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Separate forms of pathology in the cochlea of congenitally deaf white cats

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Abstract

Congenital deafness due to cochlear pathology can have an immediate or progressive onset. The timing of this onset could have a significant impact on the development of structures in the central auditory system, depending on the animal's hearing status during its critical period. In order to determine whether cats in our deaf white cat colony suffered from progressive hearing loss, they were tested repeatedly in 30-day intervals using standard auditory evoked brainstem response (ABR) methodology. ABR thresholds did not change over time, indicating that deafness in our colony was not progressive. Moreover, different forms of cochlear pathology were associated with deafness. One form (67% of the deaf ears) had a collapsed Reissner's membrane that obliterated the scala media, resembling what is called the Scheibe deformity in humans. A second form (18%) exhibited excessive epithelial growth within the bony labyrinth. A third form (15%) combined excessive epithelial growth in the apex and a collapsed Reissner's membrane in the base. Cochleae having an abnormally thin tectorial membrane and an outward bulging Reissner's membrane were associated with elevated thresholds (poor hearing).
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Key words: ABRs; Development; Hearing; Organ of Corti; Scala media

1. Introduction

Studies of the effects of deafness on the brain and its development have sought animal models upon which to generate data that is relevant to the human condition. The congenitally deaf white cat (DWC) has long been of interest because of the relationship between coat color, eye pigment, and deafness (Bamber, 1933; Wolff, 1942; Wilson and Kane, 1959; Bergsma and Brown, 1971). This well-documented syndrome presents a variable expression of distinct characteristics, including a cochlear pathology that is also found in humans where

it was first described in congenitally deaf patients (Scheibe, 1892, 1895). This particular pattern of deafness is characterized by the collapse of Reissner's membrane onto the undifferentiated organ of Corti, thinning of the stria vascularis, and malformation of the tectorial membrane. The similarities of inner ear pathology between cats and humans have promoted the DWC as a model for the Scheibe deformity (Bosher and Hallpike, 1965, 1967; Deol, 1970; Suga and Hattler, 1970; Brighton et al., 1991).

Variations in the severity of deafness and histologic damage are common (Brown et al., 1971; Rebillard et al., 1981a,b; Ryugo et al., 1998). It is important to differentiate between genetically determined deafness and acquired deafness. There is also an important distinction whether deafness begins at birth or whether it develops postnatally (Nadol and Burgess, 1982). That is, congenital cochlear dysplasia, where deafness is evident at birth (Steel and Bock, 1980; Holme and Steel, 2002) must be distinguished from postnatal cochlear

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Abbreviations: ABR, auditory evoked brainstem response; dB peSPL, dB peak equivalent sound pressure level; DWC, deaf white cat; IM, intramuscular; PN, postnatal days; SPL, sound pressure level; #/#, postnatal days of age/animal identification number

degeneration (Bosher and Hallpike, 1967; Mair, 1973; Shnerson and Willott, 1980; Li et al., 2002). These distinctions are useful to determine what, if any, medical action can be undertaken. If individuals were born with normal hearing but then experience postnatal degeneration of the cochlea, there would be hope for intervention strategies that might interrupt the pathologic process and prevent the loss of hearing. Hearing and the ability to form conscious percepts of sound require more than the transduction of mechanical energy into neural signals. The central nervous system must be able to receive, process, and transmit signals from the periphery along the pertinent pathways. In this regard, it must also be determined if abnormal changes in the central auditory system arise directly from genetic factors or from effects initiated by deafness. For example, do pathologic changes in spiral ganglion cells represent a primary or secondary consequence to the genetic defect (Rebillard et al., 1976; Pujol et al., 1977)? These same kinds of questions need to be addressed at all the central auditory stations in cases of hereditary deafness, and their answers should influence how we think about the role of hearing on brain development.

There are a number of studies that discuss early onset, progressive cochleosaccular degeneration (Scheibe, 1892, 1895; Deol, 1954, 1956a, 1968; Bosher and Hallpike, 1967; Mair, 1973; Nadol and Burgess, 1982; Holme and Steel, 1999; Keats and Berlin, 1999). The progressive nature of the deafness was inferred through data collected from an age-graded series of animals, including mice, cats, and humans. In white cats, the cochlea appeared grossly normal for the first 5 postnatal days (PN5), but a rapid degenerative process led to the complete degeneration of the organ of Corti by PN21 (Bosher and Hallpike, 1967). The histologic appearance of the organ of Corti was increasingly dysmorphic, leading to the obliteration of the cochlear duct and gradual loss of spiral ganglion cells (Mair, 1973). The demise of the organ of Corti was correlated with deafness (Scheibe, 1892, 1895). It should be noted that this notion of a progressive development of complete deafness is in sharp contrast to reports of partially hearing cats (Rebillard et al., 1981a,b; Ryugo et al., 1998). Although progressive cochlear dysplasia resembling the Scheibe deformity may be the final result for some cats, there are clearly other forms of congenital deafness that do not proceed to complete hearing loss.

In the present study, we examined the hearing and cochlear histology of white cats with a family history of congenital deafness. Our goal was to investigate the 'progressive' nature of congenital deafness in these cats. We conducted a longitudinal study of hearing and associated cochlear development in an age-graded series of kittens. In the study, however, we performed repeated measures of hearing thresholds in individual cats using auditory evoked brainstem responses (ABRs). The monthly testing of the same individual allowed us to determine directly whether there was progressive, age-related hearing loss over the course of 6–12 months. Cochlear tissue was harvested only after several ABRs were collected. We tested the hypothesis that white cats would exhibit progressive cochlear degeneration and hearing loss. Age-matched, pigmented kittens, often littermates, served as controls.

2. Materials and methods

2.1. Subjects

Thirty-two pigmented cats and 44 white cats were used in the developmental analysis. All white kittens and many pigmented kittens were born on the Hopkins farm. Consequently, the parents, litter composition, and dates of birth were known. Liberty Labs supplied pigmented adult cats, some of which were pregnant and provided additional pigmented kittens. Adult cats ranged in age from 1 to 2 years. Cats used in the study appeared healthy with normal movement, no mange, and no runny nose or eyes. On the day of birth, the age of the kitten was defined as PN0 and each successive day was numbered consecutively. All animal procedures were used in accordance with the guidelines established by the NIH and with the approval of the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

2.2. ABRs

Because we performed repeated ABR tests on all animals up to the time of sacrifice, we did not surgically open the ear canals to collect data. Instead, we waited until the external ear canal was patent to the eardrum (PN22–26). Animals were sedated with ketamine (25 mg/kg intramuscular (IM)) supplemented with xylazine (0.5 mg/kg IM) or acepromazine (0.25 mg/kg IM). Click ABRs were recorded at the time of ear canal opening, again at PN30, and then at 30-day intervals until time of sacrifice. Cats were sacrificed at varying ages ranging from birth to 1 year. ABRs were collected using a closed acoustic system with one electrode placed at the vertex and another inserted behind the pinna ipsilateral to the stimulated ear. Click levels were determined in dB peak equivalent sound pressure level (dB peSPL) referenced to 1 kHz by recording clicks using a calibrated microphone placed just inside the tip of a hollow ear bar (Burkard, 1984). The ear bar, coupled to an electrostatic speaker (Sokolich, 1977), was then placed into the external ear canal. Clicks ($n = 1000$) of

100 µs duration and alternating polarity were presented monaurally in 5-dB increments, starting at 0 dB and progressing to 95 dB peSPL. At each intensity level, ABRs were recorded for 15 ms and then averaged (Tucker Davis Technologies, Gainesville, FL, USA). Electrical activity 15 ms prior to each click stimulus was collected and averaged, and the earliest response to exceed 2 S.D. above background was defined as the threshold.

2.3. Tissue preparation

Cochleae and brains were harvested for histologic analysis at the end of the final ABR session. Following a lethal dose of barbiturate, subjects were transcardially perfused using 0.12 M cacodylate-buffered fixative containing 2% paraformaldehyde and 2% glutaraldehyde (pH 7.4). Each cochlea was perfused with the same fixative through the round and oval windows, and then placed in fixative overnight (5°C). Some cochleae were further perfused with osmium for viewing with an electron microscope. The following day, the cochleae were again perfused with fixative and dissected from the skull. In most cases, excess bone was drilled away and then the cochleae were decalcified in buffered EDTA (ethylenediaminetetraacetic acid) with 1–2% glutaraldehyde (pH 6.0–7.0). Some cochleae were placed in a microwave oven (Pelco model 3450) to accelerate decalcification (Madden and Henson, 1997).

Two embedding methods were used to prepare histologic sections of the organ of Corti. One method used a gelatin–albumin mixture. A small hole was drilled in the helicotrema and a dilute gel mixture was perfused through the round and oval windows. The cochleae were placed overnight in a humidified chamber. The following morning, a full-strength gel solution was perfused through the turns and the cochleae were placed under slight vacuum overnight. A full-strength gelatin solution with glutaraldehyde was poured over the cochleae in plastic embedding molds. After hardening at room temperature, they were stored at 4°C. They were cut on a vibratome, with sections oriented parallel to the modiolus, at a thickness of 50–60 µm. Sections were mounted on 'subbed' slides, stained with a 1% toluidine blue solution, and coverslipped.

The other procedure was used to collect thinner sections. Cochleae were dehydrated with successively higher concentrations of alcohol followed by propylene oxide and placed overnight in a 1:1 mixture of propylene oxide and araldite. The next day, cochleae were placed in 100% araldite, de-gassed under a slight vacuum, and placed with fresh araldite in molds at 60°C for 24 h. After the araldite hardened, the cochleae were cut in the mid-modiolar plane with a rotary microtome. This tissue was cut in 20-µm-thick sections, stained with a 1%
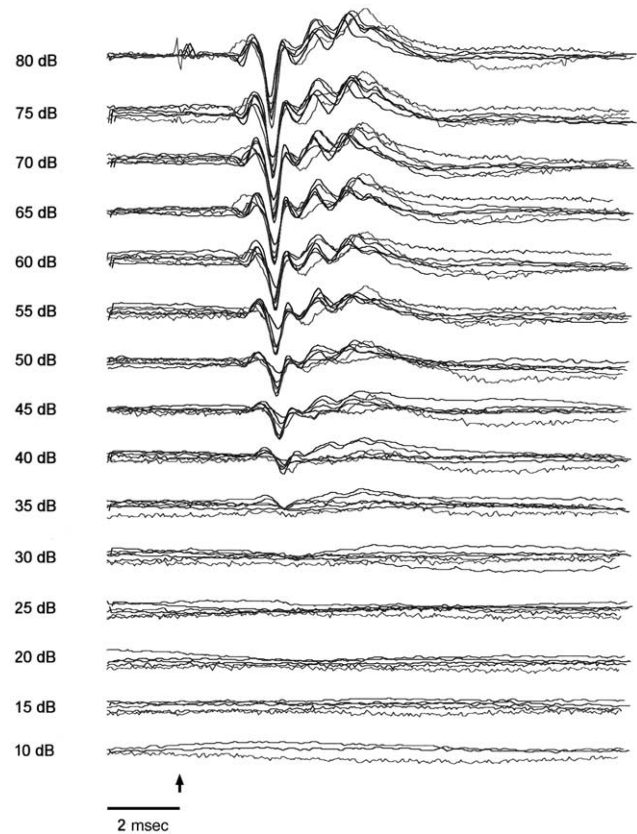

Fig. 1. Representative ABR records from a pigmented kitten with normal inner ear structure. ABRs to click stimuli (at arrow) were collected on PN23, 30, 60, 90, 120, and 150. Thresholds were determined to be between 35–40 dB SPL, and remained so at all ages. On the whole, the shape of the evoked responses remained remarkably constant over time.

toluidine blue solution, and coverslipped with Permount.

3. Results

3.1. ABR thresholds

The ABR data in this report are derived from 32 pigmented and 44 white cats and kittens of both sexes. Subjects were collected and studied over a span of 7 years. The white kittens were born in our colony, were related through two female ancestors, and represent multiple generations. All but one of the pigmented cats exhibited normal hearing when tested, whereas the white cats were quite variable. The white cats were mostly unilaterally ($n = 6$) or bilaterally ($n = 29$) deaf, although a few had elevated hearing thresholds ($n = 5$) and a few had normal hearing ($n = 4$).

ABR testing was not attempted while the external ear canals were occluded and mesenchyme filled the middle ear space (Olmstead and Villablanca, 1980). We waited

until the ear canals opened naturally in order to avoid surgical intervention. In the youngest kittens (<10 days postnatal), the canal was occluded as determined by simple observation. An otoscope with a very fine speculum was used to examine the canals of older kittens ($>$PN15), and it was determined that canal opening is a protracted process. At PN20, the canal was open nearly to the tympanic membrane but a thin, transparent flap of skin still blocked the canal. Between PN23 and PN26, this flap disappeared, and the canal was open. The time course for canal opening was similar for white and pigmented kittens.

Click ABRs were collected within a day or two of the canal opening, and then at 30-day intervals starting at PN30. Pigmented and white kittens were examined using identical parameters. We tested the hypothesis that ABR thresholds would increase with age for white cats but not for pigmented cats. The results were clear and unambiguous. If ABR thresholds were low (cats had good hearing), they stayed low. This situation held for pigmented (Fig. 1) as well as white cats (Fig. 2). If thresholds were high (cats were 'hard of hearing'), they stayed high (Fig. 3). Cats that were unresponsive

Fig. 3. Representative ABR records from a DWC with no hair cell receptors. Click stimuli (at arrow) were presented on PN25, 30, 60, 90, 120, 150, and 180. There was no evoked response up to 100 dB SPL.

to acoustic stimulation remained unresponsive. There was no change in the response magnitude with respect to increasing age. Response latency decreased with increasing stimulus level but was not correlated with age. ABR peak latencies shortened with increasing stimulus levels but not with increasing age.

There was virtually no change in the ABR thresholds at PN30 or at 30-day intervals for up to 12 months (Fig. 4). This kind of response stability was observed in all of our animals and for every ear tested (Fig. 5). Some normal ABRs came from the hearing ear of unilaterally DWCs. There were several cats with elevated thresholds whose values lay between deaf and normal hearing. The cochlear duct of these cats appeared mostly normal in histologic sections, but could be characterized by an outward bulging Reissner's membrane and a thin tectorial membrane (see also Ryugo et al., 1998). Each ear appeared relatively independent of its contralateral mate, but there was no change in hearing ability.

3.2. Cochlear histology
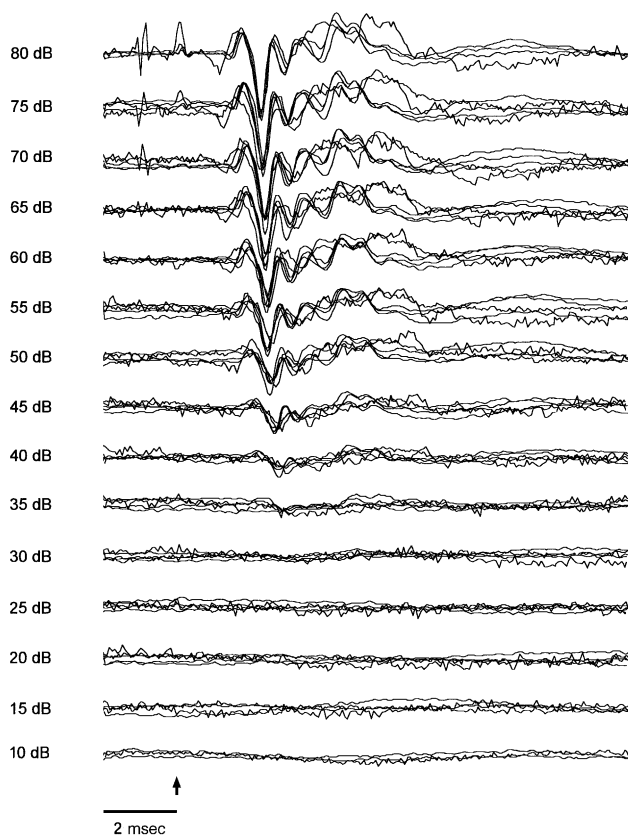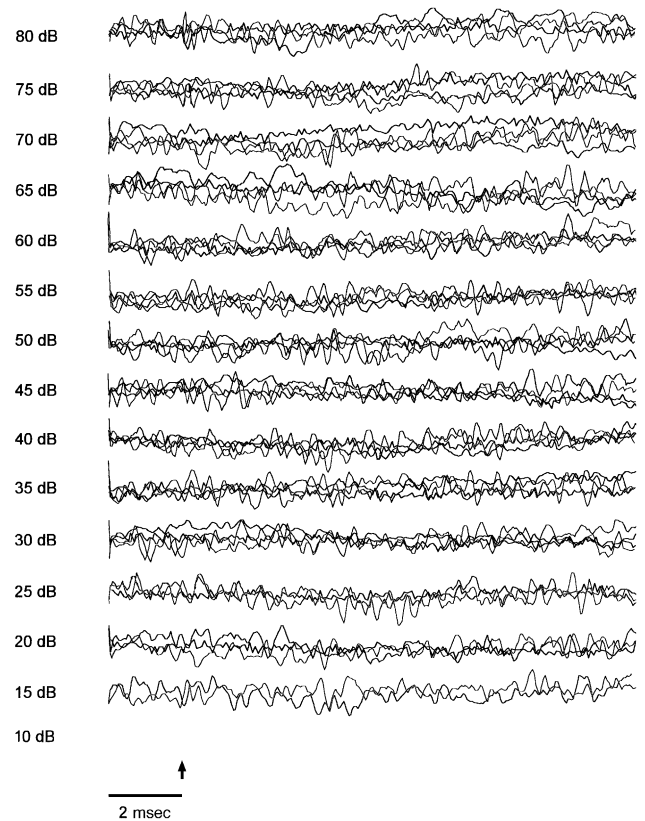
Kittens were randomly assigned to 'terminal' age

Fig. 2. Representative ABR records from a white cat with normal inner ear structure. ABRs to click stimuli (at arrow) were collected on PN24, 30, 60, 90, 120, and 150. Thresholds were determined to be between 35 and 40 dB SPL. The thresholds did not change with age.
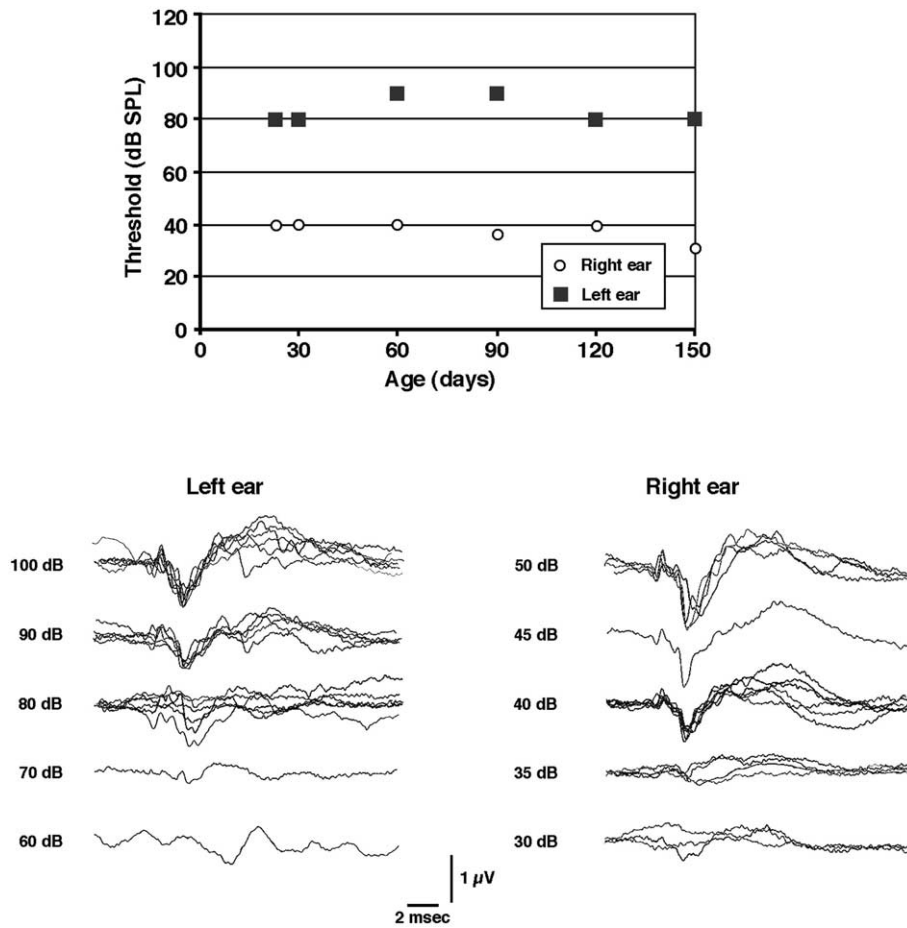
Fig. 4. ABR records from a white kitten with unilateral (left ear) deafness and corresponding inner ear pathology. ABRs were collected over 150 days. The physiologic condition of both ears remained relatively constant over this period (top). Individual records are shown for the deaf ear (left column) and the hearing ear (right column). The left inner ear featured a collapsed Reissner's membrane that obliterated the scala media and organ of Corti. In contrast, the right ear had normal morphology and normal ABR thresholds.
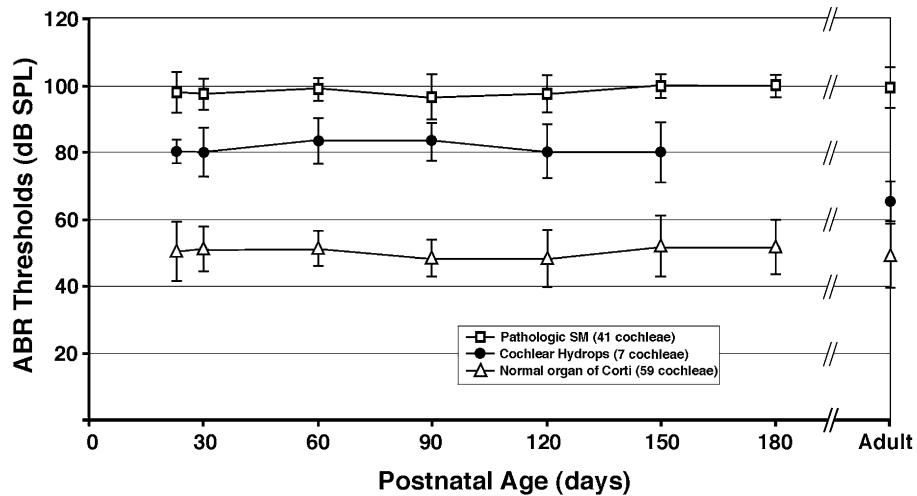

Fig. 5. Plot of all ABR thresholds. The number of data points averaged for younger animals is greater than for older animals. Subjects were tested when their ear canals opened and at 30 days postnatal. For progressive ages, there were fewer subjects due to tissue harvesting. Nevertheless, thresholds sorted out into three groups: normal, intermediate, and high. Ears with normal thresholds exhibited normal structure of the organ of Corti. Ears with no evoked responses were associated with pathologic cochlear structure. The intermediate threshold exhibited a cochlea with a bulging Reissner's membrane, indicative of hydrops. The data illustrate that deafness was not progressive, at least after the ear canal opened (PN23).

groups for cochlear analysis. Subjects were terminated after the ABR session corresponding to the age group to which they were assigned. At the end of the last ABR session, subjects were administered a lethal dose of barbiturates, perfused, and their cochleae and brains harvested. Some of the tissue from cats providing ABR data was lost during preparation. Thirty pigmented and 35 white cats contributed tissue that was processed for light microscopic study. Because not all cochleae from these cats were successfully prepared, analysis concentrated on the hearing of individual ears and their associated histology (Table 1). All but one of the pigmented cats had normal hearing thresholds and normal cochlear histology (Fig. 5).

In newborn kittens, presumably destined to have normal hearing, the scala media was well defined, with a relatively straight Reissner's membrane, a plump stria vascularis, and a distinct organ of Corti (Fig. 6). The spiral vessel was well defined and ran beneath the organ of Corti, on the opposite side of the basilar membrane. There was a full complement of inner and outer hair cells, although the tunnel of Corti was not yet formed. The greater epithelial ridge was prominent. On the basis of this morphology, we inferred that these kittens which were too young to be tested (e.g., newborns) would develop normal hearing (Fig. 6, pk-0). By 30 days postnatal, the tunnel of Corti was open, the spiral vessel resolved, and the greater epithelial ridge given way to the inner sulcus (Fig. 6, hpk-30). There was very little structural change in the organ of Corti thereafter.

The appearance of the inner ear of deaf cats was strikingly different from that of hearing cats. In cats old enough to be tested (>PN30), deafness, as indicated by a failure to elicit an acoustically evoked ABR, was coupled to cochlear pathology (Fig. 5). We noted, however, that deafness was correlated with distinct types of abnormalities. The more common form resembled that which has been previously reported (Bosher and Hallpike, 1965; Mair, 1973; Rebillard et al., 1981a,b; Ryugo et al., 1998), in which Reissner's membrane is collapsed on the organ of Corti and the scala media is obliterated (Figs. 7 and 8, left panels). The collapse occurs along the entire cochlear duct during the first 4–6 postnatal weeks, during which time the tectorial membrane gradually disappears (Fig. 8). The stria vascularis is present but is distinctly thinner than normal (compare Figs. 6 and 8). Of the 39 ears with histologically confirmed deafness, 26 exhibited unilat-

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Fig. 6. Photomicrographs illustrate normal development of the cat organ of Corti from untested newborn pigmented kitten (pk-0), PN30 hearing pigmented kitten (hpk-30), PN150 hearing pigmented kitten (hpk-150), and hearing pigmented adult (hp-adult). During this time, the spiral vessel recedes, the tunnel of Corti develops its characteristic shape, and cells of the organ of Corti assume their adult-like structure. Morphological development appears complete by PN30. Scale bar equals 50 μm. Abbreviations: OS, outer sulcus; GER, greater epithelial ridge; IS, internal sulcus; RM, Reissner's membrane; SL, spiral limbus; SM, scala media; SP, spiral prominence; SV, spiral vessel; TM, tectorial membrane.
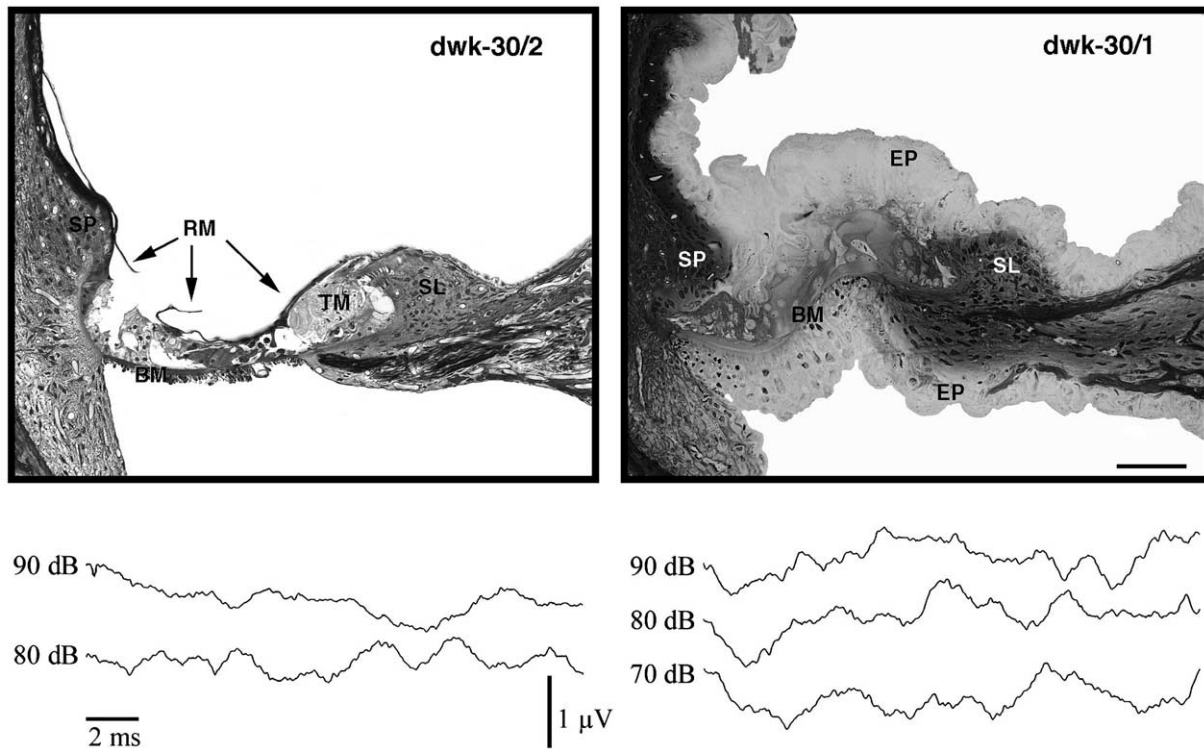
Fig. 7. Representative morphology and ABRs of two forms of cochlear pathology. Neither ear of these deaf white kittens (dwk) exhibited a click-evoked ABR at PN30. Each cochlea exhibits distinct structural differences. One form of pathology (dwk-30/2) resembles the Scheibe deformity (Scheibe, 1895) with Reissner's membrane collapsing and obliterating the scala media. The other form (dwk-30/1) reveals a kind of exuberant growth of epithelial cells that smothers the organ of Corti and stria vascularis. Photograph scale bar equals 50 μm. Abbreviations: BM, basilar membrane; EP, epithelial cell layer; RM, Reissner's membrane; SL, spiral limbus; SP, spiral prominence; TM, tectorial membrane.

eral or bilateral forms of the obliterated scala media (Table 1). It is this form of cochlear pathology that resembles the Scheibe deformity in humans (Scheibe, 1892). In one untested kitten (wk-2/1, wk: white kitten, #/#: postnatal days of age/animal identification number)), we observed what appeared to be the beginning of the collapse of Reissner's membrane. Our prediction is that this pathology in an immature cochlea heralds deafness. By the time the ear canal opens (around PN23), some of the kittens were completely unrespon-

sive to acoustic stimulation. As predicted, when their cochleae were examined at PN30 (Fig. 8, dwk-30/3, dwk: deaf white kitten), there was no scala media. The examination of cochlear structure in older deaf cats revealed that the limbus merged with abnormal cells of the organ of Corti, forming a roof over the inner sulcus or obliterating it entirely. Claudius cells also proliferate to merge with the spiral prominence and obscure the external spiral sulcus.

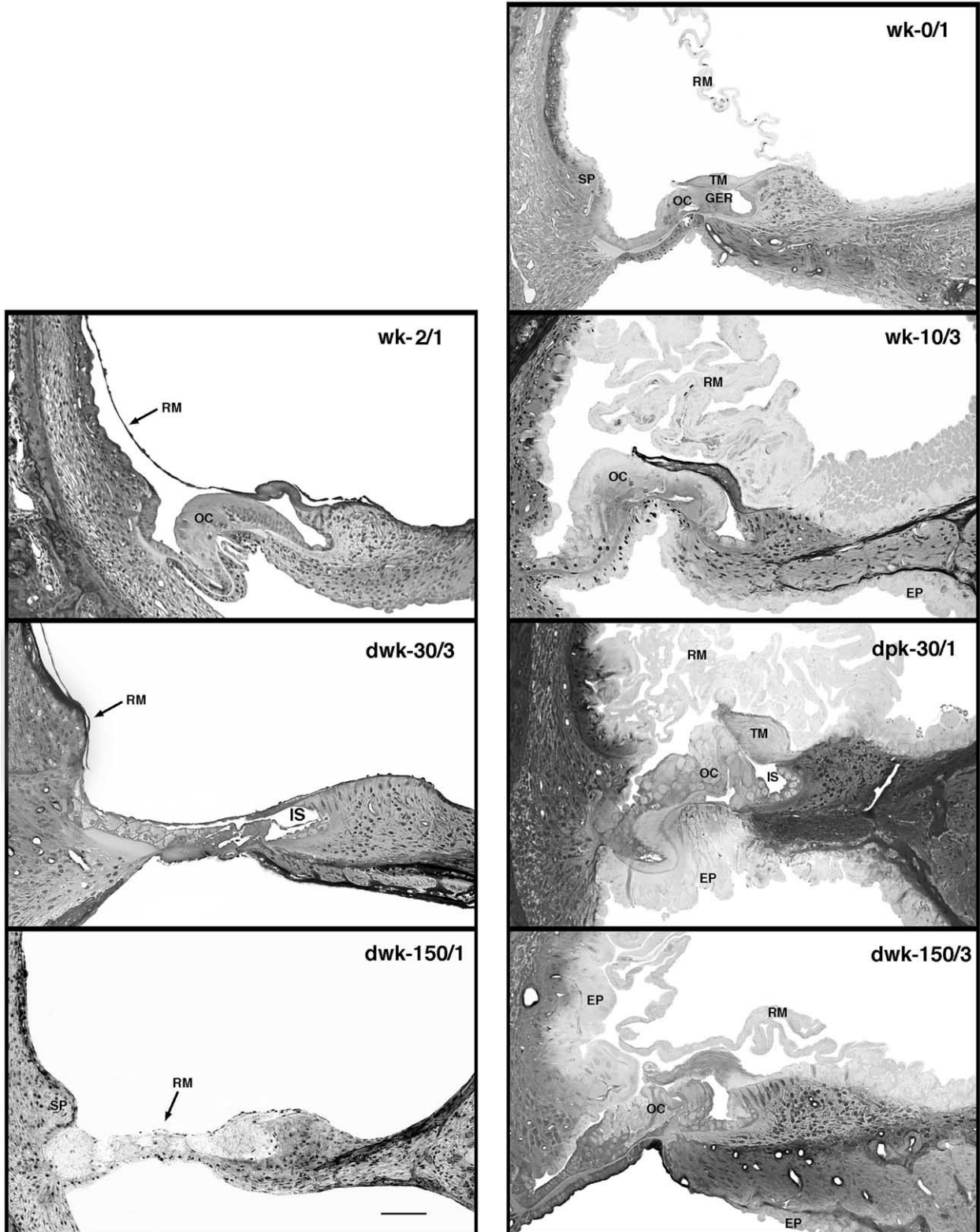The other principal form of cochlear pathology asso-

Table 1
Summary of cat and cochlear histology data base

	Pigmented cats (n = 32)		White cats (n = 44)			
	Hearing	Deaf	Hearing	Deaf		Partially deaf
				Uni.	Bilat.	
ABR tested	31	1 n = 32	4	6	29	5 n = 44
Histology	29	1 n = 35	4	3	23	5 n = 35
Cochlear morphology (years)	51N	2S	8N	26F, 7S, 6M		7H

F, collapsed Reissner's membrane that obliterated the scala media.
H, outward bulging Reissner's membrane (hydrops-like).
M, mixed pathology – spongiform in one cochlea, collapsed Reissner's membrane in the other.
N, normal cochlear histology.
S, spongiform pathology.

ciated with deafness featured an apparent proliferation of cells throughout the cochlear spiral (Figs. 7 and 8, right columns). Three white cats and one pigmented cat bilaterally exhibited this form of cochlear pathology.

One white cat was unilaterally affected. This form featured the hypertrophy of Reissner's membrane such that it became highly irregular and folded, eventually filling the scala media. The supporting cells of the organ

of Corti and epithelial cells of the basilar membrane appeared hypertrophied as well. The basilar membrane was buckled and seemed to rise up to abut the spiral prominence (Figs. 8 and 9). The tunnel of Corti never attained its characteristic triangular shape and hair cells did not differentiate. Epithelial cells that line the bony and membraneous labyrinth also hypertrophied, obscuring the stria vascularis. Overall, the tissue exhibited a 'spongiform' appearance.

Four cats exhibited both epithelial cell overgrowth and a collapsed Reissner's membrane in the same cochlea. In these cats, the mixed pathology expressed spongiform growth in the cochlear apex that gradually gave way to an obliterated scala media in the base. The ear with this type of abnormality produced no ABR to acoustic stimulation and the cats showed no behavioral signs that they could hear.

4. Discussion

The present study sought to test the hypothesis that congenital deafness in white cats was progressive. Several seminal reports on this topic reported that the earliest signs of cochlear pathology occurred after PN5–8 (Bosher and Hallpike, 1967; Mair, 1973). Progressive hair cell loss continued through the first and second years (Mair, 1973). Questions about the progressive nature of congenital deafness in white cats arose from observations that some adult cats were observed that were not completely deaf (Rebillard et al., 1981a,b; Ryugo et al., 1998). A quandary arises because hearing loss in adult white cats was reported to be complete (Mair, 1973). In this study, we demonstrated that hearing does not get progressively worse. That is, we showed that if an animal exhibited normal hearing, there was virtually no change in hearing sensitivity over time using ABR thresholds. Several animals showed partial deafness (or hearing) and their ABR thresholds likewise did not change during development.

These results differ from what had previously been reported (Bosher and Hallpike, 1967; Mair, 1973). Although our study is the only one that collected data longitudinally from the same cats, the different methods probably do not account for the different results. The
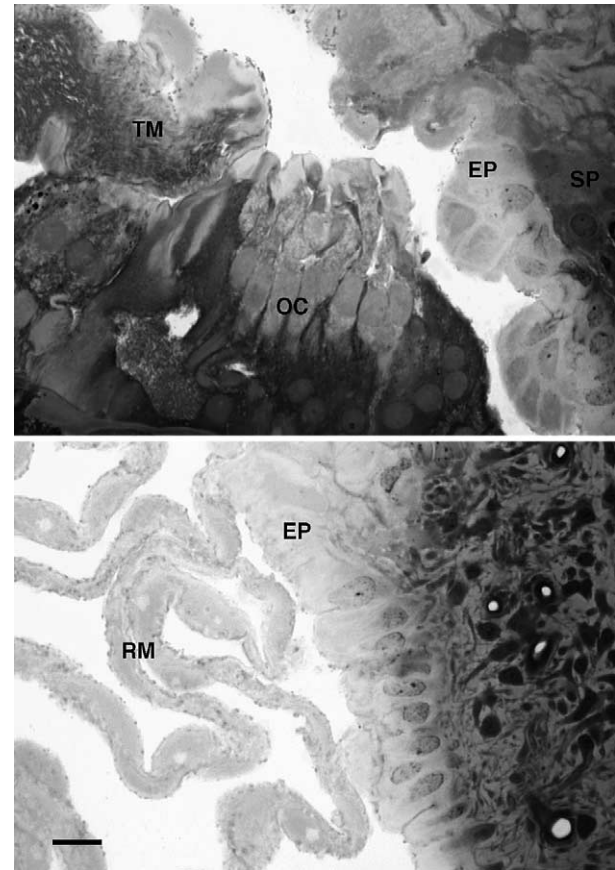

Fig. 9. Photomicrographs of spongiform pathology in the organ of Corti (top), and Reissner's membrane and lateral wall (bottom). Excessive cell growth is illustrated, especially for epithelial cells and supporting cells of the organ of Corti. Scale bar equals 20 μm. Abbreviations: EP, epithelial cells; OC, organ of Corti; RM, Reissner's membrane; SP, spiral prominence; TM, tectorial membrane.

most parsimonious interpretation is that different colonies of cats yield different types of deafness. Some colonies of cats might exhibit progressive hearing loss, whereas others might not. Within our own colony, even within littermates and occasionally within the same cochlea, we observed different manifestations of cochlear pathology. Analysis of the genetic determinants of these pathologies is beyond the goals of this report. Our main point is that deafness was not progressive in our colony.

The discovery of fundamentally different forms of

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Fig. 8. Photomicrographs illustrate the organ of Corti in cats from newborn (wk-0/1) through 5 months of age. Kittens too young to be tested are indicated as wk. A confirmed deaf kitten is indicated as dwk. In our colony, some white cats displayed abnormalities at birth that presaged deafness, and two types of structural abnormalities were clearly evident by PN10. One type (left column) was more common (80%) and featured a collapsed Reissner's membrane onto an abnormal organ of Corti. The other type (right column) occurred less frequently (20%) and exhibited a thickened yet still highly convoluted Reissner's membrane as well as enlarged epithelial cells covering scala tympani and scala media. Both abnormalities were always associated with deafness. It should be noted that both were observed in the same litter, and occasionally in the same cochlea. In three such special cases, the spongiform epithelium was found in the apex, whereas the collapsed Reissner's membrane was found in the base. Scale bar equals 50 μm. Abbreviations: EP, epithelial cells; GER, greater epithelial ridge; IS, inner sulcus; OC, organ of Corti; RM, Reissner's membrane; SP, spiral prominence; TM, tectorial membrane.

cochlear pathology in cats, sometimes in the same ear, is provocative. One form had a collapse of Reissner's membrane onto the tectorial membrane and organ of Corti, obliterating the scala media. This pattern resembled what has been described in the human as the Scheibe deformity (Scheibe, 1892), and represented most of the cases of cochlear pathology. Less frequently, there was an exuberant growth of epithelial cells on Reissner's membrane and within the scala tympani and scala media. This excessive growth smothered the organ of Corti and stria vascularis, and caused Reissner's membrane to fill what would have been scala media. The other infrequent form appeared as a combination of pathologies. Exuberant growth of epithelial cells was always in the cochlear apex and the collapse of Reissner's membrane was found in the cochlear base. Regardless of the form of pathology, however, the result was non-progressive deafness.

The genetic basis of these cochlear abnormalities is beyond the scope of the present report and, furthermore, not pertinent to the non-progressive nature of the deafness. We are, however, generating a detailed family tree for our colony and working on DNA analysis for each cat. The difficulty in this part of the project is that we were not successful in providing complete histological data for every cat. Consequently, we are unable to correlate every deaf ear to the type of cochlear pathology. At the present time, these pathologies do not appear restricted to a particular line of cats. We recognize the need for more detailed information on the genotypes and phenotypes of these cats.

The issue of how the progression of deafness affects brain development in these cats merits further discussion. Certainly, the rapid onset of deafness (Trune, 1982a,b; Powell and Erulkar, 1962) will cause alterations different from a delayed or prolonged onset (Parks, 1979; Webster and Webster, 1979; Webster, 1983b; Mostafapour et al., 2000; Rubel, 1984). These studies illustrate that the central nervous system of older animals is less susceptible to auditory deprivation in comparison to younger animals. The concept of a 'critical period' has been frequently discussed when trying to explain phenomena that are affected most severely during a restricted time window of development (Ryugo et al., 2000; Rubel and Fritzsch, 2002). These critical periods reinforce the notion that there are defined times when the physiological readiness of an organism must coincide with the occurrence of certain external experiences. Congenital deafness, for example, most certainly disrupts the acquisition of spoken language in humans by breaking the auditory feedback loop. Results from cochlear implant studies suggest that young children represent the best candidates for auditory rehabilitation because delayed implantation predicts lower levels of speech recognition (Quittner and Steck, 1991; Gantz et al., 1994; Tyler and Summerfield, 1996; Tucci and Niparko, 2000). These kinds of data emphasize the role age plays in the brain's reaction to the external environment.

In congenital deafness, the question arises as to when deafness begins to affect development of the central auditory system. In mammals, the auditory system is not fully functional at birth (Olmstead and Villablanca, 1980). By the end of the first postnatal month, cats stabilize their pinna reflex, orient appropriately to sounds in space, and learn to differentiate between sounds (Villablanca and Olmstead, 1979). These behaviors appear in accordance with the anatomical maturity of the organ of Corti (Retzius, 1884; Pujol and Hilding, 1973; Romand et al., 1980). Ganglion cell myelination (Romand et al., 1980) and spontaneous activity, rate–level functions, and tuning curve thresholds in auditory nerve fibers exhibit relatively rapid increases during the first 3 postnatal weeks, but maturation of these characteristics continues well beyond the first postnatal month (Romand, 1984; Kettner et al., 1985; Walsh et al., 1986; Walsh and McGee, 1987). The mean spontaneous discharge rate in 2-week-old kittens is approximately 10 spikes/s (Romand, 1984; Walsh et al., 1986), and at 1 month is still 20–30 spikes/s, approximately half the value found in normal adult cats (Liberman, 1978; Romand, 1984; Walsh et al., 1986). In addition, tuning curve thresholds and maximal discharge rates are not at adult levels. These data indicate that during the first month in normal developing cats, there is not a full complement of activity occurring in the auditory nerve.

It is important to note that during this early period of limited input from the auditory nerve, the various neuron types of the cochlear nucleus exhibit their most rapid growth (Brugge, 1983; Larsen, 1984). Adult size is not reached until weeks later, as different cell types grow at different rates and reach adult size at different times (Brugge, 1983). The implication is that some circuits in the auditory system develop more or less independently of each other. The question remains whether the central auditory system can distinguish between limited activity caused by immaturity versus that caused by deafness.

Spontaneous activity in the auditory nerve of congenitally deaf white adult cats is typically absent or, if present, greatly reduced (Ryugo et al., 1998). The synapses of some auditory nerve fibers manifest distinct structural abnormalities. These include depletion of synaptic vesicles, loss of intercellular cisternae, hypertrophy of postsynaptic densities, and shrinkage of postsynaptic cells (Ryugo et al., 1997, 1998; Redd et al., 2000, 2002). Similar synaptic alterations have also been observed in the endbulb synapses of congenitally deaf Shaker-2 mice (Lee et al., 2001), suggesting that these synaptic changes are indeed due to deafness.

In this context, it may be operationally unimportant to distinguish between congenital deafness whose onset is at birth and congenital deafness that has a progressive nature over the first 3 weeks. In either case, spike activity entering the central nervous system will be low during this period. Furthermore, if various auditory circuits develop in parallel rather than in series, the effects of deafness in the central nervous system might not be evident at birth. Postnatal neural activity will therefore emerge as an important variable, and manipulations of environmental sounds might prove the existence of a 'critical' or 'sensitive' period for normal structural and functional development. In the future, we plan to test the hypothesis that during early postnatal development, the structure of primary synapses in the cochlear nucleus will not differ between congenitally deaf cats and cats destined to develop normal hearing.

Acknowledgements

References

Bamber, R.C., 1933. Correlation between white coat colour, blue eyes and deafness in cats. J. Genet. 27, 407–413.

Bergsma, D., Brown, K., 1971. White fur, blue eyes, and deafness in the domestic cat. J. Hered. 62, 171–185.

Bosher, S., Hallpike, C., 1965. Observations on the histological features, development and pathogenesis of the inner ear degeneration of the deaf white cat. Proc. R. Soc. B 162, 147–170.

Bosher, S.K., Hallpike, C.S., 1967. Observations on the histogenesis of the inner ear degeneration of the deaf white cat and its possible relationship to the aetiology of certain unexplained varieties of human congenital deafness. J. Laryngol. 80, 222–235.

Brighton, P., Ramesar, R., Winship, I., 1991. Hearing impairment and pigmentary disturbance. Ann. NY Acad. Sci. 630, 152–166.

Brown, K.S., Bergsma, D.R., Barrow, M.V., 1971. Animal models of pigment and hearing abnormalities in man. Birth Defects Orig. Artic. Ser. 7, 102–109.

Brugge, J.F., 1983. Development of the lower brainstem auditory nuclei. In: Romand, R. (Ed.), Development of Auditory and Vestibular Systems. Academic Press, New York, pp. 89–120.

Burkard, R., 1984. Sound pressure level measurement and spectral analysis of brief acoustic transients. Electroenceph. Clin. Neurophysiol. 57, 83–91.

Deol, M.S., 1954. The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse. J. Genet. 52, 562–588.

Deol, M.S., 1956a. A gene for uncomplicated deafness in the mouse. J. Embryol. Exp. Morphol. 4, 190–195.

Deol, M.S., 1968. Inherited diseases of the inner ear in man in the light of studies on the mouse. J. Med. Genet. 5, 137–158.

Deol, M.S., 1970. The relationship between abnormalities of pigmentation and of the inner ear. Proc. R. Soc. Lond. 175, 201–217.

Gantz, B., Tyler, R., Woodworth, G., Tye-Murray, N., Fryauf-Bertschy, H., 1994. Results of multichannel cochlear implants in congenital and acquired prelingual deafness in children: Five year follow up. Am. J. Otol. 15, 1–8.

Holme, R.H., Steel, K.P., 1999. Genes involved in deafness. Curr. Opin. Genet. Dev. 9, 309–314.

Holme, R.H., Steel, K.P., 2002. Stereocilia defects in waltzer (Cdh23), shaker1 (Myo7a) and double waltzer/shaker1 mutant mice. Hear. Res. 169, 13–23.

Keats, B.J.B., Berlin, C.I., 1999. Genomics and hearing impairment. Genome Res. 9, 7–16.

Kettner, R.E., Feng, J.Z., Brugge, J.F., 1985. Postnatal development of the phase-locked response to low frequency tones of auditory nerve fibers in the cat. J. Neurosci. 5, 275–283.

Larsen, S.A., 1984. Postnatal maturation of the cat cochlear nuclear complex. Acta Otolaryngol. Suppl. 417, 1–43.

Lee, D.J., Cahill, H.B., Pongstaporn, T., Ryugo, D.K., 2001. Effects of congenital deafness on the cochlear nuclei of mice: Ultrastructural analysis of synaptic development. ARO Abstr. 24, 272.

Li, S., Price, S.M., Cahill, H., Ryugo, D.K., Shen, M.M., Xiang, M., 2002. Hearing loss caused by progressive degeneration of cochlear hair cells in mice deficient for the Barhl1 homeobox gene. Development 129, 3523–3532.

Liberman, M.C., 1978. Auditory-nerve response from cats raised in a low-noise chamber. J. Acoust. Soc. Am. 63, 442–455.

Madden, V.J., Henson, M.M., 1997. Rapid decalcification of temporal bones with preservation of ultrastructure. Hear. Res. 111, 76–84.

Mair, I.W., 1973. Hereditary deafness in the white cat. Acta Otolaryngol. 314, 1–48.

Mostafapour, S.P., Cochran, S.L., Del Puerto, N.M., Rubel, E.W., 2000. Patterns of cell death in mouse anteroventral cochlear nucleus neurons after unilateral cochlea removal. J. Comp. Neurol. 426, 561–571.

Nadol, J.B.J., Burgess, B., 1982. Cochleosaccular degeneration of the inner ear and progressive cataracts inherited as an autosomal dominant trait. Laryngoscope 92, 1028–1037.

Olmstead, C.E., Villablanca, J.R., 1980. Development of behavioral audition in the kitten. Physiol. Behav. 24, 705–712.

Parks, T.N., 1979. Afferent influences on the development of the brain stem auditory nuclei of the chicken: otocyst ablation. J. Comp. Neurol. 183, 665–677.

Powell, T.P.S., Erulkar, S.D., 1962. Transneuronal cell degeneration in the auditory relay nuclei of the cat. J. Anat. 96, 219–268.

Pujol, R., Hilding, D., 1973. Anatomy and physiology of the onset of auditory function. Acta Otolaryngol. 76, 1–10.

Pujol, R., Rebillard, M., Rebillard, G., 1977. Primary neural disorders in the deaf white cat cochlea. Acta Otolaryngol. 83, 59–64.

Quittner, A.L., Steck, J.T., 1991. Predictors of cochlear implant use in children. Am. J. Otol. Suppl. 12, 89–94.

Rebillard, G., Rebillard, M., Carlier, E., Pujol, R., 1976. Histo-physiological relationships in the deaf white cat auditory system. Acta Otolaryngol. 82, 48–56.

Rebillard, M., Rebillard, G., Pujol, R., 1981a. Variability of the hereditary deafness in the white cat. I. Physiology. Hear. Res. 5, 179–181.

Rebillard, M., Pujol, R., Rebillard, G., 1981b. Variability of the hereditary deafness in the white cat. II. Histology. Hear. Res. 5, 189–200.

Redd, E.E., Pongstaporn, T., Ryugo, D.K., 2000. The effects of congenital deafness on auditory nerve synapses and globular bushy cells in cats. Hear. Res. 147, 160–174.

Retzius, G., 1884. Das Gehororgan der Wirbeltiere. II: Das Gehororgan der Reptilien, der Vogel und Saugetiere. Samson and Wallin, Stolkholm.

Romand, R., 1984. Functional properties of auditory-nerve fibers during postnatal development in the kitten. Exp. Brain Res. 56, 395–402.

Romand, R., Romand, M.R., Mulle, C., Marty, R., 1980. Early stages of myelination in the spiral ganglion cells of the kitten during development. Acta Otolaryngol. 90, 391–397.

Rubel, E.W., 1984. Ontogeny of auditory system function. Ann. Rev. Physiol. 46, 213–229.

Rubel, E.W., Fritzsch, B., 2002. Auditory system development: primary auditory neurons and their targets. Ann. Rev. Neurosci. 25, 51–101.

Ryugo, D.K., Limb, C.J., Redd, E.E., 2000. Synaptic plasticity: The impact of the environment on the brain as it relates to cochlear implants. In: Niparko, J.K., Kirk, K.I., Mellon, N.K., Robbins, A.M., Tucci, D.L., Wilson, B.S. (Eds.), Cochlear Implants: Principles and Practices. Lippincott Williams and Williams, Philadelphia, pp. 33–56.

Ryugo, D.K., Pongstaporn, T., Huchton, D.M., Niparko, J.K., 1997. Ultrastructural analysis of primary endings in deaf white cats: Morphologic alterations in endbulbs of Held. J. Comp. Neurol. 385, 230–244.

Ryugo, D.K., Rosenbaum, B.T., Kim, P.J., Niparko, J.K., Saada, A.A., 1998. Single unit recordings in the auditory nerve of congenitally deaf white cats: morphological correlates in the cochlea and cochlear nucleus. J. Comp. Neurol. 397, 532–548.

Scheibe, A., 1892. A case of deaf-mutism, with auditory atrophy and anomalies of development in the membranous labyrinth of both ears. Arch. Otolaryngol. 21, 12–22.

Scheibe, A., 1895. Bildungsanomalien im häutigen Labyrinth bei Taubstummheit. Z. Ohrenheilk. 27, 95–99.

Shnerson, A., Willott, J.F., 1980. Ontogeny of the acoustic startle response in C57BL/6J mouse pups. J. Comp. Physiol. Psychol. 94, 36–40.

Sokolich, W.G., 1977. Improved acoustic system for auditory research. J. Acoust. Soc. Am. Suppl. 62, S12.

Steel, K.P., Bock, G.R., 1980. The nature of inherited deafness in deafness mice. Nature 288, 159–161.

Suga, F., Hattler, K.W., 1970. Physiological and histopathological correlates of hereditary deafness in animals. Laryngoscope 80, 81–104.

Trune, D.R., 1982a. Influence of neonatal cochlear removal on the development of mouse cochlear nucleus: I. Number, size, and density of its neurons. J. Comp. Neurol. 209, 409–424.

Trune, D.R., 1982b. Influence of neonatal cochlear removal on the development of mouse cochlear nucleus: II. Dendritic morphometry of its neurons. J. Comp. Neurol. 209, 425–434.

Tucci, D.L., Niparko, J.K., 2000. Medical and surgical aspects of cochlear implantation. In: Niparko, J.K., Kirk, K.I., Mellon, N.K., Robbins, A.M., Tucci, D.L., Wilson, B.S. (Eds.), Cochlear Implants: Principles and Practices, Lippincott Williams and Wilkins, New York, pp. 189–221.

Tyler, R.S., Summerfield, A.Q., 1996. Cochlear implantation: Relationships with research on auditory deprivation and acclimatization. Ear Hear. Suppl. 17, 38s–50s.

Villablanca, J.R., Olmstead, C.E., 1979. Neurological development in kittens. Dev. Psychobiol. 12, 101–127.

Walsh, E.J., McGee, J., 1987. Postnatal development of auditory nerve and cochlear nucleus neuronal responses in kittens. Hear. Res. 28, 97–116.

Walsh, E.J., McGee, J., Javel, E., 1986. Development of auditory-evoked potentials in the cat. I. Onset of response and development of sensitivity. J. Acoust. Soc. Am. 79, 712–724.

Webster, D.B., 1983b. Late onset of auditory deprivation does not affect brainstem auditory neuron soma size. Hear. Res. 12, 145–147.

Webster, D.B., Webster, M., 1979. Effects of neonatal conductive hearing loss on brainstem auditory nuclei. Ann. Otol. Rhinol. Laryngol. 88, 684–688.

Wilson, T.G., Kane, F., 1959. Congenital deafness in white cats. Acta Otolaryngol. 50, 269–277.

Wolff, D., 1942. Three generations of deaf white cats. J. Hered. 33, 39–43.