Morphological changes in the cochlear nucleus of congenitally deaf white cats

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Abstract

Investigations in animal models and humans have indicated that congenital deafness produces degenerative changes in the central auditory pathway. The cochlear nucleus is the first central structure that receives cochlear input, and may be considered the origin of ascending auditory pathways. In this context, we studied congenitally deaf white cats, who express early onset cochlear receptor loss, in order to assess the nature of structural changes in cells of the cochlear nucleus. It is conceivable that pathologic alterations in higher auditory structures are transneuronally distributed through this nucleus. The cochlear nuclei of nonwhite cats with normal hearing were compared to those of deaf white cats exhibiting hearing loss in excess of 70 dB SPL. The cochlear nuclei of the deaf white cats were smaller in volume by roughly 50%, with the ventral and dorsal divisions being equally affected. Cell body silhouette area was determined for spherical bushy cells of the anteroventral cochlear nucleus (AVCN), pyramidal cells of the dorsal cochlear nucleus (DCN), sensory neurons from the principal trigeminal nucleus, and motoneurons of the facial nucleus. We found no statistical difference in neuronal cell body size between nonauditory neurons of these two groups of cats, whereas auditory neurons of deaf white cats were 30.8–39.4% smaller than those of normal cats. These data imply that neuronal changes in congenitally deaf cats are specific to the auditory pathway. Although cochlear nucleus volume loss was uniform for both divisions, there was a differential effect on cell density: AVCN cell density increased by 40%, whereas DCN cell density was relatively unaffected (10% increase). Astrocyte density was also greater in the AVCN (52%) compared to that in the DCN (5%). These observations reveal a differential impact on cells in the cochlear nucleus to congenital deafness, suggesting selective processing impairment at this level. If similar patterns of degeneration occur in humans, such pathologies may underlie reduced processing of input from cochlear implants in congenitally deaf adults.

Keywords: Auditory system; Cochlear implant; Congenital deafness; Glial cell; Transneuronal degeneration

1. Introduction

Afferent activity is essential for the normal development and maintenance of central sensory systems (reviewed by Tucci and Rubel [80]). Modifications of auditory nerve input, for example, have been induced by drugs, nerve section, and/or cochlear ablation, and can produce dramatic changes in the structure and function of the central auditory pathway [32,45,50,52,54,59,78]. These experimentally-manipulated effects include neuronal loss or shrinkage, alterations in axonal projection patterns, and changes in physiological response properties. Congenitally deaf animals, however, offer a naturally occurring model of deafness and may offer an alternative approach to studies of auditory system plasticity associated with cochlear dysfunction [35,88].

The deaf white cat is one of several animals that exhibits a syndrome of sensorineural hearing loss associated with pigmentary disorders that is inherited in an autosomal dominant fashion [42,88,90,91]. Cochleosaccular degeneration has been demonstrated as the pathophysiological event producing sensorineural hearing loss in the congenitally deaf white cat [2], a condition that mimics the Scheibe deformity of humans and produces sensory degeneration in the first week of life [76]. The Scheibe deformity is also associated with measurable degenerative effects in the human auditory pathway [46]. This defect is commonly...
associated with both syndromic and non-syndromic forms of deafness [11,17,48,81].

It should be noted that the deaf white cat is distinct from the albino cat. Deaf white cats show an absence of melanocytes [5], whereas albinos have a normal distribution of melanocytes but lack the enzyme tyrosinase and so are incapable of producing melanin pigment [89]. Furthermore, albino cats are not deaf but exhibit atrophic changes along the auditory pathway as a result of pigment-related alterations of inner ear development [16].

Cochlear damage produces neuronal changes at the level of cochlear nucleus, but the remaining pathways may be abnormal as well [29,50,68]. Because the cochlear nucleus has been considered the source for the ascending central auditory pathways [39], the nature and extent of its changes may provide clues as to the pattern of transneuronal alterations further along the system. Ultimately, the alterations initiated at this first central synaptic station may impose significant limits to the (re)habilitative potential of prosthetic devices that are used to bypass a nonfunctioning cochlea.

The cochlear nucleus receives the synaptic terminations of cochlear nerve fibers, and in turn gives rise to ascending projections that initiate the central auditory pathways. This nucleus may be divided into several subdivisions on the basis of regional differences in morphologic and physiologic properties [10,39,51,58,60]. Individual auditory nerve fibers are known to give rise to a wide variety of terminals that in turn appear associated with particular populations of cells [20,39,63]. In the present study, we analyzed the cellular structure in the cochlear nucleus of deaf white cats and compared that to what we found in normal hearing cats. We were particularly interested in the potential differential effects on neuronal subpopulations in the ventral compared to the dorsal cochlear nucleus because of their cytologically distinct organization and correspondingly separate pattern of axonal connections. We specifically examined spherical bushy cells that receive large endings directly on their cell bodies (endbulbs of Held) and pyramidal cells that receive small boutons upon their basal dendrites [39,69]. Our working hypothesis was that the magnitude of cellular alterations produced by a cessation of sound-driven neural activity would be reflected by the pattern of synaptic organization of the afferent inputs where axosomatic effects would be greater than axodendritic effects.

2. Materials and methods

2.1. Subjects

Five pigmented normal cats (weighing between 1.8–3 kg) and five cats with white fur (weighing between 2.5–4 kg), anomalous iris pigmentation, and a family history of deafness were used in this study. Each group consisted of one male and four females. These cats were healthy and all external ear canals were examined with an otoscope to make certain that the tympanic membranes were intact and that there were no ear mites or infection.

2.2. Animal preparation

Cats were anesthetized using i.m. injections of ketamine hydrochloride (0.3 ml/kg body wt.) and xylazine hydrochloride (0.1 ml/kg body wt.), followed by i.p. injections of dial-urethane (0.5 cc/kg) where each cc contained 100 mg of dialyl barbituric acid (Sigma Chemical Co.), 400 mg of urethane (Aldrich Chemical Co.), and 400 mg of N-ethylurea (Fluka). Once the animals were areflexic, a tracheotomy was performed to facilitate respiration, the cat was placed in a head holder, and soft tissue surrounding the skull removed. The posterior fossa was opened with rongeurs, the dura reflected over the cerebellum, and the cerebellum retracted to expose the auditory nerve as it passed from the internal auditory meatus to the brainstem. Recording micropipettes were placed into the auditory nerve under direct visual control with the aid of an operating microscope.

Control cats exhibited click thresholds at 0–5 dB SPL and normal single unit audiograms between 0.1–40 kHz. The white cats exhibited elevated hearing thresholds. Three of these white cats had no acoustically-driven responses up to 100 db SPL, and the other two had some responses to tones below 10 kHz but with thresholds above 62 db SPL [70]. These cats will be referred to as deaf white cats.

2.3. Histology

The data presented in this report were derived entirely from Nissl-stained tissue, but this same tissue was also processed for demonstrating HRP to be used in a separate study. No HRP data are reported here but the entire histologic procedure is briefly described. At the end of each recording session, micropipettes filled with 30% (w/v) horseradish peroxidase (HRP) were inserted into the nerve and HRP was injected by passing 5 μA of positive current (7 s on, 7 s off; 50% duty cycle) for 5 min. Approximately 24 h after the injections, cats were administered a lethal dose of Nembutal and perfused through the heart with 0.1 M phosphate-buffered saline (pH 7.6) containing 0.5% sodium nitrite, followed by 1.5 l of 0.1 M phosphate-buffered fixative (pH 7.6) containing 2% glutaraldehyde and 2% paraformaldehyde. The brain was postfixfixed overnight at 5°C while still in the skull, removed from the skull the following morning, and the brainstem with cochlear nucleus blocked for cutting on a VibriQmate. Coronal sections at a thickness of 50 μm were collected in serial order, histochemically processed for HRP using standard techniques [19], mounted on ‘subbed’ slides, air-
dried overnight, stained with 0.5% cresyl violet, dehydrated, cleared and coverslipped with Permount.

2.4. Data analysis

Alternate sections, stained with cresyl violet, were selected for morphometric analysis from the most rostral AVCN and through the middle of the DCN. A minimum of 100 spherical bushy cells, 100 pyramidal cells, 50 facial motoneurons, and 50 sensory neurons of the principal trigeminal nucleus, pars ventralis, of each cat were drawn with the aid of a light microscope and drawing tube (100× oil immersion objective, NA = 1.25; total magnification ×2500). Spherical bushy cells and pyramidal cells were identified using the criteria defined by Osen [51]. Nonauditory cells were identified by their membership in nuclei defined by Berman [4]. All cells exhibiting well-defined cytoplasm, a clear nuclear outline and a nucleolus, and contained within the microscopic field of view were drawn. The border between cell body and primary dendrite was arbitrarly determined by extending the cell body perimeter on both sides of the dendritic stalk until they intersected. The somatic and nuclear cross sectional areas of these cells were then determined using computer-aided planimetry (SigmaScan, Jandel Scientific). After initial inspection, observations could not be "blinded" to the experimental groupings because cytologic features were so characteristic of each group.

Spherical bushy cells, pyramidal cells, and astrocytes (as microscopically defined by Penfield [56]) were first identified in histologic sections of the AVCN and DCN for cell density measurements. A box with each side equal to 100 μm in length was superimposed over a single microscope field and all identified neurons exhibiting a nucleus and contained within the box were drawn (40× oil immersion objective, NA = 1.0). The number of neurons and astrocytes per unit area was also determined.

The volume of the cochlear nuclei was determined by projecting stained histologic tissue sections (40× total magnification, Tri-simplex) onto paper and tracing the nuclear outlines. Serial sections of the ventral cochlear nucleus (VCN) and DCN for five normal and five deaf cats were drawn, the area of each nuclear division determined using computer-aided planimetry (SigmaScan), each area multiplied by the section thickness (50 μm), and the resulting volumes summed to produce the total nuclear volume.

These data were compared between deaf white cats and normal hearing subjects. All counts and measurements are represented by mean values and standard deviations. Group means were subject to statistical analysis using Student's two-tailed t-test for unpaired data. All experiments were performed in accordance with NIH guidelines and the Animal Care and Use Committee of the Johns Hopkins University.

3. Results

3.1. Physiological assessment of deafness

Pigmented cats exhibited normal audiograms and thresholds with respect to previously established criteria [25,37]. That is, their N1 thresholds to clicks were below 5 dB SPL, and below 10 dB SPL to short tone bursts of 0.5–15 kHz. All pigmented cats exhibited startle and orientation responses to noises presented to their backs. In contrast, white cats with a family history of deafness exhibited no such responses to similar noises. Three white cats were deemed profoundly deaf because tones between 0.1–40 kHz and exceeding 100 dB SPL were incapable of evoking single unit spike activity in the auditory nerve. Two white cats exhibited single unit thresholds in excess of 62 dB SPL for tones below 10 kHz but were unresponsive to tones between 10–40 kHz and exceeding 100 dB SPL. On the basis of these electrophysiological criteria as well as behavioral observations, the pigmented cats were placed into a hearing group and the white cats were placed into the group of deaf white cats. Single unit data have been presented in abstract form [70] and a manuscript describing the results more fully is in preparation. Underlying this grouping is the observation that deafness as expressed in the deaf white cat reduces evoked spike activity in the auditory nerve.

3.2. Control measurements

Neurons were selected from the facial motor nucleus (Fig. 1) and from the principal trigeminal sensory nucleus, pars ventralis (Fig. 2). These neuron populations represent two distinct types of nonauditory cells, and should indicate to what extent congenital deafness affects neurons outside the auditory system. There was no difference in the silhouette area of facial motoneurons (Fig. 3), as the mean in pigmented cats was 1061 ± 385 μm² compared to 1039 ± 332 μm² for deaf white cats. In the trigeminal nucleus (Fig. 3), there was also no difference in somatic silhouette area between control cats (544 ±122 μm² ) and deaf subjects (541 ± 93 μm²). These observations are consistent with the idea that differences in cell size represent a consequence of congenital impairment of cochlear function.

3.3. Nuclear volume

The volume of the VCN and the DCN was determined for normal and deaf white cats. Normal cats exhibited nuclear volumes of 18 ± 1.2 mm³ for the VCN and 6 ± 1.2 mm³ for the DCN. These values are wholly within the range of previously published determinations for normal adult cats (18.7 ± 3.2 mm³ for the VCN and 6.9 ± 1.2 mm³ for the DCN; [69]). In contrast, the volumes of these divisions for the deaf white cats were 8.7 ± 1.4 mm³ for
the VCN and $3 \pm 0.6$ mm$^3$ for the DCN. The VCN of
the deaf white cat is 52% smaller than that of the normal cat,
and the DCN is 50% smaller.

3.4. Cell measurements and cell density

Light microscopic examination of the cochlear nuclei
was conducted to test the hypothesis that cell size in the
defef white cat would be smaller than that in normal cats.
The extent of change was predicted to be greater for
spherical bushy cells which receive large, axosomatic end-
ings compared to that for pyramidal cells which receive
small, axodendritic endings (Fig. 4). This hypothesis is
founded on the observation that unlike spherical bushy
cells, pyramidal cell activity is apparently derived from
sources other than the auditory nerve and that such sources
may mitigate the impact of deafness on pyramidal cells
[33].

3.5. Spherical bushy cells

In Nissl-stained cochlear nuclei from pigmented cats,
the typical complement of neurons was observed as previ-
ously described [10,51]. In similarly prepared tissue from
the deaf white cats, the cochlear nucleus was obviously
smaller and neurons were more lightly stained. Spherical
bushy cells, identified on the basis of well-established
criteria [13] were selected from the five most anterior
sections of the AVCN. These cells exhibited round-to-oval
somata, a round centrally located nucleus, a prominent cap
of perinuclear Nissl substance, and many Nissl bodies
(Fig. 5). Average silhouette area of spherical bushy cells in
pigmented cats was $612 \pm 98 \mu$m$^2$, whereas that in deaf
white cats was $371 \pm 72 \mu$m$^2$ (Fig. 6). The size of spheri-
cal bushy cells in deaf white cats was decreased by 39.4%
($P < 0.001$) compared to that in hearing cats (Table 1).
Nuclear silhouette area in spherical bushy cells of normal
cats was $139 \pm 18 \mu$m$^2$, whereas that of deaf white cats
was $114 \pm 19 \mu$m$^2$. Nuclear size in deaf white cats was
smaller by 18.4%.

3.6. Pyramidal cells

In the DCN, pyramidal cells were identified on the
basis of their large somatic size, their distribution in layer
II, and their elongated orientation perpendicular to the

Fig. 1. Photomicrographs of typical facial motoneurons from a normal hearing cat (A) and a deaf white cat (B). There is no systematic difference in the
size, shape and staining features of these neurons when comparing the two groups of cats. These sections are 50 \mu m thick and stained with cresyl violet.
Scale bar = 0.05 mm.
Table 1
Mean and standard deviation of cell area of spherical bushy cells (SBCs) and pyramidal cells (PCs), density of astrocytes, SBCs and PCs in AVCN and DCN

<table>
<thead>
<tr>
<th>Subject</th>
<th>SBCs Area (mm²)</th>
<th>SBCs Diam. (mm)</th>
<th>PCs Area (mm²)</th>
<th>AVCN Glia (cell/0.05 mm²)</th>
<th>DCN Glia (cell/0.05 mm²)</th>
<th>VCN Volume (mm³)</th>
<th>DCN Volume (mm³)</th>
<th>SBCs Density (cell/0.05 mm²)</th>
<th>PCs Density (cell/0.05 mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWC-1</td>
<td>358 ± 88</td>
<td>21.7 ± 2.6</td>
<td>544 ± 67</td>
<td>230 ± 42.8</td>
<td>171 ± 7</td>
<td>8.8</td>
<td>3.6</td>
<td>24 ± 4.6</td>
<td>7 ± 1.7</td>
</tr>
<tr>
<td>DWC-2</td>
<td>377 ± 69</td>
<td>22.5 ± 2</td>
<td>554 ± 92</td>
<td>192.7 ± 16.6</td>
<td>166 ± 7.2</td>
<td>10.9</td>
<td>3.8</td>
<td>23 ± 1.7</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>DWC-3</td>
<td>352 ± 61</td>
<td>21.2 ± 1.9</td>
<td>541 ± 75</td>
<td>176.7 ± 9.3</td>
<td>144.7 ± 8</td>
<td>7</td>
<td>2.3</td>
<td>25.7 ± 4.6</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>DWC-4</td>
<td>382 ± 77</td>
<td>22.2 ± 2.7</td>
<td>486 ± 112</td>
<td>198 ± 6.6</td>
<td>169.3 ± 23</td>
<td>8.7</td>
<td>3.1</td>
<td>24.7 ± 1.2</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>DWC-5</td>
<td>367 ± 72</td>
<td>22.3 ± 2.2</td>
<td>539 ± 97</td>
<td>185.7 ± 32.6</td>
<td>174.7 ± 26.5</td>
<td>8</td>
<td>2.7</td>
<td>28 ± 2.6</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>Group mean</td>
<td>371 ± 72</td>
<td>22 ± 2.3</td>
<td>535 ± 97</td>
<td>197 ± 29</td>
<td>165 ± 3</td>
<td>8.7 ± 1.4</td>
<td>3 ± 0.6</td>
<td>25 ± 3.3</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>Control 1</td>
<td>621 ± 100</td>
<td>27.7 ± 2.4</td>
<td>780 ± 204</td>
<td>99.3 ± 4</td>
<td>154.7 ± 8.3</td>
<td>17.8</td>
<td>5.4</td>
<td>14.7 ± 3</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>Control 2</td>
<td>572 ± 90</td>
<td>27 ± 2.1</td>
<td>793 ± 202</td>
<td>92 ± 15.6</td>
<td>138.7 ± 8.1</td>
<td>18.8</td>
<td>7.1</td>
<td>14 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Control 3</td>
<td>619 ± 74</td>
<td>29 ± 1.7</td>
<td>774 ± 188</td>
<td>104.3 ± 20.6</td>
<td>163.7 ± 15.3</td>
<td>17.9</td>
<td>5.7</td>
<td>15 ± 0.6</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Control 4</td>
<td>565 ± 105</td>
<td>26.3 ± 2.5</td>
<td>740 ± 159</td>
<td>94.3 ± 7</td>
<td>160.3 ± 3.5</td>
<td>20.1</td>
<td>7.1</td>
<td>15.7 ± 2.1</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>Control 5</td>
<td>648 ± 85</td>
<td>30 ± 2.2</td>
<td>787 ± 122</td>
<td>83.6 ± 8.5</td>
<td>169 ± 2.6</td>
<td>17</td>
<td>4.4</td>
<td>14.7 ± 0.6</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>Group Mean</td>
<td>612 ± 98</td>
<td>28 ± 2.2</td>
<td>775 ± 172</td>
<td>95 ± 13</td>
<td>157 ± 20</td>
<td>18 ± 1.2</td>
<td>6 ± 1.2</td>
<td>15 ± 1.6</td>
<td>6.6 ± 0.8</td>
</tr>
</tbody>
</table>

% Change  -39.4%  -31.5%  -30.8%  52%  -52%  -50%  40%  10%
surface. In addition, pyramidal cells exhibit a centrally-placed, round, pale nucleus, and prominent Nissl bodies that are scattered throughout the cytoplasm and into the primary dendrites (Fig. 7). Pyramidal cells were analyzed from the midfrequency regions because they tend to be more uniform in shape compared to those from very low or very high frequency regions [6]. The silhouette area of pyramidal cells in normal pigmented cats was $775 \pm 172 \mu m^2$ compared to $535 \pm 97 \mu m^2$ in deaf white cats (Fig. 6). These measurements revealed a 30.8% difference in size ($P < 0.001$). Nuclear silhouette area was $164 \pm 34 \mu m^2$ for normal pigmented cats and $134 \pm 24 \mu m^2$ for deaf white cats. There is a 16.7% difference in nuclear size between the two groups of cats.

Fig. 2. Photomicrographs of representative sensory neurons from pars ventralis of the principal trigeminal nucleus of a normal hearing cat (A) and a deaf white cat (B). These micrographs illustrate that there is no systematic difference in the size, shape and staining features of the cells when comparing these two groups of cats. The sections are 50 \mu m thick and stained with cresyl violet. Scale bar = 0.05 mm.

Fig. 3. Histograms comparing nonauditory neuronal cell size of deaf white cats and control cats. No statistically significant changes were observed in somatic area ($P > 0.75$) for facial motoneurons (left) or sensory neurons (right) of the principal trigeminal nucleus, pars ventralis.
3.7. Neuron density

It was readily obvious using light microscopy that the packing density of neurons in the VCN was, on average, greater in the deaf white cat than in the normal pigmented cat. The number of spherical bushy cells was $25 \pm 3.3$ per $0.05 \text{ mm}^2$ in the AVCN for deaf white cats compared to $15 \pm 1.6$ per $0.05 \text{ mm}^2$ for normal cats. In the DCN, however, the number of pyramidal cells was $7.3 \pm 0.9$ per $0.05 \text{ mm}^2$ in layer II for the deaf white cat compared to $6.6 \pm 0.8$ per $0.05 \text{ mm}^2$ for normal cats. This analysis revealed that in deaf white cats (compared to normal cats), spherical bushy cell density was 40% greater and pyramidal cell density was 10% greater (Fig. 8).

3.8. Astrocyte density

One unanticipated observation was that astrocytes in the AVCN were more numerous in deaf white cats compared to that in control subjects. These small cells ($5–6 \mu\text{m}$ in diameter) were histologically identified as astrocytes [56] on the basis of an irregularly-shaped oval nucleus peppered with small grains of chromatin and no nucleolus, and their lack of visible cytoplasm. These macroglial cells were counted in the same sections containing the analyzed spherical bushy cells in the AVCN and pyramidal cells in the DCN (Fig. 9). In the AVCN of pigmented cats, the mean number of astrocytes was $95 \pm 13$ per $0.05 \text{ mm}^2$, whereas that in deaf white cats was $197 \pm 29$ per $0.05 \text{ mm}^2$. The number of glial cells in the AVCN of deaf white cats was more than double ($P < 0.001$) that in hearing cats. Within the DCN, the mean number of glial cells of pigmented cats was $157 \pm 20$ per $0.05 \text{ mm}^2$, compared to $165 \pm 30$ per $0.05 \text{ mm}^2$ in deaf white cats. The number of glial cells in the DCN was not statistically different ($P = 0.395$) between the two groups of cats.

4. Discussion

The cochlear nuclei of congenitally deaf cats exhibit striking differences in cellular morphology when compared to those of normal hearing cats. Cochlear nucleus volume in deaf cats is approximately 50% smaller than that of normals, and both the VCN and the DCN express this difference equally. Contributing to this volume difference is a size difference in the constituent neurons: spherical bushy cells, characteristic of the VCN and pyramidal cells, representative of the DCN are 40% and 31% smaller, respectively, in deaf white cats compared to normals. Cells of the principal sensory nucleus of the trigeminal and facial motoneurons exhibited no size differences when comparing the two groups of cats, suggesting that morphologic manifestations of congenital deafness in the deaf white cat are specific to the auditory pathway.

The notion of congenital deafness merits additional discussion at this time. There is a broad definition of ‘congenital deafness’ where failure of auditory development can result from genetic causes, from damage during embryonic life, or from birth trauma [44]. Some of these factors usually produce deafness before birth but may also function to produce an onset which is delayed until after birth. Congenital deafness may therefore be classified as hereditary, intrauterine, or perinatal. In the case of the deaf white cat, cochlear morphology appears normal at 4 days of age, but structural pathology becomes prominent at 8 days after birth [42] and cochlear degeneration is virtually complete by 3 weeks [9]. It is in the above context that the deaf white cats are considered to exhibit congenital deafness.

The 40% difference that we observed in spherical cell body size is consistent with but not identical to previous studies on postsynaptic neurons in the congenitally deaf cats [35,88]. Our investigation was undertaken in part because the above mentioned studies used different methods and reported effects of different magnitude. West and Harrison [88] measured 10 ‘c’ cells (equivalent to spherical bushy cells) each from two deaf white cats using light microscopy and reported a 38.8% difference in cell size. In contrast, Larsen and Kirchhoff [35] measured 50 spherical cells from each of 6 deaf white cats using electron microscopy and report a 50% reduction in cell size relative to controls. It should be noted that the measurement of silhouette area from ultrathin sections using a nucleolar criterion requires that the nucleolus not stray far from the plane of the cell’s equator. If in deaf white cats, however, the nucleolus of spherical bushy cells becomes even slightly
Fig. 5. Photomicrographs of spherical bushy cells from a normal hearing cat (A) and a deaf white cat (B). Sections are 50 µm thick and stained with cresyl violet. Higher magnifications of these cells are shown for normal (C) and deaf (D) cats. Note the increased neuronal density and the abundance of glial cells in the AVCN of the deaf white cat compared to that in the normal cat. Scale bars: A and B = 0.05 mm; C and D = 0.02 mm.
eccentric compared to that in normal cats, the method employed in ultrathin sections will systematically produce a perimeter smaller than the actual equator, and thus, a larger difference. The present light microscopic results correspond more closely to prior light microscopic observations than to those of the electron microscopic study.

Fig. 6. Histograms comparing cell size of deaf white cats and control cats. These histograms illustrate size for spherical bushy cells and pyramidal cells. Spherical bushy cells exhibited, on average, a 39.4% reduction in somatic size, whereas pyramidal cells showed a 30.8% reduction in somatic size.

Fig. 7. Photomicrographs of typical pyramidal cells from a normal hearing (A) and a deaf white cat (B). The large cells occupying the central region of each panel are the pyramidal cells. The cytoplasm of pyramidal cells in the normal cat exhibit more distinct Nissl bodies, whereas that of the deaf white cat is more diffuse in appearance. Sections are 50 μm thick and stained with cresyl violet. Scale bar = 0.05 mm.
4.1. Types of deafness and effects of duration

Short durations of deafness induced by chemical deaf-ferentation or cochlear ablation result in a similar atrophic phenomenon but of a lesser magnitude. AVCN cell size is smaller by 15–38% when the duration ranges from days to less than a year [24,41,54,55,59,72,79]. In contrast, cell size differences range from 38–50% when deafness endures beyond a few years [34,88]; present study). In human subjects, greater reductions in VCN cell size were reported in genetic deafness compared to cases of acquired deafness having equivalent periods of hearing loss [46]. It may be that congenital cochlear impairment is not the mere equivalent to experimentally-controlled deafness. That is, congenital pathologies in the cochlea may produce not only hair cell degeneration with the accompanying transneuronal changes, but also simultaneous alterations of cellular organization and connections throughout the auditory pathway.

4.2. Influence of synaptic strength

If the relative ‘weight’ of synaptic inputs (e.g., size of terminal and spatial proximity to spike generator) has any bearing upon the magnitude of the neuronal change when auditory input is lost, then cell size of spherical bushy cells should be more severely compromised than that for pyramidal cells. Spherical bushy cells receive large endings from the auditory nerve directly upon their cell bodies that cover up to 80% of the surface [39], whereas pyramidal cells receive small endings from the auditory nerve upon their basal dendrites [22,69]. Furthermore, when the cochlea is damaged, all spontaneous activity ceases immediately in spherical bushy cells, whereas spontaneous activity of pyramidal cells is unaffected [33]. Because pyramidal cells maintain inputs from many other sources through granule cells [28,47,86,87,92], they may be better able to resist sensorineural atrophy following loss of the cochlea.

Our data are partially consistent with such an hypothesis because spherical bushy cells were reduced in size by nearly 40%, whereas pyramidal cells were reduced by roughly 31%. The difference in magnitude of change, however, is smaller than expected in light of the differences in the pattern of auditory nerve inputs. Given that congenitally deaf white cats exhibit hair cell degeneration that begins within a few weeks of birth and continues throughout the first year [42], and since our deaf white cats were at least 6 years of age, perhaps a difference in size
between these two cell types might be greater if examined in younger animals. On the other hand, what is perceived as a small difference may carry functional consequences that have yet to be assessed.

4.3. Changes in neuronal and astrocytic density

Although both VCN and DCN in deaf white cats were smaller by 50% compared to that in normal hearing cats, there were obvious differences in cell density. Spherical bushy cell density was 40% greater in deaf white cats. Pyramidal cell density was increased in deaf white cats by only 10%. As previous studies have reported that cochlear destruction produces relatively little cell loss [59], our results can be interpreted as follows. Volume loss in the VCN accounts for pyramidal cells confined to layer II, but the number of cells per unit length does not change. In contrast, the VCN is not layered and so a change in spherical bushy cell density is readily detected because cells are redistributed throughout the nucleus consequent to the overall volume loss. This analysis indicates that loss in nuclear volume is due mostly to reduced neuropil volume rather than to reduced cell numbers.

We also observed a 52% increased density in macroglial cells in the AVCN of congenitally deaf cats compared to normal cats, but only a 5% difference was evident in the DCN. On the basis of light microscopic criteria [56], the macroglial increase is attributable to astrocytes. Previous studies reported that degenerative changes in the central nervous system result in neurogliosis. For example, the number of glial cells in the nucleus magnocellularis of the chicken increased following ipsilateral cochlea removal [40,65]. Glial cell proliferation was also reported in response to nerve fiber degeneration following experimentally-induced acoustic trauma on the cochlea of gerbils [62].

Neuroglia are the most abundant cell type in the mature nervous system, where they represent 80–90% of the number of brain cells [74]. These cells are hypothesized to subserve communication activities of nerve cells [56], involved in such diverse processes as support, nourishment, and repair following destructive lesions (reviewed by Peters et al. [57]). The differential glial response of the VCN versus the DCN is reminiscent of observations made previously when the cochleas of mature cats were ablated [59]. These authors noted a transient glial response in the DCN but a prolonged response in the VCN. The cause of this differential glia response remains to be determined, but it may be hypothesized that it is consequent to cellular degeneration (not simply atrophy) that is more prominent for spherical bushy cells than for pyramidal cells.

4.4. Activity and cell size

Alterations in neuronal structure have been observed as a result of disruptions in the presence or quality of inputs, the availability of effectors, or direct damage [23,67]. As a first approximation, cell size has been used to assess atrophic alterations [31,36,59,66]. Changes in somatic size have typically accompanied alterations in dendritic morphology [53,73], metabolic activity [8,18,72,75], and physiologic properties [38,71,79]. By inference, changes in cell body size further correlate with alterations in connectivity [26,61] and the physiologic organization of receptive field properties [30]. At this point, however, it is not possible to differentiate between the effects of reduced synaptic activity as opposed to the potential loss of a trophic factor secreted by the auditory nerve fiber. Regardless, cell size is experimentally malleable, such that the absence of activity produces a reduction in size but the restoration of activity returns size to normal levels [79,85]. In this context, cell size can reflect the relative health of neurons during normal development, the ambient activity levels, or degenerative effects following experimental manipulations.

Nuclear area was also analyzed, prompted by observations that the induction of nuclear proto-oncogenes is the first change in gene activity that can be observed in response to extracellular stimulation [3]. Any reduction in transcription factor activity might have a direct effect on nuclear size, and given the two separate neuron populations, the question arose as to whether there might be differential effects on the nucleus. It seems, however, that nuclear size was affected to the same extent for spherical bushy and pyramidal cells.

4.5. Cytoplasmic consequences of activity loss

It is also noteworthy that neurons in the cochlear nuclei of the deaf cats stained lightly when using basic dyes. This result is reminiscent of the appearance of 'ghost' cells, where cochlear removal produced cells exhibiting cytoplasmic ribosomal loss [64]. Ribosomal loss accompanies a reduction in protein synthesis [75] which presages cell death [7]. Such alterations in the basic machinery of cells may underlie compromised neuronal function resulting from long-term loss of normal sensory activity. Not only are some pathologies introduced at the earliest stages of central auditory processing, but they could extend throughout the auditory neuraxis.

4.6. Clinical implications

Potential success in auditory (re)habilitation lies in the ability to introduce properly coded stimulation to an auditory pathway with sufficient neuronal structure and function to process that information. With respect to cochlear
implants, this issue is particularly compelling in determining candidacy for the congenitally deaf. In contrast to the promising results found in children, initial clinical trials have confirmed that profoundly deaf adults who have gained the most auditory benefit from cochlear prostheses are those who were post-linguistically deafened. The 1995 NIH Consensus Development Conference on Cochlear Implants [1] recognized that cochlear implants in prelingually deafened adults provide sound awareness, but minimal improvement in speech recognition. This conclusion is supported by ongoing trials of cochlear implants demonstrating few, if any, objective gains from preoperative to postoperative conditions in these subjects [12,21,82,83,93].

Is there an early developmental period during which synaptic connectivity is normal for congenitally deaf individuals? It has been proposed that there is a 'critical period' for the development of brainstem auditory nuclei, and that without adequate sound stimulation during this period, neurons fail to fully develop [84]. Furthermore, spherical bushy cells are reported to reach their normal size when acoustic stimulation is restored to young animals within 45 days of the deprivation [85]. There are, however, other data that differ in their conclusions. Electrical stimulation of the auditory nerve in deafened kittens or guinea pigs, simulating the effects of cochlear implantation, has produced conflicting results on cell size redemp-

One way to avoid the experimentally-induced pathology in the central auditory system is to use the congenitally deaf animal. Our notion is that during an early critical period, implantation of a prosthetic device could help the central auditory system realize adequate cytoarchitectonic organization and synaptic connections. Furthermore, this simulation of sound, no matter how artificial, might provide the basic functional percepts necessary for the eventual acquisition of speech and language. It seems that any cochlear input that is systematically tied to specific 'acoustic' percepts could function as a native tongue along the same lines as, for example, Arabic or English. After this critical period, however, irreversible degenerative changes could diminish the efficacy of cochlear implants. Clinical experience with present cochlear implant technology is consistent with this interpretation [12,21,82,83,93]. The morphological alterations observed in the present study may result in reduced processing capabilities and poor benefit for adult cochlear implant patients who have been deaf since birth. Further studies are needed to examine the auditory nervous system in the deaf white cat at different ages, so that implantation guidelines might be better formulated which take into account the impact of short versus long-term deafness.

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