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Quantitative Distribution of Choline Acetyltransferase Activity in Rat Trapezoid Body*

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Abstract

There is evidence for a function of acetylcholine in the cochlear nucleus, primarily in a feedback, modulatory effect on auditory processing. Using a microdissection and quantitative microassay approach, choline acetyltransferase activity was mapped in the trapezoid bodies of rats, in which the activity is relatively higher than in cats or hamsters. Maps of series of sections through the trapezoid body demonstrated generally higher choline acetyltransferase activity rostrally than caudally, particularly in its portion ventral to the medial part of the spinal trigeminal tract. In the lateral part of the trapezoid body, near the cochlear nucleus, activities tended to be higher in more superficial portions than in deeper portions. Calculation of choline acetyltransferase activity in the total trapezoid body cross-section of a rat with a comprehensive trapezoid body map gave a value 3–4 times that estimated for the centrifugal labyrinthine bundle, which is mostly composed of the olivocochlear bundle, in the same rat. Comparisons with other rats suggest that the ratio may not usually be this high, but it is still consistent with our previous results suggesting that the

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centrifugal cholinergic innervation of the rat cochlear nucleus reaching it via a trapezoid body route is much higher than that reaching it via branches from the olivocochlear bundle. The higher choline acetyltransferase activity rostrally than caudally in the trapezoid body is consistent with evidence that the centrifugal cholinergic innervation of the cochlear nucleus derives predominantly from locations at or rostral to its anterior part, in the superior olivary complex and pontomesencephalic tegmentum.

Keywords

Cochlear nucleus; acetylcholine; auditory; hearing; acetylcholinesterase

1. Introduction

Although not one of the major neurotransmitters involved in auditory information processing in the cochlear nucleus, acetylcholine appears to have an important modulatory role. Early studies in whole animals suggested modest effects of acetylcholine on cochlear nucleus neuronal activity, in both anteroventral cochlear nucleus (AVCN) and dorsal cochlear nucleus (DCN) (Comis and Whitfield, 1968; Comis, 1970; Martin and Adams, 1979; Caspary et al., 1983), which affected ability to discriminate signals in noise (Pickles and Comis, 1973). More recent experiments in brain slices have reported effects of acetylcholine agonists on neural activity in the cochlear nucleus. The acetylcholine agonist carbachol depolarized most T stellate cells of the VCN in slices from immature mice (Fujino and Oertel, 2001) and most spherical bushy cells of the AVCN in slices from immature gerbils (Goyer et al., 2016). In the DCN, there is evidence that carbachol activates granule cells in slices from both immature (Köszeghy et al., 2012) and mature rats (Chen et al., 1999) and cartwheel cells in slices from immature mice (He et al., 2014). Since acetylcholine receptors can change during early development (Morley, 2005), one cannot assume that the findings for immature animals apply to adults, but it is likely that they do, at least to some extent.

Previous studies have documented a moderate cholinergic projection from brainstem regions to the cochlear nucleus, including the superior olivary complex and the laterodorsal tegmental nucleus and pedunculopontine nucleus of the pontomesencephalic tegmentum (Godfrey et al., 1983, 1987b,c, 1990; Osen et al., 1984; Mellott et al., 2011). In the rat, there are branch projections from olivocochlear neurons to the cochlear nucleus (Horvath et al., 2000), but a remarkably large proportion of the cholinergic projections from the superior olivary complex appears to follow a ventral route to the cochlear nucleus, via the ventral acoustic stria, more commonly known as the trapezoid body (Osen et al., 1984; Godfrey et al., 1987c; Sherriff and Henderson, 1994). Our previous measurements of choline acetyltransferase (ChAT) enzyme activity in the rat trapezoid body (Godfrey and Matschinsky, 1981; Godfrey et al., 1983, 1987c) have shown an average value much lower than that of the olivocochlear bundle (Godfrey et al., 1984) but much higher than in the auditory nerve root or spinal trigeminal tract (Godfrey and Matschinsky, 1981) and higher than in the cat or hamster trapezoid body (Godfrey et al., 1977, 1983, 1987c, 2013). However, immunohistochemistry for ChAT in rats has not revealed immunopositive trapezoid body fiber bundles (Godfrey, 1993; Yao and Godfrey, 1998).

In view of previous indications that ChAT activity is not uniformly distributed within the trapezoid body (Godfrey et al., 1977, 1987c), and because of the evidence that the trapezoid body is an important route for cholinergic projections to the cochlear nucleus in rats, we undertook a more detailed mapping of the distribution of ChAT activity in the rat trapezoid body.

A preliminary report of the results of this study has been presented (Godfrey et al., 1988).

2. Materials and Methods

This study employed methods similar to those described in our previous publications (Godfrey and Matschinsky, 1976, 1981; Godfrey et al. 1977, 1983, 1984, 1987a,b,c).

2.1. Preparation of sections containing the trapezoid body

Sections for the major part of this study were available from adult male rats weighing 213–509 grams, many of which had been used in previous studies (Godfrey et al., 1984, 1987a). After decapitation, brains were rapidly frozen within 30 minutes in Freon ('Freeze-it') cooled to its freezing point (-130°C) with liquid nitrogen. Brains were then mounted with brain paste onto wooden dowels (Lowry and Passoneau, 1972). Using an American Optical Cryocut Microtome at -20°C , 10 or 20 μm -thick transverse or 20 μm -thick horizontal or sagittal frozen sections were cut through the entire or almost entire extent of the cochlear nucleus and trapezoid body.

In most cases, 20 μm -thick sections were segregated into 3 alternating sets. One set of every third section was placed into 5-hole aluminum racks and into a vacuum tube for overnight freeze-drying (Lowry and Passonneau, 1972). The other 2 sets of every third section were melted onto glass slides. One of these sets was stained with thionin and eosin and the other for acetylcholinesterase (AChE) activity (Karnovsky and Roots, 1964; El-Badawi and Schenk, 1967; Godfrey et al. 1984).

In some cases, 10 μm -thick transverse sections were segregated into just 2 alternating sets, one for freeze drying and the other for AChE staining.

2.2 Measurements of histological landmarks

Stained transverse sections were studied for identification of histological landmarks relevant to trapezoid body extent and location. These included left sides of the brain stem from 3 albino rats, right sides from 2 albino rats, both sides from 6 albino rats, right sides from 4 Long Evans hooded (LEH) rats, and both sides from 7 Brown Norway rats, all available from earlier studies, as well as both sides from 6 adult Fischer 344 x Brown Norway (F344BN) rats available from a more recent study (Godfrey et al., 2017). Landmarks were also identified in horizontal sections from the left side of one rat, the right side of a second, and both sides of a third, and in sagittal sections from the right sides of 2 rats and the left side of a third (for which the opposite sides provided horizontal or transverse sections). The sections with the particular landmarks were identified, and the distances between landmarks were calculated by multiplying the difference in section number by the section thickness since all sections of each tissue block were numbered and saved.

2.3 Isolation of tissue samples

Freeze-dried sections were stored under vacuum (pressure about 50 μ m mercury or less) at -20°C in vacuum tubes (Lowry and Passonneau, 1972). To obtain samples, a vacuum tube was allowed to warm to room temperature, slowly opened, and the desired section removed from its recorded position in the aluminum rack while viewing with a Wild dissecting microscope. The vacuum tube, with all other sections, was then re-evacuated. Once the desired trapezoid body portions, including any nearby regions to be included in the same experiment, were separated from the section, the remainder was replaced within its position in the aluminum rack in the vacuum tube and the tube re-evacuated.

By means of a drawing tube attached to the Wild dissecting microscope, at 25X magnification a tracing was produced of the outline of the freeze-dried section and regional boundaries seen within it. The drawing tube was also used to project stained sections onto the tracing of the freeze-dried section to confirm the locations of regional boundaries seen in the freeze-dried section and add boundaries not seen in the freeze-dried section, including those of AChE-stained fiber bundles. The trapezoid body portion of a chosen section, and sometimes other nearby portions, was microdissected into small pieces using micro knives, made from razor blade fragments, and hairpoints (Lowry and Passonneau, 1972). During the dissection, the drawing tube was used to record all sample cuts onto the tracing of the freeze-dried section, thereby producing a map of all sample locations (Godfrey and Matschinsky, 1976).

Samples were weighed on quartz-fiber microbalances (Lowry and Passonneau, 1972) and then loaded to the bottoms of 400 μ l-capacity polyethylene micro-tubes for assay. Sample weights typically were between 0.05 and 0.5 μ g.

2.4. Enzyme assay

Choline acetyltransferase (ChAT) activity was measured in the same way as in our previous studies (Fonnum, 1969; Godfrey and Matschinsky, 1976, 1981; Godfrey et al. 1977, 1983, 1984, 1987a,b,c). Samples were incubated for 30 minutes at 38°C in 5 μ l of medium containing 100 mM sodium phosphate buffer, pH 7.4, 80 μ M [^{14}C]acetyl coenzyme A, 6 mM choline chloride, 80 μ M neostigmine methyl sulfate, 0.1 % (w/v) bovine serum albumin, 0.1 % (v/v) Triton X-100, and 0.3 M sodium chloride. The incubation was stopped and [^{14}C]acetylcholine product extracted by adding, with mixing, 41 μ l of ice cold 15 mg/ml sodium tetraphenyl boron in 3-heptanone. After centrifugation to separate the aqueous and heptanone phases, a 35 μ l aliquot of the heptanone layer was washed with 100 μ l of 0.5 mg/ml sodium tetraphenyl boron in 10 mM sodium phosphate buffer to eliminate a tissue blank, then a 30 μ l aliquot of the heptanone layer was transferred to a scintillation vial containing 10 ml of scintillation fluid (1/3 'Scintiverse' and 2/3 'Scintilene') for counting radioactivity by liquid scintillation counting. Recovery of acetylcholine product by the extraction was determined to be 100 % by including [^{14}C]acetylcholine chloride instead of [^{14}C]acetyl coenzyme A in some assay tubes without samples.

2.5. Selection of sections for ChAT analysis

Measurements of ChAT activity were made in series of transverse, sagittal, and horizontal sections through the trapezoid body. The transverse sections turned out to be the most suitable for recognizing landmarks and gaining a 3-dimensional appreciation for the distribution of ChAT activity within the trapezoid body, so most of the analysis was done on transverse sections. Only the portion of the trapezoid body between the spinal trigeminal tract dorsally and the brain surface ventrally was analyzed because of the possibility of mixing with neuronal somata or other fiber tracts more medially. The medial border of the cochlear nucleus limited the sampling laterally, and the medial extent of the spinal trigeminal tract limited the sampling medially.

Maps of ChAT activities in series of horizontal sections from 2 albino rats and a series of sagittal sections from one albino rat were also analyzed. The regional locations of samples within the trapezoid body of these sections were determined by projecting them onto transverse sections shown in the atlas of Paxinos and Watson (1998), with the aid of landmarks measured within the stained sections of these series.

2.6. Materials

[1-¹⁴C]Acetyl coenzyme A (51.9 mCi/mmol) was obtained from New England Nuclear, Boston, MA (NEC-313). [1-¹⁴C]Acetylcholine chloride (55.3 mCi/mmol) was obtained from Amersham International plc, Amersham, UK (CFA .310, Batch 37). Other chemicals were obtained from Sigma or Fisher.

'Freeze-it' was obtained from Curtin-Matheson. Aluminum racks for storing freeze-dried sections were made in the lab as needed (Lowry and Passonneau, 1972). Vacuum tubes were obtained from Ace Glass, Inc., Vineland, NJ. Microtubes (400 µl-capacity) were obtained from Bio-Rad.

2.7. Data analysis

Activities of ChAT were expressed as micromoles of acetylcholine product formed per kg dry weight of tissue per min (µmol/kg dry wt/min). Averaged data are presented as mean ± standard error of the mean (SEM). Data were tested for statistical significance by single factor analysis of variance, followed by 2-sided t-tests where analysis of variance indicated statistically significant differences among groups at $P < 0.05$.

3. Results

3.1. Measurements of trapezoid body extent and location

Distances between histological landmarks in transverse sections from 4 different strains of rats were measured (Table 1). The average caudal-rostral extent of the trapezoid body was similar among all rat strains except F344BN, in which it was shorter, with no obvious relation to the 3 different ages (6, 22, and 33 months) of these adult rats. The caudal limit of the trapezoid body was usually close to the caudal location of the auditory nerve connection to the cochlear nucleus (caudal auditory nerve root). The rostral location of the auditory nerve connection to the cochlear nucleus (rostral auditory nerve root) was close to the

midpoint of the trapezoid body extent, ranging from 40 % of the trapezoid body extent in LEH rats and 44 % in albino rats to almost 50 % in F344BN and Brown Norway rats. This rostral auditory nerve root location was also close to the caudal limit of the superior olivary complex (represented by the caudal limit of the lateral superior olive in Table 1). The rostral auditory nerve root landmark was chosen as a dividing point between caudal and rostral portions of the trapezoid body in grouping data for analysis of ChAT activity distributions because of its central location, ease of determination in the series of sections, and relation to the location of the superior olivary complex, which is the source of most of the cholinergic innervation of the cochlear nucleus (Osen et al., 1984; Godfrey et al., 1987c; Sherriff and Henderson, 1994; Mellott et al., 2011). The rostral extent of the trapezoid body, on average, was about 0.3 mm short of the rostral limit of the large-cell (non-granular region) portion of the anteroventral cochlear nucleus (AVCN). Fibers rostral to the trapezoid body were continuous with the middle cerebellar peduncle, corresponding to pontocerebellar fibers, and fibers caudal to it were continuous with the inferior cerebellar peduncle, corresponding to spinocerebellar fibers.

3.2. Observations on sections stained for AChE activity

In sections stained for AChE activity, the trapezoid body showed relatively light staining, but it was noticeably darker than the little if any staining of the spinal trigeminal tract dorsal to it (Fig. 1). Three groups of nerve fibers that passed through the trapezoid body on their way to exit the brain showed darker staining (Fig. 1). One of these, the cholinergic facial nerve root, located just medial to the spinal trigeminal tract, showed clearly darker staining than the trapezoid body. The centrifugal pathways to the auditory and vestibular parts of the inner ear, the olivocochlear and vestibular efferent pathways, referred to collectively as the centrifugal labyrinthine bundle, located in the vestibular nerve root between the spinal trigeminal tract and AVCN, showed much darker staining. Similarly dark staining was shown by fiber bundles that passed through the spinal trigeminal tract and trapezoid body at similar rostral-caudal levels as those where the centrifugal labyrinthine bundle was seen. These correspond to what previous researchers have identified as preganglionic fibers related to the parasympathetic innervation of salivary glands (Shute and Lewis, 1960; Brown and Howlett, 1968, 1971). The relative darkness of the AChE stain in the trapezoid body, spinal trigeminal tract, facial nerve root, and centrifugal labyrinthine bundle is in qualitative agreement with previous measurements of AChE activity in these pathways (Godfrey and Matschinsky, 1981; Godfrey et al., 1984).

In order to measure ChAT activity in the trapezoid body fibers, it was necessary to avoid including facial, centrifugal labyrinthine, or parasympathetic fibers in the samples assayed. Previous measurements showed that the ChAT activities in the facial and centrifugal labyrinthine bundles are extremely high, more than 50 times the average activity in the trapezoid body (Godfrey et al., 1983, 1984). Avoiding the facial and centrifugal labyrinthine fibers was accomplished by avoiding sampling in the locations where these fiber bundles were seen in adjacent AChE-stained sections. Thus, we avoided sampling medial to the medial limit of the spinal trigeminal tract or in the vestibular nerve root where these bundles were present. Avoiding bundles of parasympathetic fibers was more difficult because they had a more scattered presence in the trapezoid body region, and some of the bundles were

less than 10 μm in diameter, smaller than our thinner section thickness (10 μm , as in rat 62582C). Thus, a bundle seen in an adjacent AChE-stained section might not be present in the freeze-dried section dissected, or a bundle present in the freeze-dried section might not be seen in either of the adjacent AChE-stained sections. For example, the parasympathetic bundle seen at the 1.00 mm location in Fig. 1 was not present in the same location 20 μm away at the 1.02 mm location. Overall, however, the adjacent AChE-stained sections turned out to be valuable guides for which parts of freeze-dried sections probably contained parasympathetic fiber bundles, so that samples in these locations could be excluded from trapezoid body data, and the small number of parasympathetic bundles within the rostral-caudal extent of their occurrence minimized their effect on the measurements of trapezoid body ChAT activities.

3.3. Quantitative distribution of ChAT activity in the trapezoid body

Maps of ChAT activities in 8 trapezoid bodies from 7 rats revealed a non-uniform distribution of these activities. The general distributions were summarized by grouping sample locations into caudal and rostral portions of the trapezoid body, defined as those caudal or rostral to the rostral limit of the auditory nerve root. Samples in each of these portions were further divided into medial or lateral locations based on their location closer to the cochlear nucleus or closer to the medial limit of the spinal trigeminal tract (Table 2). Activities of ChAT in the caudal medial part of the trapezoid body were significantly less than in its other parts. ChAT activities were similar to those of albino rats in two LEH rats but lower in one LEH rat.

The detailed map of the trapezoid body of rat 62582C is shown in Figure 2. In this rat, sectioning at 10 μm thickness, with alternate freeze-dried and AChE-stained sections, enabled the highest resolution evaluation of the ChAT activity distribution and the best possibility of detecting non-trapezoid body AChE-positive fiber bundles. The average values for caudal lateral, caudal medial, rostral lateral, and rostral medial parts of the trapezoid body in this rat were similar to the averages across all rats (Table 2). In addition, it can be seen that, in lateral locations, ChAT activities were usually higher in more superficial portions (closer to the brain surface) of the trapezoid body than in deeper portions (farther from the brain surface, closer to the spinal trigeminal tract), whereas this trend was not evident in the most medial locations, where the activities were often higher in deeper portions. The higher ChAT activities in superficial portions of the trapezoid body laterally were also seen in two other rats where the dissections were done in sufficient detail. Mean $\pm$ SEM (no. samples) values for the 3 rats were, for superficial and deep portions, respectively: for rat 62582C, 146 ± 9 (37) and 82 ± 9 (39); for rat 40885A, 184 ± 21 (12) and 145 ± 15 (12); and for rat 50185D, 130 ± 5 (32) and 77 ± 10 (27).

Plots of the average ChAT activities in lateral and medial parts of the trapezoid body, calculated as total acetylcholine formation per min per total sample weight, vs. caudal-to-rostral location for rat 62582C (Fig. 3), show the gradient from lower activity caudally to higher rostrally in the medial part of the trapezoid body, less of such a trend in the lateral part, and the higher ChAT activities laterally than medially in the caudal portion of the trapezoid body. Rostrally, there is much more fluctuation of ChAT activity both laterally and

medially from one section to the next, suggesting that there may be less homogeneously distributed cholinergic fibers within this rostral region, such as if they were gathered more into bundles. Data for rat 40885A showed similar trends, although with fewer data points.

3.4. Comparison of total trapezoid body ChAT activity to total centrifugal labyrinthine bundle ChAT activity

Our previous measurements implied that, in the rat, about 65 % of the ChAT activity in the cochlear nucleus is associated with pathways entering via the trapezoid body, about 20 % with branches of the olivocochlear system (Godfrey et al., 1987c), and the rest with neurons within the cochlear nucleus (Godfrey, 1993; Vetter et al., 1993). Although the average ChAT activity in the trapezoid body is much lower than that in the olivocochlear bundle, the trapezoid body is much larger. We compared the total cross-sectional ChAT activity in the trapezoid body to the total cross-sectional activity in the centrifugal labyrinthine bundle, containing the olivocochlear bundle as its major component (Warr, 1975), in rat 62582C. The total activity in the trapezoid body was estimated by multiplying the average ChAT activity in lateral and medial parts of the trapezoid body in each section (as calculated for Fig. 3) times the dorsal-ventral width of the lateral and medial part of the tract in the same section times the 60 μm distance between sections, then adding all these values. The total ChAT activity in the centrifugal labyrinthine bundle was calculated by multiplying the average activity reported previously (Godfrey et al., 1984) times an estimated cross-sectional area of the tract (assuming an elliptical cross-section, with one diameter equal to the width of the tract measured where it is well represented in AChE-stained sections and the other diameter based on the number of sections containing the tract at that location times thickness of sections). These calculations gave the ChAT activity times cross-sectional area of the trapezoid body to be 36 $\mu\text{mol/kg dry wt/min*mm}^2$ laterally and 27 $\mu\text{mol/kg dry wt/min*mm}^2$ medially, whereas that of the centrifugal labyrinthine bundle was 8 $\mu\text{mol/kg dry wt/min*mm}^2$. This results because the much higher ChAT activity in the centrifugal labyrinthine bundle of rat 62582C (6434 $\mu\text{mol/kg dry wt/min}$), is multiplied by a much smaller cross-sectional area (0.0012 mm^2) than that of the trapezoid body in the same rat (0.31 mm^2 laterally and 0.30 mm^2 medially). Since the ChAT activity in the trapezoid body of rat 62582C is similar to the average for the rats studied (Table 2) and its extent similar to the average of those measured for rats (Table 1), its ChAT activities per cross-sectional area should be representative of the values in rats. The ChAT activity in the centrifugal labyrinthine bundle of rat 62582C is about three-fourths of the average value for 7 rats (Godfrey et al, 1984), and its thickness is similar to some of those shown in that publication but somewhat less than others. Thus, the comparison of ChAT activity times cross-sectional area in rat 62582C may overestimate the ratio of the trapezoid body value to the centrifugal labyrinthine bundle value in rats in general, but it is still roughly consistent with the estimates of the relative contributions of these pathways to ChAT activity in the cochlear nucleus, i.e., 65 % from trapezoid body and 20 % from olivocochlear system (Godfrey et al., 1987c).

4. Discussion

Our results are consistent with a majority of cholinergic innervation of the rat cochlear nucleus reaching it via a ventral route, through the trapezoid body. Based on the distribution of ChAT activity within the trapezoid body, cholinergic fibers appear to be located throughout but at higher density superficially and in rostral parts. They may enter the trapezoid body medial to the spinal trigeminal tract, partly from a rostradorsal direction. This would be consistent with their origins being primarily in the superior olivary complex, especially its ventral nucleus of the trapezoid body (VNTB) (Sherriff and Henderson, 1994; Yao and Godfrey, 1998; Mellott et al., 2011). The cholinergic projections to the cochlear nucleus from the pontomesencephalic tegmentum may also contribute to the ChAT activity in the trapezoid body, but at present the route by which they project is not clear (Mellott et al., 2011).

Other than the fiber bundles crossing through the trapezoid body to exit the brain, stained fiber bundles following the trajectory of trapezoid body fibers are not evident in sections stained for AChE activity, but the trapezoid body as a whole shows some staining that is clearly darker than that of the adjacent spinal trigeminal tract, as also reported previously (Osen et al., 1984). The labeling seen with immunohistochemistry for ChAT shows a similar appearance to that of the staining for AChE activity, slightly darker than that of the spinal trigeminal tract (Godfrey, 1993, Fig. 1).

Two groups of ChAT-positive somata have been described in the VNTB: larger more laterally located somata whose axons comprise the medial component of the olivocochlear bundle and smaller more medially located somata whose axons project to the cochlear nucleus via the trapezoid body (Sherriff and Henderson, 1994). Although the ChAT-positive VNTB neurons projecting to the cochlear nucleus via the trapezoid body show distinct somatic immunoreactivity for ChAT (Adams, 1989; Sherriff and Henderson, 1994; Yao and Godfrey, 1998), their AChE activity (Osen et al., 1984; Adams, 1989; Yao and Godfrey, 1998) and vesicular acetylcholine transporter immunoreactivity (Yao and Godfrey, 1998) appear less prominent than those of olivocochlear neurons. The functions of the two groups of VNTB projections to the cochlear nucleus are not well understood, but it seems likely that they would differ from each other, such as by acting at different types of muscarinic or nicotinic receptors. Based on our previous measurements (Godfrey et al., 1987b,c), both groups of projections innervate all major cochlear nucleus regions, but the contributions to ChAT activity from the trapezoid body pathway are especially dominant in the AVCN and the DCN fusiform soma layer. The projection to the fusiform soma layer may be involved in acetylcholine effects on granule, cartwheel, and fusiform cell activities (Chen et al., 1999; Köszeghy et al., 2012; He et al., 2014; Stefa nescu and Shore, 2017), which may be altered in tinnitus (Jin et al., 2005; Manzo or et al., 2013; Godfrey et al., 2013; Stefanescu and Shore, 2017). In this connection, it is interesting that the ChAT activities in the superficial DCN of the rat tend to be elevated in the deeper portion of the molecular layer and more superficial portion of the fusiform soma layer (Godfrey et al., 1987a, Fig. 2), where cartwheel cells are especially concentrated (Wouterlood and Mugnaini, 1984, Fig. 23).

It must be emphasized that our results for ChAT activity can be assumed to apply only to rat. Activities of ChAT in the trapezoid body in other animals, such as cat and hamster, are much lower than those in rat, and the contribution of projections via the trapezoid body to ChAT activity in the cochlear nucleus is less in cats (Godfrey et al., 1990) than in rats (Godfrey et al., 1987c), suggesting that there may be considerable cross-species variation in the magnitude and characteristics of cholinergic system effects in the cochlear nucleus. The much higher ChAT activity in the trapezoid body of the rat, as compared to cat or hamster, correlates with higher ChAT activity in the non-granular regions of the rat cochlear nucleus as compared to cat or hamster (Godfrey et 1987b,c, 1990, 2013). After cochlear damage by cochlear ablation or noise trauma, increases of ChAT activity and immunoreactivity have been measured in the cochlear nucleus (Jin et al., 2005, 2006; Meidinger et al., 2006; Godfrey et al., 2013). It remains to be finally determined to what extent olivocochlear, VNTB-trapezoid body, or pontomesencephalic tegmentum innervation may be involved in these changes that may underlie tinnitus symptoms, but the results of Meidinger et al. (2006) strongly favor involvement of the VNTB olivocochlear neurons for effects in the ventral cochlear nucleus.

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Abbreviations in text

AChE	acetylcholinesterase
AVCN	anteroventral cochlear nucleus
ChAT	choline acetyltransferase
DCN	dorsal cochlear nucleus
F344BN	Fischer 344 x Brown Norway
LEH	Long Evans hooded
SEM	standard error of the mean
VNTB	ventral nucleus of the trapezoid body

References

- Adams JC, 1989 Non-olivocochlear cholinergic periolivary cells. Soc. Neurosci. Abstr 15, abstr. 445.1.
- Brown JC, Howlett B, 1968 The facial outflow and the superior salivatory nucleus: an histochemical study in the rat. J. Comp. Neurol 134, 175–192. [PubMed: 4889608]
- Brown JC, Howlett B, 1971 Rat hindbrain thiocholine reactions relevant to the localization of the inferior salivatory nucleus. Acta Anat 79, 333–359. [PubMed: 4329262]
- Caspary DM, Havey DC, Faingold CL, 1983 Effects of acetylcholine on cochlear nucleus neurons. Exp. Neurol 82, 491–498. [PubMed: 6628633]

- Chen K, Waller HJ, Godfrey TG, Godfrey DA, 1999 Glutamatergic transmission of neuronal responses to carbachol in rat dorsal cochlear nucleus slices. *Neuroscience* 90, 1043–1049. [PubMed: 10218803]
- Comis SD, 1970 Centrifugal inhibitory processes affecting neurons in the cat cochlear nucleus. *J. Physiol* 210, 751–760. [PubMed: 5499823]
- Comis SD, Whitfield IC, 1968 Influence of centrifugal pathways on unit activity in the cochlear nucleus. *J. Neurophysiol* 31, 62–68. [PubMed: 5640740]
- El-Badawi A, Schenk EA, 1967 Histochemical methods for separate, consecutive and simultaneous demonstration of acetylcholinesterase and norepinephrine in cryostat sections. *J. Histochem. Cytochem* 15, 580–588. [PubMed: 4863647]
- Fonnum F, 1969 Radiochemical micro assays for the determination of choline acetyltransferase and acetylcholinesterase activities. *Biochem. J* 115, 465–472. [PubMed: 4982085]
- Fujino K, Oertel D, 2001 Cholinergic modulation of stellate cells in the mammalian ventral cochlear nucleus. *J. Neuroscience* 21, 7372–7383.
- Godfrey DA, 1993 Comparison of quantitative and immunohistochemistry for choline acetyltransferase in the rat cochlear nucleus. In: Merchán MA, Juiz JM, Godfrey DA, Mugnaini E (Eds.), *The Mammalian Cochlear Nuclei: Organization and Function* Plenum Press, New York, pp. 267–278.
- Godfrey DA, Matschinsky FM, 1976 Approach to three-dimensional mapping of quantitative histochemical measurements applied to studies of the cochlear nucleus. *J. Histochem. Cytochem* 24, 697–712. [PubMed: 781128]
- Godfrey DA, Matschinsky FM, 1981 Quantitative distribution of choline acetyltransferase and acetylcholinesterase activities in the rat cochlear nucleus. *J. Histochem. Cytochem* 29, 720–730. [PubMed: 7252132]
- Godfrey DA, Beranek KL, Carlson L, Parli JA, Dunn JD, Ross CD, 1990 Contribution of centrifugal innervation to choline acetyltransferase activity in the cat cochlear nucleus. *Hear. Res* 49, 259–280. [PubMed: 2292500]
- Godfrey DA, Carlson L, Parli JA, Ross CD, 1988 Distribution of choline acetyltransferase activity in the trapezoid body of the rat. *Soc. Neurosci. Abstr* 14, 490.
- Godfrey DA, Carlson L, Ross CD, 1987a Quantitative inter-strain comparison of the distribution of choline acetyltransferase activity in the rat cochlear nucleus. *Hear. Res* 31, 203–210. [PubMed: 3436848]
- Godfrey DA, Chen K, O'Toole TR, Mustapha IAA, 2017 Amino acid and acetylcholine chemistry in the central auditory system of young, middle-aged and old rats. *Hear. Res* 350, 173–188. [PubMed: 28505527]
- Godfrey DA, Kaltenbach JA, Chen K, Ilyas O, 2013 Choline acetyltransferase activity in the hamster central auditory system and long-term effects of intense tone exposure. *J. Neurosci. Res* 91, 987–996. [PubMed: 23605746]
- Godfrey DA, Park JL, Rabe JR, Dunn JD, Ross CD, 1983 Effects of large brain stem lesions on the cholinergic system in the rat cochlear nucleus. *Hear. Res* 11, 133–156. [PubMed: 6619002]
- Godfrey DA, Park JL, Ross CD, 1984 Choline acetyltransferase and acetylcholinesterase in centrifugal labyrinthine bundles of rats. *Hear. Res* 14, 93–106. [PubMed: 6746425]
- Godfrey DA, Park-Hellendall JL, Dunn JD, Ross CD, 1987b Effect of olivocochlear bundle transection on choline acetyltransferase activity in the rat cochlear nucleus. *Hear. Res* 28, 237–251. [PubMed: 3654392]
- Godfrey DA, Park-Hellendall JL, Dunn JD, Ross CD, 1987c Effects of trapezoid body and superior olive lesions on choline acetyltransferase activity in the rat cochlear nucleus. *Hear. Res* 28, 253–270. [PubMed: 3654393]
- Godfrey DA, Williams AD, Matschinsky FM, 1977 Quantitative histochemical mapping of enzymes of the cholinergic system in cat cochlear nucleus. *J. Histochem. Cytochem* 25, 397–416. [PubMed: 69653]
- Goyer D, Kurth S, Gillet C, Keine C, Rübsamen R, Kuenzel T, 2016 Slow cholinergic modulation of spike probability in ultra-fast time-coding sensory neurons. *eNeuro*, 3, ENEURO.9186–16.2016.

- He S, Wang Y-X, Petralia RS, Brenowitz SD, 2014 Cholinergic modulation of large-conductance calcium-activated potassium channels regulates synaptic strength and spine calcium in cartwheel cells of the dorsal cochlear nucleus. *J. Neuroscience* 34, 5261–5272.
- Horvath M, Kraus KS, Illing RB, 2000 Olivocochlear neurons sending axon collaterals into the ventral cochlear nucleus of the rat. *J. Comp. Neurol* 422, 95–105. [PubMed: 10842220]
- Jin Y-M, Godfrey DA, Sun Y, 2005 Effects of cochlear ablation on choline acetyltransferase activity in the rat cochlear nucleus and superior olive. *J. Neurosci. Res* 81, 91–101. [PubMed: 15931674]
- Karnovsky MJ, Roots L, 1964 A 'direct coloring' thiocholine method for cholinesterases. *J. Histochem. Cytochem* 12, 219–221. [PubMed: 14187330]
- Köszeghy Á, Vincze J, Rusznák Z, Fu Y, Paxinos G, Csernoch L, Szücs G, 2012 Activation of muscarinic receptors increases the activity of the granule neurones of the rat dorsal cochlear nucleus—a calcium imaging study. *Pflugers Arch. – Eur. J. Physiol* 463, 829–844. [PubMed: 22547003]
- Lowry OH, Passaneau JV, 1972 *A Flexible System of Enzymatic Analysis* Academic Press, New York.
- Manzoor NF, Chen G, Kaltenbach JA, 2013 Suppression of noise-induced hyperactivity in the dorsal cochlear nucleus following application of the cholinergic agonist, carbachol. *Brain Res* 1523, 28–36. [PubMed: 23721928]
- Martin MR, Adams JC, 1979 Effects of DL- α -amino adipate on synaptically and chemically evoked excitation of anteroventral cochlear nucleus neurons of the cat. *Neuroscience* 4, 1097–1105. [PubMed: 492527]
- Meidinger MA, Hildebrandt-Schoenfeld H, Illing RB, 2006 Cochlear damage induces GAP-43 expression in cholinergic synapses of the cochlear nucleus in the adult rat: a light and electron microscopic study. *Eur. J. Neurosci* 23, 3187–3199. [PubMed: 16820009]
- Mellott JG, Motts SD, Schofield BR, 2011 Multiple origins of cholinergic innervation of the cochlear nucleus. *Neuroscience* 180, 138–147. [PubMed: 21320579]
- Morley BJ, 2005 Nicotinic cholinergic intercellular communication: implications for the developing auditory system. *Hear. Res* 206, 74–88. [PubMed: 16081000]
- Osen. KK, Mugnaini E, Dahl AL, Christiansen AH, 1984 Histochemical localization of acetylcholinesterase in the cochlear and superior olivary nuclei. A reappraisal with emphasis on the cochlear granule cell system. *Arch. Ital. Biol* 122, 169–212. [PubMed: 6517650]
- Paxinos G, Watson C, 1998 *The Rat Brain in Stereotaxic Coordinates*, fourth ed. Academic Press, San Diego.
- Pickles JO, Comis SD, 1973 Role of centrifugal pathways to the cochlear nucleus in detection of signals in noise. *J. Neurophysiol* 36, 1131–1137. [PubMed: 4761723]
- Sheriff FE, Henderson Z, 1994 Cholinergic neurons in the ventral trapezoid nucleus project to the cochlear nuclei in the rat. *Neuroscience* 58, 627–633. [PubMed: 7513389]
- Shute CCD, Lewis PR, 1960 The salivatory centre in the rat. *J. Anat* 94, 59–73. [PubMed: 14446346]
- Stefanescu RA, Shore SE, 2017 Muscarinic acetylcholine receptors control baseline activity and Hebbian stimulus timing-dependent plasticity in fusiform cells of the dorsal cochlear nucleus. *J. Neurophysiol* 117, 1229–1238. [PubMed: 28003407]
- Vetter DE, Cozzari C, Hartman BK, Mugnaini E, 1993 Choline acetyltransferase in the rat cochlear nuclei: immunolocalization with a monoclonal antibody. In: Merchán MA, Juiz JM, Godfrey DA, Mugnaini E (Eds.), *The Mammalian Cochlear Nuclei: Organization and Function* Plenum Press, New York, pp. 279–290.
- Warr WB, 1975 Olivocochlear and vestibular efferent neurons of the feline brain stem: Their location, morphology and number determined by retrograde axonal transport and acetylcholinesterase histochemistry. *J. Comp. Neurol* 161, 159–181. [PubMed: 47866]
- Wouterlood FG, Mugnaini E, 1984 Cartwheel neurons of the dorsal cochlear nucleus: a Golgi-electron microscopic study in rat. *J. Comp. Neurol* 227, 136–157. [PubMed: 6088594]
- Yao W, Godfrey DA, 1998 Immunohistochemical evaluation of cholinergic neurons in the rat superior olivary complex. *Microsc. Res. Tech* 41, 270–283. [PubMed: 9605344]

Highlights:

Choline acetyltransferase (ChAT) catalyzes synthesis of transmitter acetylcholine

ChAT activity in the trapezoid body serves as a marker of cholinergic nerve fibers

ChAT activity is higher in rat trapezoid body than in cat or hamster trapezoid body

ChAT activity is higher rostrally than caudally in rat trapezoid body

The trapezoid body of rat carries about 3 times the ChAT as its olivocochlear bundle

Figure 1.

Photomicrographs of 10 µm-thick transverse sections stained for acetylcholinesterase (AChE) activity at 4 caudal-to-rostral locations relative to the caudal limit of the right trapezoid body in rat 62582C, shown as millimeter distance at upper left of each photo. Black double-arrowhead line between upper and lower photos represents 1 mm in all 4 sections. In photos, dorsal is up and lateral to the right. AChE-stained fiber bundles that cross the trapezoid body (T) include some crossing below the spinal trigeminal tract (S), which have been reported to consist of parasympathetic salivatory system fibers (P, Shute and Lewis, 1960; Brown and Howlett, 1968, 1971). Fiber bundles within the vestibular nerve root consist of olivocochlear and centrifugal vestibular fibers that innervate the auditory and vestibular portions of the inner ear labyrinth, collectively termed the centrifugal labyrinthine bundle (L, Godfrey et al., 1984). The large fiber bundle medial to the spinal trigeminal tract is the facial nerve root (F). Since the compositions of all photos are similar, the abbreviation

for a feature is given in just one, either 0.96 mm or 1.16 mm, where a feature is well represented. Other abbreviations are A, anteroventral cochlear nucleus; C, cerebellum (molecular layer, with granular layer to its right), and V, vestibular ganglion.

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Figure 2.

Distribution of choline acetyltransferase (ChAT) activity in a series of 28 10- μm thick transverse freeze-dried sections spaced at 60 μm intervals through the right trapezoid body of rat 62582C. Dorsal direction is up and lateral to the right in each section, and 1 mm scale is at the top. The distance rostral to the caudal limit of the trapezoid body is indicated in millimeters (mm) to the left of every other section, along with the relative percentage distance from the caudal to the rostral trapezoid body limit. The total extent of the right trapezoid body in this rat was 1.65 mm. Solid black lines indicate sample cut boundaries.

Dashed black lines indicate regional boundaries, including the ventral boundary of the spinal trigeminal tract (STT) dorsal to the trapezoid body. Activities are color-coded as indicated at lower left. Gray areas correspond in location to prominent parasympathetic fiber bundles staining darkly for acetylcholinesterase (AChE) activity in adjacent sections, some of which are shown in Fig. 1 (0.96 mm and 1.00 mm locations). ChAT activities for 6 samples in those areas averaged, as mean $\pm$ SEM, 1057 ± 215 $\mu\text{mol/kg dry wt/min}$. The activity for the one sample in the gray area of the section at 0.95 mm was 1211 $\mu\text{mol/kg dry wt/min}$, and the activities for two samples in the gray area of the section at 1.01 mm were 371 and 1114 $\mu\text{mol/kg dry wt/min}$. Gray dots and lines within some samples indicate the presence of AChE-positive fiber bundles in adjacent stained sections. Zones where the auditory nerve root (ANR) is present and where the facial nerve root (FNR) and AChE-positive parasympathetic fiber bundles (PF) cross the trapezoid body are indicated on the right. Fibers of the centrifugal labyrinthine bundle (Godfrey et al, 1984) cross the lateral part of the trapezoid body between 0.84 and 1.12 mm rostral to the caudal limit of the trapezoid body -almost exactly the same sections as those in which the parasympathetic fiber bundles cross more medially. The outline of the anteroventral cochlear nucleus (A) is included in the most rostral section.

Figure 3.

Plots of average choline acetyltransferase (ChAT) activity vs. percentage of the caudal-to-rostral extent of the right trapezoid body of rat 62582C (maps shown in Fig. 2) and the left trapezoid body of rat 40885A. Averages for samples located laterally, close to the cochlear nucleus, are shown by filled circles, and averages for samples located more medially, between the lateral samples and the medial limit of the spinal trigeminal tract, are shown by empty circles. An extra filled circle at the 76 % location for rat 62582C is the average of lateral samples in one section, not shown in Fig. 2, wherein there were no data for medial

samples. Averages were calculated by multiplying individual sample weights in each zone by their ChAT activities, adding the resulting values, then dividing by the total weight of the samples included. Samples corresponding in location to where acetylcholinesterase (AChE)-positive fiber bundles were seen in adjacent AChE-stained sections were excluded from the averages. Some measurements were made for samples located in fiber tracts caudal ($\% < 0$) and rostral ($\% > 100$) to the trapezoid body, corresponding to spinocerebellar and pontocerebellar fibers, respectively.

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Table 1.

Distances (mm) in transversely sectioned rat brains, as mean $\pm$ SEM (no. measurements) *

Rat strain (no.)	TB extent	caudal ANR - caudal TB	rostral ANR - caudal TB	rostral TB - rostral ANR	rostral ANR - caudal LSO	rostral AVCN - rostral TB
Albino (11)	1.71 $\pm$ 0.04 (10)	0.23 $\pm$ 0.03 (15)	0.75 $\pm$ 0.04 (12)	0.95 $\pm$ 0.07 (10)	-0.06 $\pm$ 0.14(13)	0.26 $\pm$ 0.06 (10)
LEH (4)	1.76 $\pm$ 0.05 (4)	0.06 $\pm$ 0.07(4)	0.71 $\pm$ 0.06(4)	1.08 $\pm$ 0.09 (3)	-0.02 $\pm$ 0.21(4)	0.26 $\pm$ 0.08 (4)
F344BN (6)	1.41 $\pm$ 0.03 (12)	0.13 $\pm$ 0.03(12)	0.69 $\pm$ 0.04(12)	0.71 $\pm$ 0.04 (12)	0.02 $\pm$ 0.10 (12)	0.31 $\pm$ 0.03 (12)
Brown Norway (7)	1.69 $\pm$ 0.05 (14)	0.14 $\pm$ 0.04(14)	0.83 $\pm$ 0.04(14)	0.86 $\pm$ 0.03 (14)	0.32 $\pm$ 0.07 (14)	0.45 $\pm$ 0.04 (14)
All combined (28)	1.62 $\pm$ 0.03 (40)	0.16 $\pm$ 0.02(45)	0.76 $\pm$ 0.02(42)	0.85 $\pm$ 0.03 (39)	0.09 $\pm$ 0.06 (43)	0.34 $\pm$ 0.03 (40)

* Abbreviations are: ANR, auditory nerve root; AVCN, anteroventral cochlear nucleus; F344BN, Fischer 344 x Brown Norway; LEH, Long Evans hooded; LSO, lateral superior olive; TB, trapezoid body. The numbers of measurements are in some cases less than the numbers of series of sections available because some landmarks were not present in all series, particularly for albino rats.

ChAT activities ($\mu\text{mol/kg dry wt/min}$) in trapezoid bodies of rats, as mean $\pm$ SEM (no. samples)*

Table 2.

Rat	Side	Sectioning	Caudal lateral	Caudal medial	Rostral lateral	Rostral medial
62582C	right	transverse	95 $\pm$ 7 (37)	51 $\pm$ 5 (42)	128 $\pm$ 10 (54)	110 $\pm$ 10 (54)
40885A	left	transverse	143 $\pm$ 12 (14)	85 $\pm$ 11 (18)	186 $\pm$ 21 (12)	173 $\pm$ 41 (12)
20981LEHR	right	transverse	92 $\pm$ 13 (4)	51 $\pm$ 13 (7)	127 (2)	117 $\pm$ 29 (4)
20981LEHRLC	right	transverse	104 (2)	108 $\pm$ 23 (4)	185 $\pm$ 20 (6)	126 $\pm$ 26 (6)
20981LEHRLF	right	transverse	82 $\pm$ 14 (6)	31 $\pm$ 6 (8)	65 $\pm$ 14 (5)	50 $\pm$ 18 (7)
50185D	left	horizontal	101 $\pm$ 8 (40)	65 $\pm$ 6 (24)	116 $\pm$ 10 (19)	95 $\pm$ 7 (10)
40885C	right	horizontal	133 $\pm$ 10 (4)	85 $\pm$ 6 (13)	94 $\pm$ 11 (4)	147 $\pm$ 18 (14)
40885C	left	sagittal	98 $\pm$ 7 (18)	59 $\pm$ 7 (15)	102 $\pm$ 9 (20)	124 $\pm$ 15 (12)
Averages for 8 trapezoid bodies			106 $\pm$ 7	67 $\pm$ 9	125 $\pm$ 15	118 $\pm$ 13
P for comparison to caudal medial			0.004		0.006	0.006

* Data are for total numbers of samples in each region not located where there are AChE-positive fiber bundles in adjacent stained sections. Rats with LEH in name are Long Evans hooded strain; others are albino. For comparison, ChAT activities in the facial motor root in several of the rats, as mean $\pm$ SEM (no. of samples) were: 5940 $\pm$ 223 (4) for 62582C, 5450 $\pm$ 293 (4) for 20981LEHR, 4825 $\pm$ 117 (9) for 20981LEHRLC, 6370 $\pm$ 337 (5) for 20981LEHRLF, 8977 $\pm$ 330 (4) for 50185D, and 5556 $\pm$ 381 (3) for 40885C (right side); and ChAT activity in the spinal trigeminal tract was 8 $\pm$ 3 (10) in 40885C (left side).