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Effects of surgical lesions on choline acetyltransferase activity in the cat cochlea

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Abstract

Although it is well established that the choline acetyltransferase (ChAT, the enzyme for acetylcholine synthesis) in the mammalian cochlea is associated with its olivocochlear innervation, the distribution of this innervation in the cochlea varies somewhat among mammalian species. The quantitative distribution of ChAT activity in the cochlea has been reported for guinea pigs and rats. The present study reports the distribution of ChAT activity within the organ of Corti among the three turns of the cat cochlea and the effects of removing olivocochlear innervation either by a lateral cut aimed to totally transect the left olivocochlear bundle or a more medial cut additionally damaging the superior olivary complex on the same side. Similarly to results for guinea pig and rat, the distribution of ChAT activity in the cat outer hair cell region showed a decrease from base to apex, but, unlike in the guinea pig and rat, the cat inner hair cell region did not. As in the rat, little ChAT activity was measured in the outer supporting cell region. As previously reported for whole cat cochlea and for rat cochlear regions, transection of the olivocochlear bundle resulted in almost total loss of ChAT activity in the hair cell regions of the cat cochlea. Lesions of the superior olivary complex resulted in loss of ChAT activity in the inner hair cell region of all cochlear turns only on the lesion side but bilateral losses in the outer hair cell region of all turns. The results are consistent with previous evidence that virtually all cholinergic synapses in the mammalian cochlea are associated with its olivocochlear innervation, that the olivocochlear innervation to the inner hair cell region is predominantly ipsilateral, and that the olivocochlear innervation to the outer hair cells is bilateral.

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(The experimental procedures for this study were completed while most of the authors were members of the Department of Physiology (GJW, DAG, JAP, CDR) or Anatomy (JDD) at Oral Roberts University School of Medicine, Tulsa, Oklahoma)

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Keywords

Olivocochlear; inner hair cells; outer hair cells; acetylcholine; auditory; hearing

1. Introduction

The olivocochlear system, or olivocochlear bundle (OCB), has long been recognized to provide the primary efferent innervation of the cochlea to affect the sensitivity of its coding of auditory signals (Warr, 1992; Guinan, 2006).

The OCB originates bilaterally from the superior olivary complex (SOC) in the brain stem and consists of lateral and medial efferent systems. The lateral olivocochlear (LOC) system consists of neurons with somata laterally located in the SOC and thin, unmyelinated axons, while medial olivocochlear (MOC) neurons have somata more medially and rostrally located in the SOC and thicker, myelinated axons. Both MOC and LOC axons travel dorsomedially in close proximity to the fourth ventricle upon leaving the SOC. At this point, about 70 % of MOC fibers in the cat decussate to the opposite side of the brain stem and about 30 % remain ipsilateral. A few MOC neurons have bilateral projections. About 80 % of LOC fibers remain ipsilateral, and about 20 % cross to the opposite side in the cat. All of these fibers traverse the facial nerve root before forming the compact OCB in the vestibular nerve root. The LOC fibers comprise about 60 % of the OCB in the cat (Warr, 1992).

In the cochlea, the LOC fibers have been shown to predominantly synapse with the type I afferent nerve fibers underneath the inner hair cells (IHC), whereas the MOC fibers primarily synapse on the bases of the outer hair cells (OHC). The LOC fibers form the inner spiral bundle and the tunnel spiral bundle under the inner hair cells of the organ of Corti of the cat (Ginzberg and Morest, 1984; Liberman et al., 1990). In the cat, the fiber population of the inner spiral bundle peaks in mid-cochlear regions; synapses per auditory nerve fiber peak in more apical parts, but the number of auditory nerve fibers decreases apically (Liberman et al., 1990). The MOC neurons travel as upper tunnel radial fibers in the organ of Corti before reaching the OHC. In the cat, few if any MOC fibers travel in the inner spiral bundle (Liberman et al., 1990). Efferent innervation of the OHC is greatest in the mid-to-high frequency region of the cochlea, with a decline in innervation both apically and basally (Liberman et al., 1990; Warr, 1992).

Choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, has been shown to be a definitive marker for cholinergic neurons (Godfrey et al., 1985). The high ChAT activity in the OCB is consistent with all LOC and MOC fibers being cholinergic (Godfrey et al., 1984), and the OCB fibers exhibit immunoreactivity to ChAT (Altschuler and Fex, 1986; Eybalin, 1993). Some LOC neurons have also shown immunoreactivity to the neurotransmitters enkephalin and calcitonin gene-related peptide in conjunction with ChAT, whereas MOC neurons have shown immunoreactivity only to ChAT (Altschuler and Fex, 1986; Eybalin, 1993).

Quantitative assessment of the density of acetylcholine metabolic enzymes (acetylcholinesterase and ChAT) in the cochleas of the guinea pig (Godfrey et al., 1976) and

rat (Godfrey and Ross, 1985; Godfrey et al., 1986) have shown high activities of these enzymes in the IHC and OHC regions. In the cat, ChAT in the cochlea as a whole has been associated with its OCB innervation (Jasser and Guth, 1973), but there has not previously been an analysis of the hair cell regions of the organ of Corti. To specifically evaluate the cholinergic OCB innervation of the hair cell regions of the cat cochlea, ChAT activity was measured in microdissected samples of the organ of Corti of 13 cats, some of which had surgical cuts in the brain stem that damaged the olivocochlear system. A preliminary presentation of the results has been made (Wiet et al., 1989).

2. Methods

2.1. Preparation of temporal bones

These experiments were carried out on temporal bones obtained during a previous study on the cochlear nucleus of cats (Godfrey et al., 1990a). The temporal bones were isolated and quickly frozen after the removal and freezing of brain tissues. The cats are identified by the same letters as in the previous study, except for two cats for which brain tissue was not included in that study (Table 1).

The results presented here are derived from 13 cats of both sexes weighing 2–4 kg. Placement of surgical cuts and animal treatment, which was in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, have been described, and maps of the lesion locations have been published (Godfrey et al., 1990a) except for cats C and E, with sham lesions, and cat D2, which was not included in the previous study. The lesion locations for the 9 lesioned cats included in the previous study are shown in abbreviated form in Fig. 1, and a more comprehensive map of the lesion location for cat D2 is shown in Fig. 2. The cats can be separated into 4 groups: 3 sham-lesioned cats, with exposure of the left cochlear nucleus but no cut made (C, E, I), 5 cats with transection of the left OCB (A, D2, F, H, J), 3 cats with left superior olivary complex (SOC) damage as well as transection of most or all of the left OCB (B, G, K), and 2 cats with cuts within the left cochlear nucleus to separate its rostral from its caudal parts (intra-cochlear nucleus (intraCN) cuts) (D, L). Post-surgery survival times were 7–10 days. Brain parts and temporal bones were frozen in Freon (Curtin Matheson “Freeze It”) cooled to its freezing point (–130 °C) with liquid nitrogen. Temporal bones were trimmed close to their cochlea portions before freezing approximately an hour post-mortem. Cochleas were freeze-dried (Thalmann, 1976) for 9–14, usually 10 days at –40°C and then stored under vacuum at –20°C until dissected.

2.2. Microdissection of samples for assay

A three-dimensional approach was used in dissecting the freeze-dried temporal bones using a specially designed small mammal temporal bone holder similar to but larger than that used for rat and guinea pig (Wiet et al., 1986). First, the outer osseous covering was thinned using a dental burr and picked away using microforceps. Next, pieces of intracochlear structures were removed. For this study, the organ of Corti was subdivided into IHC, OHC, and outer supporting cells (OSC, Hensen’s cell) regions (Matschinsky and Thalmann, 1967; Thalmann, 1972, 1976; Godfrey et al., 1986; Wiet et al., 1986, 1990). The hair cell regions

were viewed, identified, and cut from above, but it was difficult to be sure how close the bottoms of the samples came to the basilar membrane. Since the OCB terminals would be in the lower portions of the hair cell samples, the extents of their inclusion in the samples likely varied depending on where exactly the samples detached from the underlying tissue. This presumably accounts for some variability in the measurements of ChAT activity, as in our previous studies (Godfrey et al., 1976; Godfrey and Ross, 1985).

2.3. Measurement of sample weights and ChAT activities

Samples were weighed on quartz fiber microbalances (Lowry and Passonneau, 1972) and loaded to the bottoms of 400 microliter capacity polyethylene tubes. The weights of the samples of the 3 portions of the organ of Corti sampled, as mean $\pm$ SEM (number of samples) were 0.088 ± 0.004 (223) μg for IHC region, 0.132 ± 0.005 (222) μg for OHC region, and 0.110 ± 0.006 (207) μg for OSC region. ChAT activity was measured using the same procedure used in previous studies (Fonnum, 1969; Godfrey and Ross, 1985; Godfrey et al., 1990a) and expressed per dry weight of tissue. All samples from both left and right cochleas of each cat were included together in the same assay, along with reagent blank tubes and homogenate of rat whole brain as a tissue standard. All blanks, samples, and homogenates were incubated for 30 minutes in 5 microliters of phosphate-buffered medium, pH 7.4, containing [1- ^{14}C]acetyl coenzyme A and choline as substrates, and [^{14}C]acetylcholine product was subsequently extracted with sodium tetraphenyl boron dissolved in 3-heptanone and quantified by liquid scintillation counting. A correction for 2.5 % carryover of counts from one tube to the next during the pipetting was incorporated into the calculations.

2.4. Data analysis

Data were tested for normality by Kolmogorov-Smirnov and Shapiro-Wilk tests using IBM SPSS Statistics version 23 software. Most were consistent with normal distributions, but about a fourth of the groups were not. Statistical significances of differences were evaluated by both one-way analysis of variance (ANOVA) followed by t-tests (parametric) and Kruskal-Wallis followed by Mann-Whitney tests (non-parametric), which almost always gave the same results for statistical significance. Modified box plots of data were produced using SigmaPlot software.

3. Results

3.1 Results for blank tubes and homogenate standards

Empty tubes were occasionally included in assays when samples were lost (usually during the weighing procedure). The average $\pm$ SEM ChAT activity for 33 such tubes, using an arbitrary value of 0.1 μg as the sample weight, was -5 ± 2 $\mu\text{mol/kg/min}$. For the rat brain homogenate included in all the assays, the mean $\pm$ SEM ChAT activity for 39 measurements was 98 ± 2 $\mu\text{mol/kg wet wt/min}$.

3.2 Sham lesions

In the cats with sham lesions (Cats A, E, and I), in which the left cochlear nucleus was exposed but no cut made into the OCB, SOC, or cochlear nucleus, ChAT activity in the

cochlea subregions was similar on right and left sides (Fig. 3), with no statistically significant differences. Also, there was no statistically significant difference among the three turns of the cochlea in the IHC region. A gradient of ChAT activity, decreasing from basal to apical turn, was measured in the OHC region. The OSC region showed low activity overall but with higher ChAT activity in 4 of the 49 samples. Sizeable variations in ChAT activities of cochlear samples resulted from occasional low activities in hair cell samples and occasional high activities in OSC samples. The low activities in hair cell samples did not likely result from problems with the assay since they never occurred with homogenate samples, which showed much less variation, as noted above. Rather, they likely resulted from some hair cell samples containing little OCB tissue because of how the sample pieces broke off from underlying tissue. The relatively high activities in some OSC samples did not likely result from problems with the assay since reagent blanks included in the assays never showed high activities, as noted above. Rather, they likely resulted either from the cut between OHC and OSC regions occasionally including some of the tissue beneath hair cells in OSC samples or some OCB fibers traveling in the OSC region, as has been reported previously (Wright and Preston, 1976; Stopp and Comis, 1978; Godfrey and Ross, 1985; Liberman et al., 1990).

3.3 Cuts within the cochlear nucleus - IntraCN cuts

In the cats with transverse cuts placed within the left cochlear nucleus (Cats D and L), ChAT activity in the cochlea was not expected to be affected. Data were obtained for both cochleas of Cat L but only for the left cochlea of Cat D. It was expected that results for these cats would be similar to those for the sham cats because there was no cut through the OCB. For Cat L, the average $\pm$ SEM ChAT activities of the IHC regions, for all turns combined, in the left and right cochleas were 1075 ± 203 and 1014 ± 218 $\mu\text{mol/kg dry wt/min}$, respectively. The average $\pm$ SEM ChAT activities of the OHC regions, with all turns combined, for the left and right cochleas were 370 ± 26 and 410 ± 95 $\mu\text{mol/kg dry wt/min}$, respectively. Since the results for the cats with intraCN cuts were similar to those for the cats with sham lesions, and since there were no statistically significant differences between left and right cochleas, the data for both cochleas of the 3 sham cats and Cat L and for the left cochlea of Cat D were combined to provide control data for comparison with those from the cats with OCB damage (Table 2). For the combined control data, as in the sham cats, which provided most of the control data, no significant gradient of ChAT activity was measured in the IHC region, but a gradient from base to apex was measured in the OHC region. The difference between first and second turns (OHC1 vs. OHC2) was not statistically significant, but that between the first or second turn and the third turn was highly significant ($P < 0.001$ by ANOVA and t-test and $P = 0.001$ by Kruskal-Wallis and Mann-Whitney test in both cases).

Three samples were obtained from the vicinity of the stapedius muscle of the right middle ear, which was present in the freeze-dried temporal bone piece of Cat L. The ChAT activity for these samples, as mean $\pm$ SEM, was 1602 ± 93 $\mu\text{mol/kg dry wt/min}$. It was not clear at the time of the dissection whether these were samples of stapedius muscle or of its innervating facial nerve. However, since our previous measurements of cat stapedius muscle homogenate ChAT activity averaged approximately 120 $\mu\text{mol/kg/min}$ on a dry weight basis (Godfrey et al., 1990b), whereas those of cat facial nerve root averaged 1673 $\mu\text{mol/kg dry}$

wt/min (Godfrey et al., 1990a), we concluded that the samples must have been from the facial nerve. The stapedius muscle homogenates would include the facial motoneuronal innervation as well as the muscle fibers. Estimates of stapedius muscle ChAT activity remote from its motoneuronal innervation have not been directly measured in cat but were obtained in rat (our unpublished work) by cutting and freeze drying sections of quickly frozen stapedius muscle and dissecting samples at a distance from acetylcholinesterase-positive spots, presumed to be motor endplates, seen in adjacent stained sections. The average ChAT activity for 4 such samples, as mean $\pm$ SEM, was 118 ± 23 μ mol/kg dry wt/min. Since the ChAT activity of rat stapedius muscle homogenate (Godfrey et al., 1990c) is about 5 times that of cat stapedius muscle homogenate (Godfrey et al., 1990b), the estimated ChAT activity of cat stapedius muscle not including its facial nerve innervation would be about 24 μ mol/kg dry wt/min.

3.4 Cuts that transected the OCB - OCB cuts

Cats A, D, F, H, and J all had lateral transections through the left OCB. Large differences between right and left sides were seen in both the IHC and OHC regions (Fig. 4). The OHC and IHC regions of the left cochlea showed minimal ChAT activity, while the OHC and IHC regions of the right cochlea revealed results comparable to those found in the control cats (Table 2). ChAT activity in the OSC region on both sides was generally low, with no difference between left and right sides.

3.5 Cuts that damaged the SOC – SOC lesion

Cats B, G, and K had lesions that affected more medial parts of the left side of the brain stem, transecting most or all of the left OCB and including damage to the left SOC. As with the more lateral OCB cuts, statistically significant differences were seen between the right and left sides of the IHC and first and second turn OHC regions, as well as between the left sides and controls (Fig. 5, Table 2). The decreases in the left, lesion-side, IHC regions were large, but not quite as complete as in the cats with the more laterally placed OCB cuts, possibly suggesting that a few OCB fibers were missed by the more medial cuts. The ChAT activities in the contralateral IHC regions were similar to those in the control cats and the cats with more lateral OCB cuts. For the OHC regions, on the other hand, there were bilateral decreases in ChAT activity, although larger ipsilaterally, with the ChAT activities on both sides significantly less than those in the control cats (except for third turn right OHC region by nonparametric tests). Again, activity in the OSC regions on both sides remained low.

3.6 Comparison of cochlea and cochlear nucleus effects

In addition to its innervation of the organ of Corti in the cochlea, the OCB has been shown to send branches to the cochlear nucleus (Brown et al., 1988; Ryan et al., 1990; Warr, 1992). We looked for correlations between OCB damage effects on IHC and OHC regions and those on rostral cochlear nucleus regions (Fig. 6). Among cats in which both cochlear nucleus and cochlea data were obtained, highly significant correlations were found between IHC and OHC region ChAT activities in the left (lesion side) cochlea and ChAT activities in the left rostralateral granular region of the cochlear nucleus, and high correlations were also found between the ChAT activities of hair cell regions and rostral anteroventral cochlear

nucleus region AA of Brawer et al. (1974). The correlation coefficient (r) and significance for left region AA with left IHC region were $r = 0.79$ and $P = 0.004$, and those for left region AA with left OHC region were $r = 0.91$ and $P = 0.00008$. Correlations between the right rostralateral granular region with right IHC region or right OHC region were not statistically significant (Fig. 6), nor were correlations between the right region AA and right IHC region ($r = 0.37$) or right OHC region ($r = 0.13$). Correlations between cochlea data and other cochlear nucleus regions for which results were obtained in the same cats, dorsal cochlear nucleus molecular, fusiform soma, and deep layers and caudal posteroventral cochlear nucleus (Godfrey et al., 1990a), were also examined, although less data were available for these comparisons. Among these regions, the only ones whose average ChAT activities showed correlations with cochlear regions that approached statistical significance were left dorsal cochlear nucleus molecular and fusiform soma layers: molecular layer with IHC region, $r = 0.82$, $P = 0.02$, and with OHC region, $r = 0.75$, $P = 0.05$; fusiform soma layer with IHC region, $r = 0.92$, $P = 0.004$, and with OHC region, $r = 0.78$, $P = 0.04$.

4. Discussion

4.1 Comparisons with previous quantitative histochemical studies

The results of this study, as those of our previous study of the rat cochlea (Godfrey and Ross, 1985), imply that the OCB provides all, or nearly all, the ChAT activity measured in the cochlea. The slight remaining activity in hair cell regions may merely represent incomplete loss of ChAT activity during the 7–10 day survival period following the OCB transection, although the somewhat higher remaining activity in the 3 cats with more medially located cuts that damaged the SOC may also include activity in some fibers that were missed by these cuts. These findings are consistent with earlier results for cat whole cochlea (Jasser and Guth, 1973).

The ChAT activities measured here for the cat hair cell regions are similar in magnitude to those previously reported for the guinea pig (Godfrey et al., 1976), and the presence of a decreasing gradient of activity from base to apex in the OHC region resembles results for guinea pig and for rat (Godfrey and Ross, 1985). The absence of any base-to-apex gradient in the IHC region differs from results for rat and guinea pig, wherein the activity in the IHC region of the apical turn was less than those in the more basal turns. The magnitudes of IHC and OHC ChAT activities in the cat are less than those measured for the basal and middle turns of the rat on an absolute basis, IHC 1012 vs. 4705 and OHC 661 vs. 2660, but similar when expressed as ratios of the ChAT activity in the cholinergic facial nerve root, which has about one fourth as high activity in cat as in rat (Godfrey et al., 1984): 1673 vs. 6853 $\mu\text{mol/kg dry wt/min}$. Based on published photographs (Stopp and Comis, 1978), it has been estimated that the OCB fibers occupy roughly a tenth of the volume of IHC and OHC regions (Godfrey et al., 1986). Thus, the ChAT activity in the OCB fibers and terminals of the cat may approach 10 $\text{mmol/kg dry wt/min}$, a value similar to that measured in the rat centrifugal labyrinthine bundle, consisting of OCB and centrifugal vestibular fibers (Godfrey et al., 1984). The ChAT activity in the OCB fibers and terminals of the rat organ of Corti would presumably be even much higher. This implies that the ChAT activity in the OCB

terminals is higher than that in the fibers, as reported for other systems (Fonnum et al., 1973).

4.2 Comparisons with anatomical information

The distribution of ChAT activity in the IHC and OHC regions correlates generally but not exactly with the described OCB distribution pattern (Liberman et al., 1990; Warr, 1992). The anatomical results indicate a decrease in OCB fiber population apically in both IHC and OHC regions, but we measured decreased ChAT activity apically only in the OHC region. Perhaps the ChAT activity in OCB terminals, which should be higher than that in the fibers, compensates for a lower density of fibers. The density of OCB terminals in the apical turn IHC region should be roughly similar to that in middle and basal turns based on the results of Liberman et al. (1990), since the higher number of terminals per auditory nerve fiber appears to balance the lower density of auditory nerve fibers in the apical region.

Our results are mostly consistent with the anatomical evidence for bilateral OCB projections. The lesions which damaged the SOC did not show any evidence for the minor LOC projections to the contralateral cochlea that have been described for the cat (Warr, 1992), since ChAT activities in the contralateral IHC region were just as high as in control cats and those with lateral cuts of the OCB. On the other hand, the contralateral effects on the OHC region ChAT activity of the lesions which damaged the SOC were consistent with a major contralateral projection of the MOC to the OHC region (Warr, 1992). For the cats with the SOC lesions, the decreases relative to control ChAT activities of the basal, middle, and apical turns were 56 %, 72 %, and 96 %, as high, respectively, in the contralateral OHC region as in the ipsilateral OHC region, in good agreement with the reported 70 % contralateral projection of the MOC in the cat (Warr, 1992). Also, the higher ChAT activity in the IHC region than in the OHC region is consistent with the larger number of LOC than MOC neurons in the cat (Warr, 1992).

Our results are also consistent with the anatomical evidence for OCB branches innervating the cochlear nucleus (Brown et al., 1988; Ryan et al., 1990; Warr, 1992), particularly the evidence for OCB innervation of granular regions.

Finally, our results, in comparison with our previous studies (Godfrey et al., 1976; Godfrey and Ross, 1985; Godfrey et al., 1986), are consistent with evidence for differences across species in details of the OCB innervation of the IHC and OHC regions of the cochlea (Warr, 1992).

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Abbreviations in text

ChAT	choline acetyltransferase
IHC	inner hair cells

IntraCN	intra-cochlear nucleus
LOC	lateral olivocochlear
MOC	medial olivocochlear
OCB	olivocochlear bundle
OHC	outer hair cells
OSC	outer supporting cells
r	correlation coefficient
SOC	superior olivary complex

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Highlights

Choline acetyltransferase (ChAT) catalyzes synthesis of transmitter acetylcholine

Cat cochlea inner and outer hair cell regions contain high activities of ChAT

Cat cochlea ChAT is associated with its innervation by the olivocochlear bundle

ChAT in the cat outer hair cell region has less activity in its more apical parts

ChAT in the cat inner hair cell region has similar activity in all three turns

Figure 1.

Tracings showing lesion location (gray area) in one transverse section from each of 9 cats, identified by large bold letter at lower left of each section, for which more complete maps have been presented previously (Godfrey et al., 1990a). Regional abbreviations are: A, anteroventral cochlear nucleus (thin zones lateral to A are granular regions); a, auditory nerve; C, corticospinal (pyramidal) tract; Cb, cerebellum; D, dorsal cochlear nucleus; F, facial motor nucleus; FG, facial genu; FR, facial nerve root; I, interstitial nucleus; L, lateral superior olivary nucleus; M, medial superior olivary nucleus; MT, trigeminal motor nucleus; N, medial nucleus of the trapezoid body; P, posteroventral cochlear nucleus; S, spinal trigeminal tract; T, trapezoid body; V, vestibular nerve root. Dorsal is up, left side is left, and medial and lateral directions are indicated with the 4 mm scale. Section numbers traced for the cats are: A 269, B 163, D 203, F 190, G 224, H 226, J 173, K 300, and L 214. Portions of the centrifugal labyrinthine bundle, containing the OCB, visualized in adjacent sections stained for acetylcholinesterase activity, are included for cats F, H, and J, as black strips marked by arrows. The lesion in cat D, which extended through the cochlear nucleus in the traced section, was not present at more rostral locations where the OCB exits the brain in the vestibular nerve root.

Figure 2.

Tracings of transverse sections showing the lesion location (gray area) in Cat D2.

Abbreviations and details as in Fig. 1. Section numbers are shown at lower left of each section, numbered sequentially from caudal (lower numbers) to rostral (higher numbers) for the 20 μ m thick sections.

Figure 3.

Modified box plots of cochlea data for sham-lesioned cats (C, E, I). Each vertical bar represents the range of data from the first to the third quartile, thus including the middle half of the data points. The horizontal line within each vertical bar marks the median data value. The lower fourth of the data points fall below the bottom of the vertical bar and the higher fourth above the top. Data are plotted for the left (L) and right (R) sides of each cochlear turn (1 basal, 2 middle, 3 apical) for the inner and outer hair cell regions and outer supporting cell region.

Figure 4.
Modified box plots of cochlea data for cats with lateral cuts of the left OCB (A, D2, F, H, J).
Details as in Fig. 3.

Figure 5.

Modified box plots of cochlea data for cats with cuts of the left OCB also damaging the left SOC (B, G, K). Details as in Fig. 3.

Figure 6.

Plots of average ChAT activities of IHC and OHC cochlear regions of individual cats vs. average ChAT activity for the rostralateral granular region of the same cat for the cats having both sets of data. The IHC and OHC data are the averages of all values for all three cochlear turns for each region. The rostralateral granular region data are from Fig. 4 of Godfrey et al. (1990a). Linear regression lines are included. Correlation coefficients and P values for significance are, for IHC left 0.918 and $P = 0.00007$; for OHC left 0.946 and $P = 0.00001$; for IHC right -0.031 not significant; for OHC right 0.456 not significant.

Table 1

Information about the lesions in the cats that provided cochlear tissues

Cat	Lesion
A	Complete lateral cut through left brain stem, just medial to the cochlear nucleus, completely transecting the left OCB, shown in Fig. 1 of Godfrey et al. (1990a)
B	Large damage to the left side of the brain stem, completely transecting the left OCB and encroaching partially on the left SOC, shown in Fig. 7 of Godfrey et al. (1990a)
C	Sham lesion; left cochlear nucleus exposed but no cut made, not included in Godfrey et al. (1990a)
D	Cut within left cochlear nucleus, shown in Fig. 8 of Godfrey et al. (1990a)
D2	Complete lateral cut through left brain stem, just medial to the cochlear nucleus, completely transecting the left OCB, not included in Godfrey et al. (1990a) but shown in Fig. 2
E	Sham lesion; left cochlear nucleus exposed but no cut made, not shown in Godfrey et al. (1990a)
F	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
G	Cut through left brain stem, transecting the left OCB and encroaching partially on the left SOC, transecting its contralateral projections, shown in Fig. 7 of Godfrey et al. (1990a)
H	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
I	Sham lesion; left cochlear nucleus exposed but no cut made, shown in Fig. 1 of Godfrey et al. (1990a)
J	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
K	Cut through left brain stem, transecting the left OCB and encroaching partially on the left SOC, transecting its contralateral projections, shown in Fig. 7 of Godfrey et al. (1990a)
L	Cut within left cochlear nucleus, shown in Fig. 8 of Godfrey et al. (1990a)

Table 2

Statistical significances of differences for cat cochlea regions, lesioned side (Left) vs. contralateral (Right) and each vs. Control

Control Mean $\pm$ SEM (N) (Sham + IntraCN)						
	IHC1	975 $\pm$ 122 (24)	IHC2	1049 $\pm$ 161 (25)	IHC3	1427 $\pm$ 152 (25)
	OHC1	738 $\pm$ 81 (24)	OHC2	583 $\pm$ 74 (26)	OHC3	231 $\pm$ 57 (28)
	OSC1	219 $\pm$ 130 (22)	OSC2	61 $\pm$ 41 (25)	OSC3	5 $\pm$ 3 (24)
	Mean $\pm$ SEM (N)			P values for significance (t-test, Mann-Whitney test) ^a		
Lesion	Region	Left	Right	Left vs. Right	Left vs. Control	Right vs. Control
OCB cut	IHC1	106 $\pm$ 21 (15)	1169 $\pm$ 220 (14)	<0.001, 0.001	<0.001, <0.001	ns, ns
	IHC2	78 $\pm$ 20 (15)	1219 $\pm$ 184 (14)	<0.001, <0.001	<0.001, <0.001	ns, ns
	IHC3	33 $\pm$ 8 (15)	1137 $\pm$ 104 (16)	<0.001, <0.001	<0.001, <0.001	ns, ns
	OHC1	23 $\pm$ 6 (14)	880 $\pm$ 176 (15)	<0.001, <0.001	<0.001, <0.001	ns, ns
	OHC2	118 $\pm$ 64 (14)	473 $\pm$ 64 (15)	<0.001, <0.001	<0.001, <0.001	ns, ns
	OHC3	7 $\pm$ 2 (15)	141 $\pm$ 34 (15)	0.001, <0.001	<0.001, <0.001	ns, ns
SOC lesion	OSC1	22 $\pm$ 10 (11)	36 $\pm$ 16 (14)	ns, ns	ns, ns	ns, ns
	OSC2	2 $\pm$ 4 (12)	34 $\pm$ 21 (15)	ns, ns	ns, ns	ns, ns
	OSC3	2 $\pm$ 1 (15)	7 $\pm$ 3 (16)	ns, ns	ns, ns	ns, ns
	IHC1	238 $\pm$ 80 (9)	1383 $\pm$ 267 (12)	0.001, 0.001	<0.001, 0.001	ns, ns
	IHC2	124 $\pm$ 57 (9)	1488 $\pm$ 333 (8)	0.004, 0.009	<0.001, 0.002	ns, ns
	IHC3	407 $\pm$ 162 (9)	1483 $\pm$ 266 (9)	0.004, 0.005	<0.001, 0.001	ns, ns
	OHC1	96 $\pm$ 39 (9)	378 $\pm$ 70 (9)	0.004, 0.012	<0.001, <0.001	0.002, 0.010
	OHC2	49 $\pm$ 11 (10)	200 $\pm$ 37 (9)	0.003, 0.009	<0.001, <0.001	<0.001, 0.004
	OHC3	27 $\pm$ 9 (9)	35 $\pm$ 13 (9)	ns, ns	0.001, ns	0.002, ns
	OSC1	13 $\pm$ 4 (9)	162 $\pm$ 82 (6)	ns, ns	ns, ns	ns, ns
	OSC2	23 $\pm$ 8 (10)	5 $\pm$ 4 (8)	ns, ns	ns, ns	ns, ns
	OSC3	20 $\pm$ 8 (9)	6 $\pm$ 4 (9)	ns, ns	ns, ns	ns, ns

^aData sets that diverged from normality by Kolmogorov-Smirnov and/or Shapiro-Wilk tests were those for Control OHC3 and all OSC; OCB cut IHC3 Left, OHC1&2 Left, and OSC1 Left & Right and 2 Right; and SOC lesion IHC1,2&3 Left and OHC1&2 Left & 3 Right. Differences were tested for statistical significance by both parametric and non-parametric tests: single-factor analysis of variance followed by t-tests and Kruskal-Wallis followed by Mann-Whitney tests; ns = not statistically significant at $P < 0.05$.