ventrolaterally (large arrows) from the dorsal motor nucleus of the vagus (dmv), beneath the solitary tract (TS). Additional small ChAT-positive cells (small arrows) are found among the fibers of the descending root of the vestibular nerve in the inferior vestibular nucleus. These are illustrated at higher magnification in (a). Scale bars indicate 25 μm for (a) and 100 μm for (b).
FIG. 45. The distribution of ChAT-positive cells present in the lumbar spinal cord is illustrated in (a). Small, weakly stained cells are present in the dorsal horn (b) and larger cells are found adjacent to the spinal canal (c), in addition to the large multipolar cells of the ventral horn (d). Scale bars indicate 250 μm for (a) and 50 μm for (b,c,d).
tract. The dorsal motor nucleus of the vagus was quite small at this level, and lay dorsal to the hypoglossal nucleus (Fig. 37). Also, the accessory cells that were present dorsally in the nucleus of the solitary tract and the inferior vestibular nucleus had disappeared at this level.

At more caudal levels, ChAT-positive cells spread out ventrolaterally from the hypoglossal nucleus and appeared to be continuous with ventral horn cells and the cells found around the central canal and in the dorsal horn of the cervical cord (Figs. 28-40).

Spinal Cord

Sections from the lumbar and cervical cord were examined, and the patterns of ChAT staining observed at these levels were similar. In the ventral horn both giant, weakly stained cells, and medium-sized, strongly stained cells were present. Both cell types also appeared to have large ChAT-positive puncta on their surface. Another group of medium-sized, strongly staining cells was present just lateral to the central canal. These, like those in the ventral horn, were often multipolar. A small population of ChAT-positive cells was also observed in the dorsal horn. These were small, weakly stained and mostly bipolar in shape. They were most commonly observed in laminae V and VI, and were more common in the cervical than in the lumbar cord (Fig. 45).

DISCUSSION

The distribution of ChAT-immunoreactive cells reported here appears to be more extensive than that which has been previously described in rodent [3, 16, 21, 32, 36, 42, 47, 48, 54, 72, 82, 94, 95] and primate [55, 60, 73, 74] brains. The present observations confirm some, but not all of the original findings of Kimura et al. [43] in their study of the feline brain using a rabbit antiserum raised against human ChAT. In addition, many of the ChAT-positive cell groups which were discovered in the present study were not noted in previous reports. In the following discussion, we shall therefore compare our observations with those previously reported in the cat using immunohistochemistry and AChE histochemistry. Similarities and differences between what we have observed and what has been noted in other species will also be described.

The Cortex

No ChAT-immunoreactive neurons were detected in the neocortex or hippocampus of the cat. This is in agreement with the original report of Kimura et al. [43] and contrasts with the situation in the rat, where various groups have reported the presence of cortical ChAT-immunoreactive cells [20, 23, 32, 33, 36, 49]. To date, similar cells have not been seen in primate brain [55, 73, 74]. This cortical population of ChAT-positive neurons may therefore represent a peculiar feature of the rodent brain, and in the cat and primate, all cortical cholinergic innervation may be of subcortical origin.

The Striatum

The ChAT-immunoreactive neurons present in the feline caudate nucleus, putamen and nucleus accumbens appear to correspond to the large aspiny interneurons described in detail in various species [3, 9, 16, 26, 32, 36, 42, 43, 45, 54, 55, 67, 68, 76]. The distribution of ChAT-immunoreactive neuropil observed in the present study appears similar to that recently described by Graybiel et al. [29].

The Rostral Column

As noted previously in the cat [43, 94], and in agreement with what is observed in other species [3, 16, 21, 32, 36, 42, 46, 48, 53-55, 72-74, 82, 94, 95], the major ChAT-immunoreactive cell group in the telencephalon is the magnocellular basal forebrain complex. However, the topography of the rostral cholinergic column appears to be different in the cat than in the monkey or rodent. In the cat, there appears to be a large dorsal extension of the rostral column into the internal capsule. A similar dorsal extension of the nucleus basalis has been noted in the human brain [60, 71].

In their pharmacohistochemical study of AChE-positive cells in the cat forebrain, Parent and O'Reilly-Fromentin [66] noted the presence of large, very intensely AChE-positive cells lying dorsal to the globus pallidus within the internal capsule (see also Parent et al. [65]). Bear et al. [5] have found, using combined cresyl violet and AChE staining, that virtually all the neurons within the cat internal capsule are intensely AChE-positive. Kimura et al. [43] first noted the presence of large ChAT-positive cells within the feline internal capsule, and suggested that these could either belong to and connect with components of the neostriatum, or more caudally unite with the ChAT-positive cells of the medullary stria of the pallidum or the interstitial cells of the ansa lenticularis. Our observations would be consistent with this latter suggestion.

The Islands of Calleja

The ChAT-immunoreactive cells found capping the islands of Calleja appear to correspond to the supra-insular AChE cells noted by Parent and O'Reilly-Fromentin [66] in their pharmacohistochemical study of the cat forebrain. It is clear that these insular ChAT-positive cells are much smaller than the magnocellular cholinergic neurons of the rostral column. Fallon et al. [24] have described medium and large cells in the islands of Calleja complex of the rat that show strong AChE staining after a DFP challenge. Other groups have also noted AChE- or ChAT-positive cells associated with the islands in the rat [21, 72]. In our own studies of AChE staining in DFP pretreated rats, and in rat sections processed together with the present material for ChAT immunohistochemistry, we too observed similar cells. These appeared to be much larger than the ChAT-positive cells found in the medial cat islets, and were similar in size to the striatal cholinergic cells. In the primate brain, ChAT-positive cells have been noted around or within the islands, however, they have been included together with the striatal cholinergic interneurons [55]. The situation in the primate islets may therefore be similar to that in the rat.

The small ChAT-immunoreactive cells in the cat islands of Calleja, and the larger cells observed in association with the rat and primate islets may have localized projections onto the granule cells of the islets. The neuropil of the islands stains very strongly for AChE in these species [24, 73]. In addition, dense ChAT fiber staining can also be seen covering the granule cells (Fig. 10; see also Mesulam et al. [55]). Thus these cells may be under local cholinergic control.

The Amygdala

In the rat, the major source of cholinergic input to the amygdaloid complex appears to arise in the basal telencepha-
The Hypothalamus

There is evidence that cholinergic mechanisms may regulate hypothalamic function. Parent and Butcher [64] noted that the supraoptic and paraventricular nuclei stained for AChE following a DFP challenge, however, this reaction does not appear to be intense [26]. Instead, recent results indicate that the cholinergic input to the supraoptic nucleus arises in the basal forebrain cholinergic neurons of the rostral column extending from the horizontal limb of the diagonal band and the substantia innominata laterally into the anterior amygdaloid area. In addition, smaller cells, similar in size to those in the adjacent putamen, are present in the longitudinal association bundle of Johnston beneath the putamen and above the central and basolateral nuclei. ChAT-immunoreactive cells are also present in the dorsal anterior amygdaloid area, within the intercallated masses and along the dorsal aspect of the central nucleus [71].

An important observation from the present study was the presence of intrinsic ChAT-immunoreactive neurons within the amygdaloid complex. Some of the magnocellular cholinergic neurons of the rostral column extend from the horizontal limb of the diagonal band and the substantia innominata laterally into the anterior amygdaloid area. In addition, smaller cells, similar in size to those in the adjacent putamen, are present in the longitudinal association bundle of Johnston beneath the putamen and above the central and basolateral nuclei. ChAT-immunoreactive cells were also detected in the stria terminalis, medial and ventral to these cell groups. At more caudal levels some cells are found within the lateral subdivision of the central nucleus. Recently, small ChAT immunoreactive cells have also been detected in the basolateral nucleus of the rat [12].

Parent and O'Reilly-Fromentin [66] have noted some moderately and a few intensely AChE-positive cells in the dorsal anterior amygdaloid area, within the intercallated masses and along the dorsal aspect of the central nucleus. These appear to correspond to the ChAT-immunoreactive cells we have observed. Recently, Saper and Chelimsky [71] have described magnocellular AChE-positive neurons in the human brain surrounding the dorsal aspects of the central and basolateral amygdaloid nuclei.

The Thalamus

In agreement with earlier studies in the cat [43] and other species, no ChAT-immunoreactive cells were detected in the thalamus. Instead, a highly organized pattern of immunoreactive fibers was evident. The distribution of these terminal fields appears similar to that which has been recently noted in the rat brain [83] and in the visual thalamus of the cat [18, 84]. Much of this thalamic cholinergic innervation may arise from the caudal cholinergic cell column [75, 83].

The Superior Colliculus and Parabigeminal Nucleus

Recent immunohistochemical studies with various monoclonal antibodies to ChAT have demonstrated the presence of immunoreactive habenular neurons in the rat [16, 32, 36, 39] and primate [55]. We have now detected a similar cell cluster in the feline medial habenula. In addition, scattered ChAT-positive cells were detected within the lateral habenula in this species. These habenular ChAT-positive neurons appear to provide the major cholinergic innervation to the interpeduncular nucleus, although additional cholinergic input appears to arise from some of the magnocellular neurons of the diagonal band [18, 98]. The staining pattern we have observed within the interpeduncular nucleus appears to be similar to that reported by Kimura et al. [43] and agrees with the topography of the feline habenulo-interpeduncular projection [31].

The Habitual-Interpeduncular System

Recent immunohistochemical studies with various monoclonal antibodies to ChAT have demonstrated the presence of immunoreactive habenular neurons in the rat [16, 32, 36, 39] and primate [55]. We have now detected a similar cell cluster in the feline medial habenula. In addition, scattered ChAT-positive cells were detected within the lateral habenula in this species. These habenular ChAT-positive neurons appear to provide the major cholinergic innervation to the interpeduncular nucleus, although additional cholinergic input appears to arise from some of the magnocellular neurons of the diagonal band [18, 98]. The staining pattern we have observed within the interpeduncular nucleus appears to be similar to that reported by Kimura et al. [43] and agrees with the topography of the feline habenulo-interpeduncular projection [31].

The Superior Colliculus and Parabigeminal Nucleus

Dense ChAT-positive fiber networks were detected in the superficial and intermediate gray layers of the superior colliculus. These appear to correspond to the AChE staining patterns observed by Graybiel [27] in this species. The ChAT and AChE in the superficial gray layer may well arise from the parabigeminal nucleus. Graybiel [28] has shown that this cell group projects to this layer. We have found that essentially all of the neurons in the cat parabigeminal nucleus display ChAT immunoreactivity. This is consistent with recent reports that the reptilian isthmic nucleus, the homologue of the mammalian parabigeminal nucleus, is ChAT-immunoreactive [10, 17] and that lesions of this nucleus decrease tectal ChAT activity [69]. Desan (personal communication cited in [69]) has also found ChAT-immunoreactive cells in the parabigeminal nucleus of the cat, while Mufson et al. [58] have retrogradely labelled ChAT-positive cells in this nucleus in the mouse following injections of tracer into the superior colliculus.

The parabigeminal nucleus does not appear to project to the intermediate gray layers of the superior colliculus which contain AChE positive [28] and ChAT-immunoreactive patches. AChE-positive neurons have been observed following DFP in this area in the cat [37] and we have observed some ChAT-immunoreactive cells within the superficial layers of the superior colliculus. Intrinsic ChAT-positive cells have also been recently noted in the goldfish tectum [89]. The tectal ChAT-immunoreactive cells could thus contribute to the fiber patches seen in the cat. A more likely source for the ChAT patches may be the NADPH-diaphorase containing cholinergic neurons of the caudal column [93]. These cells are well known to have ascending projections to the tectum and thalamus [75, 81, 83] and we have observed patches showing neuropil staining for NADPH-diaphorase within the intermediate gray layers of the cat superior colliculus (in preparation).
The Oculomotor Nucleus

The oculomotor complex extended rostrally above the mamillary bodies. These anterior cells were medium size and weakly stained. They appear to correspond to ChAT-positive preganglionic parasympathetic cells recently described in the cat by Brezina et al. [11], and included in the Edinger-Westphal nucleus by Taber [87].

The Red Nucleus

The magnocellular neurons of the feline red nucleus were found to be ChAT-immunoreactive in the original study by Kimura et al. [43]. However, such cells did not display ChAT immunoreactivity in the present study. ChAT activity is quite low in the cat red nucleus [61]. Immunohistochemical studies in rodent [3, 14, 32, 54, 72] and primate [55, 73, 74] have also noted ChAT-immunoreactive cells in the red nucleus. Thus the neurons of the red nucleus do not appear to be cholinergic.

The Caudal Column

A column of ChAT-immunoreactive neurons has been reported within the pedunculopontine and laterodorsal tegmental nuclei in the rat [3, 16, 21, 54, 72, 93], cat [41, 43] and primate [55, 73, 74] and has been termed the caudal cholinergic column [72, 73, 93, 94]. This column is slightly more dispersed in the cat than in other species, particularly within the mesencephalic reticular formation. In both the rat [3, 16, 21, 54, 72] and primate [55, 72, 74], the caudal cholinergic column forms a discrete and compact cell cluster within the pars compacta of the pedunculopontine nucleus. In the cat, these cells do not coalesce to form a distinct cluster, but rather are widely dispersed throughout both the pars compacta and pars dissipata of the pedunculopontine nucleus of Taber [87].

A band of ChAT immunoreactive neurons was observed capping the dorsal nucleus of the lateral lemniscus. The cells here were similar in both size and staining pattern to those of the laterodorsal tegmental and pedunculopontine nuclei. Neurons identical in distribution and morphology were seen in sections stained for NADPH-diaphorase activity, which in the cat as in the rat and primate, selectively stains the caudal cholinergic column [93] (Reiner and Vincent, in preparation). The similarities between these ChAT-positive neurons and those of the caudal cholinergic column in terms of size, immunostaining intensity, and NADPH-diaphorase activity suggest that these neurons may represent a displaced component of the caudal column.

The Parabrachial Nuclei

At the caudal end of the brachium conjunctivum, small, lightly stained ChAT-positive neurons were observed both medial and lateral to the brachium. These neurons were easily distinguished from the ChAT-positive neurons of the caudal cholinergic column by both their size and staining intensity. Furthermore, in contrast to the strongly stained caudal cholinergic column, these parabrachial cells did not display NADPH-diaphorase activity (Reiner and Vincent, in preparation). Thus these ChAT-immunoreactive neurons may represent a group of neurons distinct from those of the caudal cholinergic column. Kimura et al. [43] described ChAT-positive and 'chinoceptive' neurons surrounding the pontine portion of the brachium conjunctivum. No homologous group of ChAT-immunoreactive neurons has been described in either the rodent or primate pontine tegmentum.

A few small ChAT-immunoreactive neurons were found in the inferior vestibular nucleus. These may be similar to the small cells extending out from the dorsal motor nucleus of the vagus into the nucleus of the solitary tract and adjacent regions. This contrasts with the report of Kimura et al. [43] who noted large or giant multipolar ChAT-positive cells in Deiters' nucleus. Such cells have not been observed in other species or by other groups.

The Superior Olive Complex

In their original study of the cat brain using rabbit antiserum raised against human ChAT, Kimura et al. [43] reported that the majority of cells in the lateral superior olive were immunoreactive, and additional cells were present in the medial nucleus, the preolivary nucleus and the nucleus of the trapezoid body. We could not confirm these observations. Instead, ChAT-immunoreactive neurons were only observed within the preolivary nucleus and the periolivary portions of the superior olive complex.

The ChAT-immunoreactive neurons found in the superior olive complex appear similar to those recently described by Altschuler et al. [2] in the guinea pig. Similar cells have been described in the cat with ACHE histochemistry, and they appear to give rise to both the medial and lateral olivocochlear efferents [96]. In the medial component in both of these species, enkephalin immunoreactivity appears to coexist with ChAT [2,25].

Motoneurons and Accessory Nuclei of the Pons and Medulla

Recent retrograde tracing studies have localized the neurons forming the superior salivatory nucleus in the cat [77]. These cells, which give rise to the parasympathetic fibers to the submandibular and sublingual glands, are found in the dorsal portion of the rostral medullary reticular formation [77]. These appear to correspond to some of the CHAT-immunoreactive neurons observed in the present study dorsolateral to the accessory facial nucleus at the caudal end of the superior olivary complex, beneath the genu of the facial nerve. These multipolar neurons were similar in size within the accessory facial nucleus and the facial nucleus itself. Some of the ChAT-positive cells in this region may also innervate the stapedius muscle in the cat [50,79].

The rostral pole of this cell group appears to be continuous with similar small ChAT-positive cells lying along the ascending and descending branches of the facial nerve, which may represent the source of innervation to the posterior belly of the digastric muscle [52,86]. These cells in turn appear to merge rostrally with a similar group of small ChAT-immunoreactive neurons found in a cluster ventral to the trigeminal motor nucleus, probably representing the motoneuron pool innervating the tensor tympani muscle [40, 79, 86]. Some of the ChAT-positive cells in this region also appear to innervate the anterior belly of the digastric muscle [57]. Thus in the cat [35, 52, 57, 86], as in the rat [85], the accessory nuclei of the trigeminal, abducens and facial nerves appear to form a continuous column of cholinergic cells.

Kimura et al. [43] reported large-to-giant ChAT-containing cells in the segmental fields of the medullary reticular formation. In agreement with the present observations, these cells were seen most often dorsal to the inferior olivary complex and medial to the lateral tegmental field. However, Kimura et al. [43] also described cholinergic-chinoceptive cells in the lateral reticular nucleus. In the
present study, no ChAT-positive cells were detected within this nucleus.

Above the inferior olive complex and the lateral reticular nucleus, the retrofacial nucleus appears to form a bridge between the caudal end of the facial nucleus and the nucleus ambiguous. This agrees with recent retrograde tracing studies [90, 91] and with the suggestion of Taber [87], that the retrofacial nucleus of the cat is a ventral continuation of the rostral nucleus ambiguous.

The small, multipolar ChAT-positive cells observed dorsal to the nucleus ambiguous beneath the dorsal motor nucleus of the vagus appear to correspond to the inferior salivatory nucleus. Neurons in this area have been shown to provide parasympathetic input to the parotid gland in the cat [78].

The nucleus ambiguous extends caudally from the inferior olivary complex to the pyramidal decussation. At this level, the ChAT-positive cells spread laterally forming the nucleus retroambiguus, which extends into the upper cervical cord [38]. The dorsal motor nucleus of the vagus also extends to the upper cervical cord, where it appears to be continuous with the nucleus dorsomedialis and the spinal nucleus of the accessory nerve [38].

The Spinal Cord

The ChAT-positive neurons of the rat spinal cord have been elegantly described by Barber et al. [4]. Our observations in the cat are in good agreement with this report. Thus cells were seen not only in the ventral horn, but sounding this nucleus. Neurons in this area have been shown to provide parasympathetic input to the parotid gland in the cat [78].

The nucleus ambiguous extends caudally from the inferior olivary complex to the pyramidal decussation. At this level, the ChAT-positive cells spread laterally forming the nucleus retroambiguus, which extends into the upper cervical cord [38]. The dorsal motor nucleus of the vagus also extends to the upper cervical cord, where it appears to be continuous with the nucleus dorsomedialis and the spinal nucleus of the accessory nerve [38].

ACKNOWLEDGEMENTS

The authors thank Dr. Felix Eckenstein for generously providing the monoclonal antibody to ChAT which made this study possible. This work was supported by grants from the Medical Research Council of Canada and the British Columbia Health Care Research Foundation. S.R.V. is a Scholar and P.B.R. a Fellow of the M.R.C.

REFERENCES


