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Choline Acetyltransferase Activity in the Hamster Central Auditory System and Long-Term Effects of Intense Tone Exposure

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Abstract

Acoustic trauma often leads to loss of hearing of environmental sounds, tinnitus, in which a monotonous sound not actually present is heard, and/or hyperacusis, in which there is an abnormal sensitivity to sound. Research on hamsters has documented physiological effects of exposure to intense tones, including increased spontaneous neural activity in the dorsal cochlear nucleus. Such physiological changes should be accompanied by chemical changes, and those chemical changes associated with chronic effects should be present at long times after the intense sound exposure. Using a microdissection mapping procedure combined with a radiometric microassay, we have measured activities of choline acetyltransferase (ChAT), the enzyme responsible for synthesis of the neurotransmitter acetylcholine, in the cochlear nucleus, superior olive, inferior colliculus, and auditory cortex of hamsters 5 months after exposure to an intense tone compared with control hamsters of the same age. In control hamsters, ChAT activities in auditory regions were never more than one-tenth of the ChAT activity in the facial nerve root, a bundle of myelinated cholinergic axons, in agreement with a modulatory rather than a dominant role of acetylcholine in hearing. Within auditory regions, relatively higher activities were found in granular regions of the cochlear nucleus, dorsal parts of the superior olive, and auditory cortex. In intense-tone-exposed hamsters, ChAT activities were significantly increased in the anteroventral cochlear nucleus granular region and the lateral superior olivary nucleus. This is consistent with some chronic upregulation of the cholinergic olivocochlear system influence on the cochlear nucleus after acoustic trauma.

Keywords

acetylcholine; cochlear nucleus; granular regions; olivocochlear bundle; tinnitus

High-intensity sound exposure has been shown to produce damage to and physiological changes in the cochlear nucleus (Kim et al., 2004; Feng et al., 2011; Kaltenbach, 2011; Vogler et al., 2011; Dehmel et al., 2012; Du et al., 2012; Pilati et al., 2012) and other central auditory regions (Seki and Eggermont, 2003; Mulders and Robertson, 2011). Changes in these brain regions can become chronic and contribute to hearing disorders such as hearing loss, tinnitus, and hyperacusis (Henry et al., 2005; Roberts et al., 2010; Eggermont, 2012). Chemical changes in the central auditory system are likely to be associated with such disorders, and some have been reported previously, including changes in energy and neurotransmitter metabolism, neurotransmitter receptors, gene expression, and markers of plasticity (Ryan et al., 1992; Abbott et al., 1999; Milbrandt et al., 2000; Michler and Illing, 2003; Zhang et al., 2003a,b; Jin et al., 2006; Godfrey et al., 2008, 2012; Wang et al., 2009, 2011; Dong et al., 2010; Kraus et al., 2011).

Choline acetyltransferase (ChAT), the enzyme that catalyzes the synthesis of the neurotransmitter acetylcholine, is found throughout neurons that employ acetylcholine as transmitter, termed *cholinergic neurons*, and is a well-established marker for such neurons (Godfrey et al., 1985). Previous studies have found evidence for a small to moderate amount of ChAT activity in the cochlear nucleus (Godfrey et al., 1983, 1990), associated primarily with fibers and terminals of its centrifugal innervation, which probably derives mostly from the superior olive (Godfrey et al., 1987, 1990; Vetter et al., 1993; Sherriff and Henderson, 1994; Yao and Godfrey, 1998; Mellott et al., 2011).

We previously found increased ChAT activity in some parts of the hamster cochlear nucleus at 8 days and at 2 months after exposure to an intense tone, particularly in granular regions and in the deep layer of the dorsal cochlear nucleus (DCN; Jin et al., 2006). These increases suggest an upregulation of cholinergic inputs to the cochlear nucleus following acoustic trauma. However, there is still a question of how long such increases endure and whether they are also found in other auditory nuclei, including those that contribute cholinergic inputs to the cochlear nucleus. To address these questions, we extended our measurements of ChAT activity for the hamster cochlear nucleus to 5 months after intense tone exposure, a time when physiological changes in the DCN are still prominent (Kaltenbach et al., 2000), and also measured ChAT activities in some higher level auditory centers, including the superior olive, inferior colliculus, and auditory cortex. Our hypothesis was that, if increases of ChAT activity in the cochlear nucleus after acoustic trauma are chronic and reflect upregulation of cholinergic inputs from the superior olive, then such increases should be apparent at this long postexposure time in the cochlear nucleus and in superior olivary nuclei that project to it, but possibly not in the inferior colliculus or auditory cortex. This study used tissue from the same group of hamsters as for our concomitant measurements of amino acid concentrations in central auditory regions (Godfrey et al., 2012).

MATERIALS AND METHODS

Other than using open-field rather than monaural intense tone exposure in the present study, the methods were the same as for our previous study (Jin et al., 2006), including microdissection of samples from freeze-dried tissue sections for measurement of ChAT activity by a radiometric microassay.

Animals and Intense Tone Exposure

This study used tissue from the same 12 male Syrian hamsters as for our study of long-term effects of intense tone exposure on amino acid concentrations (Godfrey et al., 2012). The hamsters, obtained from Charles River and weighing 145–178 g, were divided into two groups: six were exposed to a 10-kHz intense tone in the open field at a level of 127 dB SPL for 4 hr, whereas the other six were placed in a sound-attenuation room for a similar period but in the absence of an exposure tone. Tissue from four additional, similarly treated male hamsters, three exposed and one control, was included in anteroventral cochlear nucleus (AVCN, two exposed), superior olive (all four), and inferior colliculus (one control and one exposed) measurements. All hamsters survived for 140–145 days after treatment with or without tone exposure. Treatment of animals was approved by and was in accordance with existing policies and regulations of the Institutional Animal Care and Use Committee and the National Institutes of Health.

Isolation of Tissue Samples

After euthanasia of hamsters by decapitation while they were deeply anesthetized with ketamine +xylazine (25 mg +4 mg, i.m.), brains were isolated and frozen within 3–4 min in Freon (Fisher Friendly Freeze-It) chilled to its freezing point with liquid nitrogen. Frozen tissue blocks were stored in double-airtight containers at -80°C until sectioning.

Each brain was placed into a cryostat at -20°C and cut into transverse sections $20\text{ }\mu\text{m}$ thick. At the cochlear nucleus level, every section was saved, alternate ones for freeze drying and staining. Rostral to the cochlear nucleus, continuing through the auditory cortex, two sequential sections of every six were saved, one for freeze drying and one for staining. Sections to be freeze dried were placed into aluminum racks (Lowry and Passonneau, 1972) kept on blocks of dry ice in the cryostat. These racks were subsequently put into a glass vacuum tube and transferred to a freezer set at -40°C . The vacuum tube was connected to a vacuum pump through a dry ice trap, and the water was removed from the sections by evacuating overnight. The freeze-dried sections were stored in the vacuum tube under vacuum below -20°C . Adjacent sections were melted onto slides and Nissl-stained with thionin. Sections for two control hamsters in the main series were not used in the study, one because of a problem with sectioning and another because of a problem with freeze drying.

Freeze-dried tissue was dissected into samples for assay at $\times 25$ magnification in a room with relative humidity below 50%. Sample locations were mapped within each region via a drawing attachment on a Wild dissecting microscope (Godfrey and Matschinsky, 1976). Sections approximately halfway through the rostral part of AVCN, DCN, superior olive, inferior colliculus, and auditory cortex were dissected. With only a couple of exceptions, the dissection for each region followed the same plan for each hamster (Fig. 1) so that paired sample-by-sample comparisons could be made between exposed and control hamsters. Identification of regions and auditory cortex layers was aided by reference to adjacent thionin-stained sections, an atlas of the hamster brain (Morin and Wood, 2001), and a published detailed description of rat auditory cortex layers (Games and Winer, 1988). The dissection plans (Fig. 1) for the DCN, inferior colliculus, and auditory cortex were similar to those used in our study of amino acid concentrations (Godfrey et al., 2012), whereas the

plan for the main part of the AVCN was modified to include medial and lateral samples, because we have previously found differences of ChAT activity between its medial and its lateral portions (Jin et al., 2005, 2006). Sampling from the DCN avoided its most dorsomedial region, where very high ChAT activities have previously been measured in some but not all samples, complicating comparisons between control and exposed hamsters (Jin et al., 2006). These sporadically high ChAT activities are apparently related to some cholinergic, possibly strial route, olivocochlear branch fibers (Osen et al., 1984) traversing and terminating in parts of this region (Jin et al., 2006). The plan for the superior olive included samples within the major nuclei and in neighboring locations. Within the LSO, samples were cut from lateral to medial to approximate the LSO tonotopic axis (Tsuchitani and Boudreau, 1966), because we previously found a gradient of the density of ChAT-positive somata and ChAT activity along this axis in rats (Yao and Godfrey, 1998; Jin et al., 2005). For comparison with auditory regions, facial nucleus and nerve root were included in assays, as examples of purely cholinergic neuron populations, and hippocampal oriens (including alveus) and pyramidal layers, from the part of the hippocampus deep to auditory cortex, as another forebrain region besides auditory cortex.

Samples were weighed on quartz-fiber microbalances (Lowry and Passonneau, 1972) and then loaded into 400- μ l-capacity polyethylene tubes for radiometric assay of ChAT activity. Samples of both control and exposed hamsters were included in the same assays to minimize chances of any differences resulting from technical factors rather than real differences between the two groups.

ChAT Assay

The assay for ChAT activity was basically the same as that used previously (Godfrey et al., 1983, 1987, 1990; Jin et al., 2005, 2006), which was adapted from Fonnum (1969). To each sample tube, as well as two empty tubes used to measure background activity, were added 5 μ l of 92 mM sodium phosphate-buffered medium, pH 7.4, containing 80 μ M [acetyl-1- 14 C]coenzyme A, 6 mM choline chloride, 0.3 M NaCl, 1 g/liter bovine serum albumin, 1 ml/liter Triton X-100, and 80 μ M neostigmine methyl sulfate. After a 30-min incubation at 37–38°C, a 43- μ l aliquot of ice-cold 15 mg/ml sodium tetra-phenyl boron in 3-heptanone was added to each tube to end the incubation and extract [14 C]acetylcholine product from the aqueous phase. A 40- μ l aliquot of the heptanone phase from each tube was then washed with 100 μ l of 0.5 mg/ml sodium tetraphenyl boron in 10 mM sodium phosphate buffer, pH 7.8, in a second tube, to remove any extracted [14 C]acetate formed in a deacylase side reaction. After this, a 31- μ l aliquot of the heptanone phase was added to 5 ml of scintilene/scintiverse cocktail for scintillation counting. Recovery of [14 C]acetylcholine from the incubation to the scintillation fluid was 100% when the aliquot sizes were taken into account. When rat whole-brain homogenate was assayed, a ChAT activity of 116 μ mol/kg wet weight/min was measured, similar to what we have measured previously (Godfrey et al., 1983).

Data Analysis

Measured ChAT activities (as μ mol/kg dry wt/min) were plotted onto the maps of the dissected sections (Fig. 1). The data for all samples within defined regions were averaged

for control and for exposed hamsters (Table I). Data were tested by the Kolmogorov-Smirnov procedure to determine whether they were approximately normally distributed, as a criterion for applying *t*-tests for significant differences between exposed and control means. Because the data for some regions in one or both groups did not satisfy the criterion for normality at $P < 0.05$, a nonparametric test of significance, the Wilcoxon-Mann-Whitney (WMW) test, was also applied (Zar, 1984). Differences between exposed and control means with $P < 0.05$ were considered as statistically significant, although, with the large number of comparisons made, some of these might have occurred by chance. Therefore, the differences with the lowest P values with both statistical tests (Table I) were considered the most likely to be real.

Materials

Glass vacuum tubes for freeze drying and for storage of freeze-dried sections were from Ace Glass (Vineland, NJ). [Acetyl-1- ^{14}C]coenzyme A was purchased from New England Nuclear, Perkin Elmer Life Science (Boston, MA). The one lot ordered was sufficient for all the assays in this study. Other chemicals were from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

RESULTS

ChAT Activities in Control Hamsters

Activities of ChAT were much lower in all other regions than in the facial nucleus and nerve root (Table I). The facial nerve root should represent a pure population of myelinated cholinergic axons, whereas the nucleus contains the somata, dendrites, and initial portions of axons of the cholinergic facial motoneurons, as well as surrounding glia, various afferent axons, and possibly a few neurons that do not innervate muscles (Travers, 1995). The highest ChAT activity in any other region did not reach one-tenth that in the facial nerve root, suggesting that cholinergic structures are a minority of the neuronal components of all these regions. In particular, ChAT activities in some parts of the cochlear nucleus and inferior colliculus were about 1% or less of that in the facial nerve root. For regions with such low values, numbers of cholinergic structures must be very small, so that a difference of just one or a few cholinergic axons in one sample compared with another can make a large relative contribution to its ChAT activity. This may explain, as in our previous study (Jin et al., 2006), the relatively large standard errors for many of these regions, which were generally similar between the control and exposed groups.

In the cochlear nucleus, the highest ChAT activities were measured in granular regions. Within the DCN, ChAT activity increased with distance from the surface (Fig. 2). The highest ChAT activities in the superior olive were measured dorsally, in the dorsal periolivary nucleus and dorsal to it. The trapezoid body, containing the majority of fibers connecting the cochlear nucleus to the superior olive, had low ChAT activity.

Within the inferior colliculus, the highest ChAT activities were found in its lateral part (Fig. 3). The periaqueductal gray region ventromedial to the colliculus contained higher activity than any part of the inferior colliculus.

Activities in layers of the auditory cortex were higher than in any other auditory system region except for the dorsal periolivary nucleus (Table I). There was a small trend toward lower ChAT activities in deeper layers, especially layer VI, and the average activity in the external capsule deep to the auditory cortex was less than one-fifth that in layer VI.

ChAT Activities in Intense-Tone-Exposed Hamsters

An almost 40% increase of ChAT activity was measured in the granular region lateral to the AVCN of exposed hamsters, which was statistically significant (Table I). The higher exposed-hamster average in this granular region occurred in both its ventral and its dorsal portions (Fig. 4), but only the larger difference in the ventral portion reached statistical significance by both *t*-test ($P < 0.005$) and WMW test ($P = 0.035$). In all other parts of the cochlear nucleus, average ChAT activities in exposed hamsters were similar to or lower than those in controls (Fig. 4). Although the decrease in the main part of the AVCN as a whole did not reach statistical significance (Table I), the decrease in just its ventral portion (Fig. 4, two sample locations combined) was statistically significant ($P = 0.02$ by both *t*-test and WMW test) as well as the decrease in the ventrolateral sample location ($P = 0.04$ by *t*-test and 0.02 by WMW test).

A statistically significant 40% higher ChAT activity was measured in the LSO of exposed hamsters compared with controls (Table I). The most lateral portion had the highest exposed-to-control ratio (1.46) and the most medial portion the lowest (1.25), although the increases over control at individual sample locations (Fig. 4) did not reach statistical significance. The average activity in the VNTB was 32% higher in the exposed hamsters, but the difference did not reach statistical significance because of large variations of activity among samples. With the VNTB subdivided into lateral and medial portions (Fig. 4), it was seen that the higher values in exposed hamsters were in its lateral portion, where the average was twice as high as in controls, and the difference approached statistical significance ($P = 0.05$ by both *t*-test and WMW test).

A higher ChAT activity was also measured in the facial nerve root of exposed hamsters compared with controls, and this difference reached statistical significance. The exposed-to-control ratio of ChAT activities in the facial nerve root was higher in the more dorsal location (Fig. 4), where the difference between groups approached statistical significance ($P = 0.05$ by both *t*-test and WMW test).

No differences between control and exposed hamsters were found in the inferior colliculus or auditory cortex (Table I). Sample-by-sample comparisons in the inferior colliculus revealed no statistically significant differences between exposed and control hamsters by either *t*-test or WMW test.

DISCUSSION

Comparisons With Previous Measurements for Hamster

The ChAT activities for control hamster cochlear nucleus regions were generally similar to those previously reported (Jin et al., 2006). Differences among DCN layers were greater in the present study, but there was the same trend for ChAT activity to increase with depth,

unlike the case in rats and cats, in which the highest DCN ChAT activities were in the fusiform soma layer (Godfrey et al., 1983, 1987, 1990). The average ChAT activities for control hamster facial nucleus and nerve root were more than twice those we previously reported for hamsters (Jin et al., 2006), resembling those for rats (Godfrey et al., 1983). This could relate to a difference between groups of hamsters (those used previously were younger), a technical difference between different lots of radiolabeled substrate (the one lot used for all assays in the present study differed from that used for the previous measurements), and/or a difference in sample size (the samples for the previous assays were about five times as large as those for the present study). Differences among individual animals in facial system ChAT activity have been reported previously (Godfrey et al., 1977, 1984); different purities of radiolabeled substrate may affect ChAT activities (Fonnum, 1975), and conversion of more than 10% of available substrate to product by larger amounts of tissue can result in decreased ChAT reaction rates (Fonnum, 1975). Our previous results suggested increased capacity to synthesize acetylcholine, through increased ChAT activity, in the deep layer of the DCN and in granular regions up to 2 months after acoustic trauma. The results of the present study suggest that only the change in granular regions remains at 5 months after exposure.

Comparisons to ChAT Activity Distributions in Other Mammals

As reported previously (Jin et al., 2006), the distribution of ChAT activities in hamster cochlear nucleus resembles that in cat (Godfrey et al., 1990) more than that in rat (Godfrey et al., 1983, 1987; Jin et al., 2005). This may suggest that a large proportion of the ChAT activity in hamster cochlear nucleus, as in cat, is related to innervation from branches of the olivocochlear system (Godfrey et al., 1990; Jin et al., 2006). The lower ChAT activities in hamster cochlear nucleus than in rat may also correlate with fewer ChAT-positive olivocochlear neurons (Raji-Kubba et al., 2002; Sánchez-González et al., 2003). Consistent with this possibility, the average ChAT activities in hamster LSO and VNTB were less than 30% of those in rat (Godfrey et al., 1983). Also, unlike the case in rat (Jin et al., 2005), we did not find a lateral-to-medial increasing gradient of ChAT activity in hamster LSO. This is consistent with the absence of a clear lateral-to-medial population density gradient of retrogradely labeled olivocochlear neurons in hamster LSO (Sánchez-González et al., 2003).

The relatively low ChAT activities for hamster inferior colliculus are similar to those reported for cat inferior colliculus (Adams and Wentholt, 1979), but the highest values in cats were found in medial rather than lateralmost parts. Measured ChAT activities in rat inferior colliculus, although higher than those in hamster, were also relatively low compared with most other brain regions, with the highest activities close to, but not at, the lateral surface (Godfrey and Ross, 1990).

The average of the ChAT activities for hamster auditory cortex layers is comparable, with appropriate unit conversions, to values reported for whole auditory cortex of rat (Wenk et al., 1980; Ross et al., 1995) but about twice as high as that for mouse (Contreras and Bachelard, 1979). The values for hippocampus stratum oriens (+alveus) and stratum pyramidale are, with appropriate conversions, about 85% of those reported previously for rat superior hippocampal region (Fonnum, 1970).

Increases of ChAT activity in Intense-Tone-Exposed Hamsters and Functional Considerations in Relation to Tinnitus

Although we did not study the long-term effects of intense tone exposure on neural responses and spontaneous activity in the present groups of animals, we have found that animals examined 1–2 weeks after tone exposure, using conditions identical to those used in this study, show severe threshold shifts across much of the tonotopic range of the inferior colliculus (unpublished observations); when responses were observed, they tended to be weak and displayed thresholds of 84 dB SPL and above. We have previously found that hamsters exposed to a 10-kHz tone of duration and level similar to those used here showed loss of both inner and outer hair cells in a circumscribed region of the cochlear basal turn; however, damage to or loss of stereocilia was much more widespread, extending over much of the basal half of the cochlear partition (Kaltenbach et al., 1992). Furthermore, loss of inner hair cell stereocilia has been found to be sufficient for the induction of irreversible hearing loss (Liberman and Dodds, 1984). It therefore seems likely that the exposed animals used here for measures of ChAT activity would have experienced similarly dramatic threshold shifts, which would not have recovered within our 5-month postexposure survival times.

Although the effects of intense tone exposure have been reported to be more severe in some individual animals than in others (Kaltenbach et al., 2000; Wang et al., 2009; Dehmel et al., 2012), the relative variations of ChAT activities among exposed hamsters (Table I) were generally similar to those among controls. Our results suggest that, after acoustic trauma, there are small chronic increases in the capacity to produce acetylcholine for neurotransmission in at least some cochlear nucleus granular regions, particularly that lateral to the AVCN. Increased ChAT activity in the LSO and possibly also in the lateral part of the VNTB, the part where ChAT-immunopositive medial olivocochlear neuron somata are concentrated in rats (Sherriff and Henderson, 1994; Yao and Godfrey, 1998), and at the rostrocaudal level of the LSO, where we obtained our VNTB samples, in hamsters (Fig. 5 in Sánchez-González et al., 2003), further suggests that the increased ChAT activity in granular regions may represent increased activity in olivocochlear branches innervating them (Brown et al., 1988). Previous work has more strongly implicated the medial olivocochlear system (Kraus et al., 2011), deriving predominantly from the VNTB, whereas our results more strongly implicate the lateral olivocochlear system, deriving predominantly from the LSO in rodents (Warr, 1992). Many granule cells form excitatory glutamatergic synapses in the DCN molecular layer with dendrites of fusiform and cartwheel cells (Mugnaini et al., 1980; Godfrey et al., 1997), and there is evidence that acoustic trauma can affect the activity of fusiform and cartwheel cells (Chang et al., 2002; Brozoski et al., 2002; Finlayson and Kaltenbach, 2009; Pilati et al., 2012). The mechanisms involved may include alterations of acetylcholine neurotransmission (Chang et al., 2002; Kaltenbach and Zhang, 2007). Thus, increased cholinergic olivocochlear activation of granule cells following acoustic trauma could lead to increased release of glutamate onto DCN fusiform and cartwheel cell dendrites (Chen et al., 1999), eventually resulting in increased fusiform cell spontaneous activity (Brozoski et al., 2002; Finlayson and Kaltenbach, 2009), as discussed previously (Kaltenbach and Godfrey, 2008).

The tendency for changes of ChAT activity, whether increases in granular regions, LSO, VNTB, and MNTB or decreases in the main part of AVCN, to be larger in more ventral and/or lateral locations, corresponding to relatively lower frequency parts of AVCN, LSO, VNTB, and MNTB (Collinge and Schweitzer, 1991; Friauf, 1992), is potentially interesting but requires further confirmation. The AVCN results may correlate with recent evidence that changes in the AVCN are involved in tinnitus generation (Vogler et al., 2011; Gu et al., 2012).

The increased ChAT activity in the facial nerve root of the exposed hamsters was unexpected. Although the relative increase was small, it cannot be easily correlated with the very small component of the facial nerve that innervates the stapedius muscle (Lyon, 1978). Possibly, the major chronic hearing loss and tinnitus that the exposed hamsters probably experienced indirectly affected facial muscle activity.

Acoustic trauma has been reported to result in degeneration of nerve fibers and terminals in the cochlear nucleus, both ventral (Bilak et al., 1997; Kim et al., 2004; Feng et al., 2011) and dorsal (Du et al., 2012) parts, followed by later regeneration, at least in the ventral part (Bilak et al., 1997; Kim et al., 2004; Kraus et al., 2011). These changes should result in loss of substances specific to the degenerated fibers and terminals, increases of those related to glial hypertrophy and growth of new nerve fibers and synapses, and secondary changes in postsynaptic neurons. The cholinergic system changes reported here and previously (Chang et al., 2002; Jin et al., 2006; Kaltenbach and Zhang, 2007) might represent new growth of cholinergic axons and synapses in granular regions to replace degenerated fibers and terminals using other neurotransmitters. Type 2 auditory nerve fibers, which synapse with outer hair cells in the cochlea, terminate in granular regions (Brown, 1993), sometimes on the same neurons that receive olivocochlear terminals (Benson and Brown, 2004), and may be strongly impacted by acoustic trauma, which can produce profound loss of outer hair cells (Bilak et al., 1997; Kim et al., 2004).

Although our results for ChAT do not support changes in acetylcholine neurotransmission in the inferior colliculus or auditory cortex, our previous results suggest that there are changes in excitatory and inhibitory amino acid neurotransmission in those regions (Godfrey et al., 2012). The changes for the cholinergic system, combined with changes reported previously for amino acids (Abbott et al., 1999; Milbrandt et al., 2000; Godfrey et al., 2008, 2012; Wang et al., 2009, 2011; Dong et al., 2010), although not large in themselves, could be sufficient, together with possible changes in other neurotransmitter systems, to produce distortions of hearing. Depending on the excitatory or inhibitory nature of lost and newly developed synapses, particular neural circuits could have altered activity, giving rise to the consequences of acoustic trauma, such as tinnitus.

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Fig. 1.

Examples of microdissection maps, each from one hamster, for transverse sections through the four major auditory regions sampled. A similar dissection strategy was used for all hamsters except for inferior colliculus in one control and one exposed hamster and some peripheral parts of the superior olive. The 1 mm scale at left also shows dorsal (D), ventral (V), lateral (L), and medial (M) directions. Thin solid lines are tracings of the cuts made to obtain samples; thick solid lines are tracings of the edges of the freeze-dried sections; dashed lines are tracings of internal boundaries seen in the freeze-dried sections; and dotted lines are tracings of internal boundaries from adjacent thionin-stained sections. Letters and numerals within samples identify those for which data were averaged for Table I.

Abbreviations: for cochlear nucleus, AVCN, anteroventral cochlear nucleus, including main (m), subgranular (s and yellow shading), and granular (g) regions, and nearby trapezoid body (T); DCN, dorsal cochlear nucleus, including molecular (m), fusiform soma (f and yellow shading), and deep (d) layers, and nearby facial nucleus (F); for superior olive, lateral superior olivary nucleus (l), medial nucleus of the trapezoid body (n), superior paraolivary nucleus (s), ventral nucleus of the trapezoid body (v), dorsal periolivary nucleus (d), and nearby facial nerve root (F); for inferior colliculus, dorsal (d), ventral (v), lateral (l), and medial (m) parts and periaqueductal gray (P); and for auditory cortex, layers I through VI,

underlying external capsule (E), and nearby hippocampal oriens (+alveus; o) and pyramidale (p) layers. Compared with inferior colliculus subdivisions as defined for rats (Paxinos and Watson, 1998; Loftus et al., 2008), our most dorsal “d” sample, medial “d” sample of the second row, and “m” sample collectively approximate its dorsal cortex (pink shading), the ventral part plus the lateral “d” sample of the second row its central nucleus (yellow shading), and the lateral part its lateral, or external, cortex (green shading). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 2.

Distribution of average choline acetyltransferase (ChAT) activities, in $\mu\text{mol/kg dry wt/min}$, in cochlear nuclear, superior olivary, and facial samples of control hamsters, recorded on the maps shown in Figure 1. Regional identifications and boundaries and scale and directional bars are as in Figure 1. Numbers of values averaged are 4 for DCN, 2 for facial nucleus (F), 4–5 for AVCN and vicinity except 3 for granular region (g), 6 for trapezoid body (T), 4 for facial nerve root (F), 4 for LSO (l), 3 for VNTB (v), DPO (d), and SPO (s), 2 for MNTB (n), 4 for the central superior olivary location, and 7 for the location dorsal to DPO and SPO. The thin piece of AVCN subgranular region between the subgranular and granular samples was not assayed.

Fig. 3.

Distribution of average $\pm$ SEM choline acetyltransferase (ChAT) activities, in μ mol/kg dry wt/min, in inferior colliculus samples, recorded on the map shown in Figure 1. Data for three control hamsters are presented above data for three exposed hamsters in which the dissection followed the same plan. There were no statistically significant differences between the groups. The higher mean and SEM values in medially located samples for control hamsters resulted from relatively high activities in one control hamster. Regional identifications and boundaries and scale and directional bars are as in Figure 1.

Fig. 4.

Distribution of ratios of choline acetyltransferase (ChAT) activities in exposed hamsters to those in control hamsters for comparable samples of cochlear nucleus and superior olive, recorded on the maps shown in Figure 1. Regional identifications and boundaries, abbreviations, and scale and directional bars are as in Figure 1. Control data are shown in Figure 2. Numbers of values averaged for control and exposed hamsters are 4 and 6 for DCN samples, 3 and 8 for AVCN granular (g) samples, 5 and 8 for AVCN subgranular (s) samples, 4 and 8 for AVCN main (m) samples, 6 and 6 for trapezoid body (T) samples, 4 and 6 (ventral) or 9 (dorsal) for facial nerve root (F) samples, 4 and 6 for lateral superior olivary nucleus (l) samples, 3 and 7 for ventral nucleus of the trapezoid body (v) samples, 2 and 5 for medial nucleus of the trapezoid body (n) samples, 3 and 8 for superior paraolivary nucleus (s) samples, and 3 and 7 for dorsal periolivary nucleus (d) samples.

TABLE I

Activities of Choline Acetyltransferase in Central Auditory Regions of Control and Intense-Tone-Exposed Hamsters^a

Region	Control	Exposed	Exp/Cont	t-test P	WMW P
AVCN main	79 ±10 (17,4) ^a	61 ±5 (35,8)	0.77	0.113	0.156
AVCN subgranular	114 ±13 (5,4)	95 ±14 (8,8)	0.83	0.342	>0.20
AVCN granular	265 ±16 (8,4)	366 ±20 (16,8)	1.38	0.0008	0.0028
DCN molecular layer	76 ±16 (8,4)	65 ±16 (12,6)	0.86	0.650	>0.20
DCN fusiform soma layer	174 ±14 (8,4)	160 ±16 (12,6)	0.92	0.515	>0.20
DCN deep layer	216 ±20 (8,4)	170 ±20 (12,6)	0.79	0.126	0.100
Trapezoid body	35 ±10 (6,4)	51 ±13 (6,6)	1.45	0.365	>0.20
LSO	142 ±12 (14,5) ^a	199 ±11 (24,8)	1.40	0.0016	0.0093
VNTB	138 ±20 (6,3)	182 ±32 (14,7) ^a	1.32	0.252	>0.20
MNTB	164 ±66 (6,4) ^a	139 ±37 (13,8) ^a	0.77	0.747	>0.20
SPO	176 ±60 (5,3) ^a	202 ±21 (16,8)	1.15	0.691	>0.20
DPO	593 ±63 (3,3)	396 ±40 (7,6)	0.67	0.0607	0.100
Inferior colliculus dorsal	40 ±6 (13,4) ^a	44 ±4 (13,4)	1.10	0.588	>0.20
Inferior colliculus ventral	53 ±14 (16,4) ^a	47 ±5 (16,4)	0.88	0.675	0.100
Inferior colliculus lateral	102 ±8 (11,4)	102 ±9 (11,4)	1.00	0.977	>0.20
Inferior colliculus medial	59 ±17 (4,4)	31 ±10 (4,4)	0.53	0.225	>0.20
Periaqueductal gray	205 ±85 (3,3)	165 ±12 (3,3)	0.80	0.686	>0.20
Auditory corte ^a layer I	425 ±17 (10,4)	429 ±17 (11,6)	1.01	0.872	>0.20
Auditory cortex layer II	442 ±17 (10,4)	411 ±20 (12,6) ^a	0.93	0.245	>0.20
Auditory cortex layer III	369 ±32 (10,4)	376 ±27 (12,6)	1.02	0.944	>0.20
Auditory cortex layer IV	400 ±26 (10,4)	378 ±18 (12,6)	0.94	0.488	>0.20
Auditory cortex layer V	376 ±22 (10,4)	381 ±14 (12,6)	1.01	0.866	>0.20
Auditory cortex layer VI	334 ±21 (10,4)	343 ±11 (12,6)	1.03	0.594	>0.20
External capsule	61 ±15 (5,3)	88 ±24 (5,5)	1.44	0.367	>0.20
Facial nerve root	7,361 ±314 (8,3)	8,527 ±267 (15,8)	1.16	0.0118	0.0175
Facial nucleus	5,374 ±209 (8,2)				
Hipp stratum oriens	290 ±22 (8,4)				

Region	Control	Exposed	Exp/Cont	t-test P	WMW P
Hipp stratum pyramidale	635 ±33 (4,2)				

^aData for activities are $\mu\text{mol/kg dry wt/min}$, presented as mean $\pm$ SEM based on number of samples. The number of samples, followed by the number of hamsters from which they were obtained, is given in parentheses for each region of each group. AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; DPO, dorsal periolivary nucleus; Hipp, hippocampus; LSO, lateral superior olivary nucleus; MNTB, medial nucleus of the trapezoid body; SPO, superior paraolivary nucleus; VNTB, ventral nucleus of the trapezoid body. The stratum oriens samples of the hippocampus included the thin alveus layer. Statistical significances of differences between control and exposed hamsters were determined by both t-tests (t-test P) and Wilcoxon-Mann-Whitney tests (WMW P), because data for some regions, marked by a superscript “a,” did not satisfy criteria of the Kolmogorov-Smirnov test at $P < 0.05$ for being normally distributed.