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Distribution of cholinergic cells in guinea pig brainstem

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Abstract

We used an antibody to choline acetyltransferase (ChAT) to label cholinergic cells in guinea pig brainstem. ChAT-immunoreactive (ChAT-IR) cells comprise several prominent groups, including the pedunclopontine tegmental nucleus, laterodorsal tegmental nucleus, and parabigeminal nucleus, as well as the cranial nerve somatic motor and parasympathetic nuclei. Additional concentrations are present in the parabrachial nuclei and superior colliculus.

Among auditory nuclei, the majority of ChAT-IR cells are in the superior olive, particularly in and around the lateral superior olive, the ventral nucleus of the trapezoid body and the superior paraolivary nucleus. A discrete group of ChAT-IR cells is located in the sagulum, and additional cells are scattered in the nucleus of the brachium of the inferior colliculus. A group of ChAT-IR cells lies dorsal to the dorsal nucleus of the lateral lemniscus. A few ChAT-IR cells are found in the cochlear nucleus and the ventral nucleus of the lateral lemniscus.

The distribution of cholinergic cells in guinea pigs is largely similar to that of other species; differences occur mainly in cell groups that have few ChAT-IR cells. The results provide a basis for further studies to characterize the connections of these cholinergic groups.

Keywords

Acetylcholine; pedunclopontine tegmental nucleus; laterodorsal tegmental nucleus; ChAT

Acetylcholine acts on many brainstem structures. Its effects are varied, and related to its origins. Three nuclei, the pedunclopontine tegmental nucleus (PPT), laterodorsal tegmental nucleus (LDT), and parabigeminal nucleus (PBG), are traditionally identified as the main sources of cholinergic projections to other brainstem nuclei and to the thalamus. The PBG projects to the superior colliculus and visual thalamus (e.g. Jiang et al., 1996; Wilson et al., 1995). The PPT and LDT have widespread projections, the best known of which are those to the dorsal thalamus (e.g., Woolf and Butcher, 1986; Steriade et al., 1988; Oakman et al., 1999).

Recent studies have begun to reveal the role of acetylcholine in sensory processing in brainstem nuclei (e.g., Fernández de Sevilla et al., 2006; Simons et al., 2006; Uteshev and Smith,

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2006). In the auditory system, processing of sounds is affected by cholinergic projections from the superior olive to the cochlea and cochlear nucleus (Guinan, 1996; Mulders et al., 2003). A few studies have identified cholinergic effects in other brainstem auditory nuclei, such as the inferior colliculus (e.g., Habbicht and Vater, 1996), but the underlying circuitry is unknown. An important step in characterizing these circuits is to identify the source(s) of cholinergic input. This in turn requires a detailed description of cholinergic nuclei in the species under study.

The purpose of the present study is to describe the distribution of cholinergic cells in the brainstem of guinea pigs, a species widely used in auditory research. We identify cholinergic cells with antibodies to choline acetyltransferase (ChAT), the synthetic enzyme for acetylcholine and a specific marker of cholinergic cells (Levey and Wainer, 1982; Armstrong et al., 1983; German et al. 1985; Maley et al., 1988). This approach has been used in a variety of species and a general mammalian pattern has emerged (Woolf, 1991). Despite the overall similarities, there are differences that, at the least, must be attended when analyzing cholinergic circuitry. The present report provides a basis for future studies of cholinergic circuitry in guinea pig brainstem.

Experimental Procedures

Animals

All procedures were performed in accordance with the Institutional Animal Care and Use Committee and NIH guidelines. Seventeen albino (Charles River; Wilmington, MA) and ten pigmented (Elm Hill; Chelmsford, MA) adult guinea pigs weighing 296-602 grams were used. Efforts were made to minimize suffering and the number of animals used.

Perfusion

Each animal was given an overdose of sodium pentobarbital (440mg/kg ip). After cessation of breathing and loss of withdrawal reflex, the animal was perfused through the left ventricle with Tyrode's solution followed by 700 ml fixative (4% paraformaldehyde, with or without 0.2% picric acid and/or 0.1 – 0.5% glutaraldehyde) in 0.1M phosphate buffer, pH 7.4. For brains that were to be frozen for sectioning, the fixative was divided into two parts with 10% sucrose added to the second volume. The brain was removed and stored in fixative (with 30% sucrose for frozen sectioning) for 4-24 hours at 4°C.

Immunohistochemistry

After removal of cerebral cortex and cerebellum, the brainstem was cut on a Vibratome or frozen and cut on a sliding microtome into 40-50 µm sections in the transverse or sagittal plane. Sections were collected in six series. One series was mounted on slides and stained with thionin for cytoarchitectonic identification of nuclei. Two to four additional series were stained with antibodies against choline acetyltransferase (ChAT) to identify cholinergic cells. We tried several ChAT antibodies, including Chemicon AB144P, Chemicon AB5042, Chemicon AB1582, ImmunoStar 20093, and the ChAT antibody described in Schemann et al., (1993)¹. One antibody, Chemicon AB144P, gave the most intense staining and we chose it to complete these experiments.

Sections were treated in 0.4% Triton X-100 in PBS (0.9% NaCl in 0.01M phosphate buffer) for 30 minutes (all steps at room temperature unless noted). After three five-minute washes in PBS, the tissue was treated with 20% normal rabbit serum (NRS) with 0.1% Triton X-100 in PBS for 1 hour. Goat anti-ChAT polyclonal antibody (Chemicon AB 144P) was applied with

¹Schemann ChAT was a gift from Dr. Raymond Papka.

0.1% Triton X-100 and 1% NRS in PBS for 24-72 hours at 4°C. The concentration of primary antibody varied from 1:100 to 1:400. Following three five-minute washes in PBS, the tissue was incubated for one hour with a secondary antibody, (biotinylated rabbit anti-goat IgG, BA-5000, Vector Lab), at a 1:100 concentration with 1% NRS in PBS. Following three five-minute washes in PBS, the sections were incubated in avidin-biotin-peroxidase (Vectastain ABC kit, Vector), then rinsed and stained with diaminobenzidine (DAB) with nickel enhancement (Adams, 1981). Reaction times were determined empirically. The sections were mounted on gelatin-coated slides and allowed to dry. The sections were dehydrated, cleared, and then coverslipped with DPX (Aldrich Chemical Co., St. Louis, MO).

Controls

Controls were carried out with the Chemicon AB144P antibody. Staining was eliminated with preadsorption of the antibody with ChAT (Chemicon AG220) (Fig. 1A, B) or with omission of primary or secondary antibodies (Fig. 1A, C, D). Western blot analysis of brainstem tissue from two guinea pigs revealed a single band of staining (Fig. 1E).

Data Analysis

Labeled cells were plotted throughout the brainstem using a Zeiss Axioplan II microscope and Neurolucida reconstruction system (MBF Bioscience, Williston, VT). Adjacent thionin-stained sections were used to identify nuclei. We used a rat brain atlas (Paxinos and Watson, 1998) to identify brainstem regions. In addition, we used papers that describe specific nuclei in guinea pig (e.g., Hackney et al., 1990; Schofield and Cant, 1991, 1997; Leonard et al., 1995). Adobe Illustrator was used to illustrate the plotted sections. Photomicrographs were obtained using a Zeiss Axioskop microscope and Magnafire digital camera (Optronics). Adobe Photoshop was used to add scale bars and labels, size and crop images, convert to grayscale, erase background outside tissue sections, and adjust brightness and contrast.

Results

Staining was similar regardless of fixation protocol or animal pigmentation (albino or pigmented). The general pattern of staining was similar for all the ChAT antibodies we tried. In general, the antibodies stained cell bodies and often dendrites. Large cholinergic axon bundles such as the facial nerve were usually stained, but axons in other areas were rarely stained. Figures 2 (transverse plots) and 3 (parasagittal plots) show the distribution of ChAT-immunoreactive (ChAT-IR) cells. Clusters of stained cells are numerous, while additional ChAT-IR cells are scattered sparsely across many nuclei. We first describe the traditionally named cholinergic groups and the cranial nerve nuclei. Next we describe other discrete groups of ChAT-IR cells, including ChAT-IR cells in auditory nuclei. Finally we discuss areas with more diffuse distributions of ChAT-IR cells.

Traditionally-named cholinergic nuclei

ChAT-IR cells are prominent in the PPT and LDT (Fig. 4) and PBG (Fig. 5). The cells are generally smaller than those in the cranial nerve motor nuclei but larger than almost all other ChAT-IR cells in the brainstem.

The PPT extends through the midbrain from caudo-dorsal tegmentum (surrounding the superior cerebellar peduncle [scp]) to the rostro-ventral tegmentum bordering the substantia nigra (Fig. 2 sections 15-23; Fig. 3 sections 3-8). The PPT can be divided into “compact” and “dissipated” regions (Leonard et al., 1995). The compact region contains tightly clustered ChAT-IR cells and is centered dorsal to the scp (Fig. 4D, F). The larger part of the nucleus is the dissipated region, which contains less tightly clustered cells, and extends ventral and medial to the scp (Fig. 4D, G).

The LDT is located largely within the periaqueductal gray except rostrally, where it extends slightly beyond the border of the central gray (ventral LDT; Leonard, 1995) (Fig. 2 sections 14-19; Fig. 3 sections 8-10; Fig. 4B).

Cranial Nerve Motor Nuclei

The cranial nerve motor nuclei have densely packed ChAT-IR cells that are generally larger than most other ChAT-IR cells in the brainstem (Fig. 6).

Other discrete clusters of ChAT-IR cells

Other discrete groups of ChAT-IR cells are found near the scp. The medial and lateral parabrachial nuclei have a dense collection of darkly stained ChAT-IR cells (Fig. 2, sections 12-14; Fig. 3, section 7; Fig. 4D). Another substantial collection of ChAT-IR cells is found in the superficial gray layer of the superior colliculus (Fig. 7).

Auditory Nuclei

Within the auditory system, there are several structures with ChAT-IR cells (Fig. 8). In the superior olivary complex, ChAT-IR cells are present in and around the lateral superior olive, and in most periolivary nuclei, especially the ventral nucleus of the trapezoid body and the superior paraolivary nucleus (Fig. 8A-F). In the lateral lemniscus, labeled cells are scattered in the ventral nucleus of the lateral lemniscus (Fig. 8E), particularly in the ventral division. Labeled cells are not present in the dorsal nucleus of the lateral lemniscus (DLL), but are found medial, lateral and dorsal to it (Fig. 8G). The medial cells are part of PPT. The cluster lateral to the DLL is within the sagulum (Fig. 8H). Dorsal to the DLL, ChAT-IR cells form a “bridge” that stretches from the sagulum to the PPT (Fig. 8G, arrows). The ChAT-IR cells in the bridge are smaller than those in PPT or sagulum. Scattered ChAT-IR cells are present in the nucleus of the brachium of the IC (e.g. Fig. 2, section 21; Fig 3, section 2). A few scattered labeled cells are present in the deep layer of dorsal cochlear nucleus, on or near the medial margin of the ventral cochlear nucleus and in the granule cell area. In a few cases, the IC contained a few labeled cells.

Cranial Nerve Sensory Nuclei

Scattered ChAT-IR cells are present in the spinal nucleus of the trigeminal nerve (Fig. 2, section 5; Fig. 3, sections 5, 6). Scattered ChAT-IR cells are also present in the medial and superior vestibular nuclei and, in smaller numbers, in the spinal vestibular nucleus (Fig. 2, sections 1-10; Fig. 3, sections 3-10). No ChAT-IR cells are seen in the lateral vestibular nucleus.

The nucleus of the solitary tract contains ChAT-IR cells (Fig. 2, sections 3-5; Fig. 3, sections 8-10). These cells are adjacent to cells in the dorsal motor nucleus of the vagus; the latter cells are larger and more densely packed than those in nucleus of the solitary tract (Ruggiero et al., 1990).

Reticular and tegmental areas

There are scattered ChAT-IR cells in many reticular formation nuclei and some other tegmental areas. Lateral to the genu of the seventh nerve is a small, distinct cluster of ChAT-IR cells (“EVe”, Fig. 2, sections 9, 10); these are probably the vestibular “efferents”, which project to the vestibular component of the inner ear (Strutz, 1982). The cuneiform nucleus borders the PPT (Fig. 2, sections 16-17); the ChAT-IR cells are similar in the two nuclei, and it is difficult to determine whether the ones within the cuneiform nucleus form a separate group. ChAT-IR cells are present in the nucleus prepositus hypoglossi. The dorsolateral periaqueductal gray and external cuneate nucleus each have a clustering of ChAT-IR cells that is less dense than the motor cranial nerve nuclei and the named cholinergic nuclei.

ChAT-IR cells are scattered very diffusely in the gigantocellular nucleus, dorsal tegmental nucleus, nucleus X, pontine reticular nucleus, lateral paragigantocellular nucleus, intermediate reticular nucleus and raphe nuclei. The parvicellular reticular nucleus, reticulotegmental nucleus of the pons, and ventral tegmental nucleus appear devoid of ChAT-IR cells.

Discussion

The present study describes the distribution of ChAT-IR cells in guinea pig brainstem. Previous studies in guinea pigs are limited to a short description by Maley et al., (1988), who described ChAT-IR cells in the cranial nerve motor nuclei, parabrachial region, superior olive and lateral reticular nucleus. Our results confirm the earlier study and extend it with details on many additional brainstem regions. Before further comparing our results with those of previous studies, we consider several technical issues associated with immunohistochemical identification of presumptive cholinergic cells.

Technical Issues

ChAT is considered a specific marker for cholinergic cells (Levey and Wainer, 1982; Armstrong et al., 1983; German et al. 1985; Maley et al., 1988). We tried several ChAT antibodies. While the staining varied in intensity, the locations of stained cells were similar, supporting the conclusion that the stained cells were cholinergic. We continued our study with the antibody that gave the most intense staining in preliminary studies. This antibody successfully stains cholinergic axons in guinea pig peripheral nervous system (Hoover et al., 2004). Our controls validate the use of this antibody in guinea pig central nervous system. Our results are consistent with those described previously in guinea pigs (Maley, et al., 1988, who used a different antibody). In addition, we observed staining in “expected” areas such as the cranial nerve motor and parasympathetic nuclei and in the major cholinergic nuclei of the brainstem (e.g., PPT) and forebrain (e.g., striatum; data not shown). We conclude that the staining shown here is highly likely to be associated with cholinergic cells.

Comparisons with other studies

Major cholinergic nuclei—ChAT-IR cells are routinely found in the cranial nerve motor and parasympathetic nuclei. Two additional nuclei that are regularly identified as cholinergic are the PPT and LDT. These nuclei are conserved across species. They are best known as the source of cholinergic input to the thalamus (e.g. Woolf & Butcher, 1986; Steriade et al., 1988; Oakman et al., 1999). We have shown in preliminary studies that ChAT-IR cells in the PPT and LDT project to the thalamus in guinea pigs (Motts and Schofield, 2006).

The parabigeminal nucleus (PBG) is another nucleus that consistently contains ChAT-IR cells across species. It provides cholinergic input to the superior colliculus and visual parts of the thalamus (e.g. Jiang et al., 1996; Wilson et al., 1995). The present results suggest that the major cholinergic nuclei – PPT, LDT and PBG and the motor and parasympathetic nuclei – are similar in guinea pigs and other species.

Other cholinergic nuclei—Many other brainstem areas contain ChAT-IR cells. Table 1 summarizes results across species. The final section discusses some of the apparent variation. ChAT-IR cells are found in a number of auditory nuclei. Most numerous are the cells in the superior olivary complex. Many of these cells are part of the olivocochlear system (Altschuler et al., 1984; Robertson et al., 1987; Tokunaga, 1988; Warr, 1992; Warr et al., 2002) others may project to the cochlear nucleus (Sherriff and Henderson, 1994). The sagulum and, to a lesser extent, the nucleus of the brachium of the inferior colliculus, contain ChAT-IR cells. Both nuclei project to the auditory thalamus, and the sagulum also projects to the inferior colliculus. The contributions of cholinergic cells to these projections is unknown. In other species, the

sagulum comprises two or more subdivisions. The ChAT-IR cells may be restricted to a portion of the nucleus, but this awaits identification of subdivisions in guinea pigs. The ChAT-IR cells in the ventral nucleus of the lateral lemniscus may project to the cochlea, as a small number of “olivocochlear” cells have been described in this nucleus in guinea pigs (Robertson et al., 1987). In addition, ChAT-IR cells were observed in areas near the dorsal nucleus of the lateral lemniscus and, in smaller numbers, in the cochlear nucleus. The projections of the ChAT-IR cells in these areas are unknown. No ChAT-IR cells were observed in the medial superior olivary nucleus or dorsal and intermediate nuclei of the lateral lemniscus.

Cholinergic cells are common in many sensory nuclei (Table 1). The superior colliculus (SC) is also noteworthy. In guinea pigs, intensely-stained ChAT-IR cells are very numerous in the superficial gray layer of the SC. Scattered ChAT-IR cells have been noted in the superficial gray layer of the cat SC (Vincent and Reiner, 1987). Similar cells are both ChAT-IR and AChE-positive in guinea pigs (present data, Schnurr et al., 1992), but are apparently absent in other species. The significance of this difference is unclear.

Finally, ChAT-IR cells are scattered among many nuclei of the reticular formation. Some of the variation apparent in Table 1 may be due to differences in terminology or identification of the nuclei. This is particularly likely with reticular formation nuclei, which are often poorly differentiated, and also with nuclei such as the medial and lateral parabrachial nuclei. The latter nuclei are prominently stained in guinea pigs and apparently in some other species (Table 1). However, they are contiguous with the PPT, and the term parabrachial (or “peribrachial”) is sometimes applied to these groups in different ways.

In conclusion, the distribution of ChAT-IR cells in guinea pig brainstem appears largely similar to that described in other species. There are several prominent cell groups, as well as scattered cells across many of the brainstem nuclei. The scattered nature will complicate efforts to characterize the cholinergic circuitry. The present results should complement such efforts in guinea pigs by providing a detailed description of the locations of presumptive cholinergic cells.

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List of Abbreviation

- 3
oculomotor nucleus
- 4
trochlear nucleus

4n	4 th nerve
5	motor nucleus of 5 th nerve
6	abducens nucleus
7	facial nucleus
7n	facial nerve
8vn	vestibular root of 8 th nerve
10	dorsal motor nucleus of the vagus
Amb	nucleus ambiguus
APT	anterior pretectal nucleus
Aq	aqueduct
ATg	anterior tegmental nucleus
bic	brachium of the inferior colliculus
Cb	cerebellum
CG	central gray
ChAT	choline acetyltransferase
ChAT-IR	choline acetyltransferase immunoreactive
cic	commissure of inferior colliculus
CnF	cuneiform nucleus
cnr	cochlear nerve root
cp	cerebral peduncle

Cu	cuneate nucleus
cu	cuneate fasciculus
DAB	diaminobenzidine
das	dorsal acoustic stria
DCN	dorsal cochlear nucleus
DLL	dorsal nucleus of lateral lemniscus
DpG/Wh	deep gray/white layers of superior colliculus
DPO	dorsal periolivary nucleus
DTg	dorsal tegmental nucleus
ECu	external cuneate nucleus
EVe	vestibular efferents
fr	fasciculus retroflexus
g7	genu of facial nerve
Gi	gigantocellular reticular nucleus
grca	granule cell area
IC	inferior colliculus
icp	inferior cerebellar peduncle
ILL	intermediate nucleus of lateral lemniscus
InG/W	intermediate gray/white layers of superior colliculus
IO	inferior olive

IP	interpeduncular nucleus
ip	intraperitoneal
IRt	intermediate reticular nucleus
IS	inferior salivatory nucleus
LD	laterodorsal thalamic nucleus
LDT	laterodorsal tegmental nucleus
lfp	longitudinal fasciculus of pons
LG	lateral geniculate nucleus
ll	lateral lemniscus
LPB	lateral parabrachial nucleus
LPGi	lateral paragigantocellular reticular nucleus
LRt	lateral reticular nucleus
LSO	lateral superior olive
LTB	lateral nucleus of trapezoid body
LVe	lateral vestibular nucleus
m5	motor root of 5 th nerve
mcp	middle cerebellar peduncle
Me5	mesencephalic nucleus of 5 th nerve
me5	mesencephalic tract of 5 th nerve
MG	medial geniculate body

ml	medial lemniscus
mlf	medial longitudinal fasciculus
MnR	median raphe nucleus
MPB	medial parabrachial nucleus
MSO	medial superior olive
MTB	medial nucleus of trapezoid body
MVe	medial vestibular nucleus
MVeMC	medial vestibular nucleus, magnocellular
MVePC	medial vestibular nucleus, parvicellular
NRS	normal rabbit serum
op	optic nerve layer of superior colliculus
PAG	periaqueductal gray
PaR	pararubral nucleus
PB	parabrachial nucleus
PBG	parabigeminal nucleus
PBS	phosphate buffered saline
pc	posterior commissure
PCRt	parvicellular reticular nucleus
PMnR	paramedian raphe nucleus
Pn	pontine nuclei

PnC	pontine reticular nucleus, caudal
PnO	pontine reticular nucleus, oral
PnR	pontine raphe nucleus
PPT	pedunculopontine tegmental nucleus
PPTc	pedunculopontine tegmental nucleus, compact region
PPTd	pedunculopontine tegmental nucleus, dissipated region
Pr	prepositus nucleus
Pr5	principle sensory 5 nucleus
py	pyramidal tract
R	red nucleus
Rbd	rhabdoid nucleus
RMg	raphe magnus nucleus
RRF	retrotrubral field
RtTg	reticulotegmental nucleus of the pons
s5	sensory root of 5 th nerve
Sag	sagulum
SC	superior colliculus
scp	superior cerebellar peduncle
SN	substantia nigra
Sol	nucleus of the solitary tract

sol	solitary tract
sp5	spinal trigeminal tract
Sp5	spinal trigeminal nucleus
SPN	superior paraolivary nucleus
SPTg	subpeduncular tegmental nucleus
SpVe	spinal vestibular nucleus
SuG	superficial gray layer of superior colliculus
SuVe	superior vestibular nucleus
tfp	transverse fibers of the pons
tz	trapezoid body
VCN	ventral cochlear nucleus
VLL	ventral nucleus of lateral lemniscus
VTa	ventral tegmental area
VTB	ventral nucleus of trapezoid body
VTg	ventral tegmental nucleus
X	nucleus X
xscp	decussation of superior cerebellar peduncle
ZI	zona incerta

Figure 1.

Immunohistochemistry controls. A) ChAT-IR staining in the pedunclopontine tegmental (PPT) nucleus. Absence of staining is demonstrated with B) preabsorption, C) primary antibody omission, and D) secondary antibody omission controls in nearby sections from the same animal. All images are from the PPT. Scale bar = 10 μ m for A-D. E) Western blot analysis of brain tissue with ChAT antibody (Chemicon AB144P). Left lane: brainstem. Right lane: thalamus and forebrain. In each lane a single band is present at the expected 68-70 kDa molecular weight.

Figure 2.

Plots of ChAT-IR cells in transverse sections. Each dot represents one ChAT-IR cell. Sections are 50 μ m thick. Each section is numbered from caudal to rostral and every whole number increase in section number represents a spacing of 300 μ m between sections. Not every section is shown.

Figure 3.
Plots of ChAT-IR cells in parasagittal sections. Conventions as in Figure 2. Cross hatching (sections 3 & 4) indicate tissue damage.

Figure 4.

Photomicrographs showing ChAT-IR cells in traditionally-named cholinergic nuclei. A) pedunclopontine (PPT) and laterodorsal tegmental (LDT) nuclei (compare with Fig. 2, approx. section 16). D - Dorsal; L - lateral. B) Parasagittal section showing LDT and its ventral subdivision (LDTv), located ventral to the border of the periaqueductal gray (dotted line). Dorsal is up and rostral to the right in this and subsequent panels. C) LDT cells. D) Parasagittal section showing the relationship between the parabrachial nuclei (PB), the compact part of PPT (PPTc) and the dissipated part of PPT (PPTd). E) PPTd cells. F) compact PPT. G) dissipated PPT. Scale bar = 0.5 mm for A, D; 100 μ m for B; 10 μ m for C, E; and 50 μ m for F, G.

Figure 5. Low (A) and high (B) magnification photomicrographs of ChAT-IR cells in the parabigeminal nucleus. Transverse sections. Scale bar = 50 μ m for A; 10 μ m for B.

Figure 6. Low and high magnification photomicrographs of ChAT-IR cells in representative cranial nerve nuclei. A, B) trochlear nuclei, C, D) nucleus ambiguus. Transverse sections. Scale bar = 100 μ m for A, C; 10 μ m for B, D.

Figure 7. Low (A) and high (B) magnification photomicrographs of ChAT-IR cells in the superficial gray layer of the superior colliculus. Transverse sections. Scale bars = 50 μ m for A, 10 μ m for B.

Figure 8.

Photomicrographs of ChAT-IR cells in auditory nuclei A) Major nuclei of the superior olivary complex: lateral superior olivary nucleus (LSO), medial nucleus of the trapezoid body (MTB), medial superior olivary nucleus (MSO), superior paraolivary nucleus (SPN), and ventral nucleus of the trapezoid body (VTB). B) SPN cell. C, D) LSO cells. E) VTB and ventral nucleus of lateral lemniscus. F) VTB cells. G) Cells in the sagulum (Sag) lateral to DLL, in the PPT medial to DLL, and forming a “bridge” (arrows) stretching from sagulum to PPT dorsal to the DLL. H) Sagulum cells. Transverse sections. Scale bar = 100 μ m for A, E, G; 50 μ m for C; 20 μ m for F; and 10 μ m for B, D, H.

Table 1
Species differences in ChAT staining in brainstem structures

Region	Guinea pig ^{1,2}	Rat ^{3,5}	Cat ^{6,9}	Ferret ¹⁰	Baboon ¹¹	Monkey ^{12,13}	Human ^{14,15}
Sensory (non-auditory) cranial nerve nuclei							
external cuneate nu.	xx	y	o				
spinal V	x	y					
nu. of the solitary tract	xxxx	y	y				o
superior vestibular nu.	xx	y	o				o
lateral vestibular nu.	o	o	o		o		x
medial vestibular nu.	xx	y	y				o
spinal vestibular nu.	x	y	y				
OTHER							
cuneiform nu.		y		o			x/o
Kölliker-Fuse nu.		y	y	y			
locus coeruleus		y	y				x
nu. prepositus hypoglossi	x	y	y				xxx
parabrachial nu.	xxx	y	y	y		o	xxx
raphe magnus nu.		y/o	y		o	o	o
raphe obscurus nu.		y/o	y			o	o
superior colliculus	xxx	y/o	y			o	o
AUDITORY GROUPS							
dorsal cochlear nu.	x	y					
ventral cochlear nu.	x						
Superior olivary complex							x
LSO	xx	y	y				
MSO	o	y	y				
periolivary nu.							
nuclei of lateral lemniscus	xx	y	y				x
inferior colliculus	x	y	y	o			o
nucleus of the brachium of the inferior colliculus	o	o					
sagulum	xx						
RETICULAR FORMATION							
caudal pontine reticular nu.		y					
gigantocellular reticular nu.	x	y	y	o			xxx
intermediate reticular nu.	x	y					
lateral paragigantocellular nu.	x	y					
lateral reticular nu.	x	y	y				x
magnocellular tegmental field			y	o			
oral pontine reticular nu.	x	y					
paramedian reticular nu.			y				
parvocellular reticular nu.		y			y		
reticular nu. medulla pars ventralis		y					

Summary of ChAT-IR stained cell bodies across species. Symbols: "y" indicates the presence of ChAT-IR cells; "o" indicates lack of ChAT-IR cells; a blank box indicates staining in the structure was not described in the study. In guinea pig and human, the number of labeled cells in a nucleus was rated on a 4 "x" scale, with one x meaning relatively few cells and four x's meaning many labeled cells. The motor and parasympathetic nuclei of the cranial nerves and the "traditionally-named" cholinergic nuclei (PPT, LDT, PBG) are not listed because they were considered positive in all species studied. Only those nuclei that were mentioned in two or more studies are listed. Symbols separated by a "/" indicate that different findings were present in different studies. * Source:

¹ current study;

² Maley et al., 1988;

- ³ Satoh et al., 1983;
- ⁴ Tatehata et al., 1987;
- ⁵ Tago et al., 1989;
- ⁶ Ikeda et al., 1992;
- ⁷ Holmes et al., 1994;
- ⁸ Vincent & Reiner, 1987;
- ⁹ Jones & Beaudet, 1987;
- ¹⁰ Henderson, 1987;
- ¹¹ Satoh & Fibiger, 1985;
- ¹² Mesulam et al., 1984; 1998;
- ¹⁴ Mesulam et al., 1992;
- ¹⁵ Mizukawa et al., 1986.