CHANGES IN SPONTANEOUS ACTIVITY AND CNS MORPHOLOGY ASSOCIATED WITH CONDUCTIVE AND SENSORINEURAL HEARING LOSS IN CHICKENS

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The effects of a conductive or mixed (conductive and sensorineural) hearing loss on anatomical and physiological properties of the chicken auditory system were examined. Animals used in the anatomical studies underwent either a columella (ossicle) removal, which produced a moderate conductive hearing loss, or an oval window puncture, which produced a severe mixed hearing loss, at 4 days posthatch. In a companion study, multiunit spike counts were obtained from 3-week-old chickens before, during, and after consecutive tympanic membrane puncture, columella removal, and oval window puncture. Tympanic membrane puncture and columella removal (conductive hearing loss) are not associated with either cell area changes in the nucleus magnocellularis or changes in spontaneous neuronal activity. Conversely, an oval window puncture (sensorineural damage) is associated with a cell area reduction of 20%, as well as a marked decline in activity within auditory nuclei.

KEY WORDS — deprivation, development, neural activity.

INTRODUCTION

Numerous authors have suggested that a chronic, intermittent conductive hearing loss during childhood is associated with substandard performance on specific measures of auditory processing skills,[1,2] an effect that may be permanent and irreversible.[3] The conclusions drawn from these studies have been subjected to thoughtful scrutiny in reviews of this literature by Ventry,[4] Paradise,[5] and Ruben.[6] Criticisms focus on the problems of subject selection, small numbers of subjects, documentation of the original diagnosis and associated hearing impairment, and appropriateness of tests and testing techniques.

While inherent limitations do exist in the generalization of results from animal studies to a human population, such paradigms offer solutions to the experimental design problems noted above. Studies published to date suggest that peripheral manipulations that reduce exposure to environmental sound are associated with changes in neuron soma size in first through fourth order auditory neurons in the mouse, rat, and chicken[7-10] as well as dendritic morphology in brain stem auditory nuclei.[11,12] Alterations in physiological response to sound[13,14] and auditory perceptual tasks[15,16] have also been noted. One shortcoming of these studies is that the type and degree of hearing loss produced by experimental manipulations has not been well characterized. It is not clear, in all cases, that attempts to experimentally produce a purely conductive hearing loss have been successful. Cochlear damage concurrent with or secondary to the experimental manipulation has not been thoroughly examined. Furthermore, although it has been hypothesized that altered neural activity is responsible for the morphological changes in auditory neurons,[17,18] measurements of spontaneous and acoustically driven activity have not been reported in conditions of known hearing loss.

We report the results of experiments that address the following questions. 1) Is a documented conductive or sensorineural hearing loss associated with changes in second order auditory neuron morphology? 2) Are changes from baseline neural activity associated with conductive or sensorineural disorders? These questions were examined using chickens as experimental subjects. These animals have been used extensively in our laboratory for anatomical, physiological, and developmental studies of the auditory system. The advantages of their use are detailed elsewhere.[19]

METHODS

SURGICAL MANIPULATIONS

Columella Removal. The chicken has one middle ear ossicle, the columella. Removal of the columella is a simple procedure, accomplished by creating a small hole in the tympanic membrane, identifying the shaft of the columella, and removing the ossicle

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Portions of this work were presented at the fall meeting of the Association for Research in Otolaryngology, Las Vegas, Sept 15, 1984, and at the Eighth Midwinter Meeting of the Association for Research in Otolaryngology, St Petersburg, Florida, Feb 4, 1985. Portions of this study were previously published in Tucci DL, Rubel EW; Afferent influences on brain stem auditory nuclei of the chicken: effects of conductive and sensorineural hearing loss on n. magnocellularis; *J Comp Neurol* 1985;238:371-81.

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from the middle ear space. Removing the columella in this manner does not produce observable leakage of perilymph. Chickens were 4 days posthatch at the time of surgery. After survival times of 2 (n = 6), 4 (n = 4), 8 (n = 4), 15 (n = 5), 30 (n = 6), 45 (n = 6), or 60 (n = 5) days, animals were perfused with either Heidenhain's solution or a mixture of 2% glutaraldehyde and 2% formaldehyde in phosphate buffer. Brains were dehydrated, embedded in paraffin, coronally sectioned at 10 μm, mounted on slides, and stained with thionin. Columella removal was always performed on the right ear, so that the left served as control. In addition, two to three animals that hatched with the experimental animals served as controls in each survival group.

Oval Window Puncture. In seven 4-day-old animals, removal of the columella was followed by insertion of the forcep tip (No. 5 Dumont) 1 to 2 mm through the oval window. Animals were allowed to survive 60 days and were then processed as described above. Because we have examined cochlear hair cells in ears following this manipulation and found no evidence of hair cell damage by light microscopy,[20] we assume that this puncture results in a perilymph fistula rather than in free mixing of endolymph and perilymph.[21,22] It is possible that restricted damage to the tegmentum vasculosum is produced by this procedure. Hair cell counts may not reveal subtle but physiologically important defects in these structures.

CELL AREA MEASUREMENTS

Eighth nerve fibers project from the ipsilateral basilar papilla (cochlea) providing monaural input to second order neurons in the nucleus magnocellularis (NM). This nucleus receives no known input from contralateral auditory structures. In each experimental and control animal, the cross-sectional areas of NM cells were measured on each side of the brain. All cell area measurements were made by one experimenter, and only neurons with a visible nucleolus, a clear nucleus, and stained cytoplasm were measured. All neurons in the NM meeting these criteria were measured from three consecutive sections (spaced at 40-μm intervals). The measurements were repeated at two positions in the NM, 30% and 70% of the anterior-to-posterior extent of the nucleus. These positions correspond roughly to the 2,000- and 500-Hz regions, respectively, of this tonotopically organized nucleus. Cell measurements were made with a Zeiss Videoplan Image Analysis System. A total of 16,008 cells were measured for this study. Repeat measurements, made by both the experimenter (ten brains) and a naive observer (six brains), varied from the original by less than 5%.

HEARING THRESHOLD MEASUREMENTS

For the purposes of this report we will use the accepted clinical terminology to define conductive and sensorineural hearing loss. A conductive hearing loss is an elevation of air-conducted stimulus thresholds, with normal responses to bone-conducted signals. Sensorineural hearing loss is present when thresholds to both air- and bone-conducted signals are elevated.

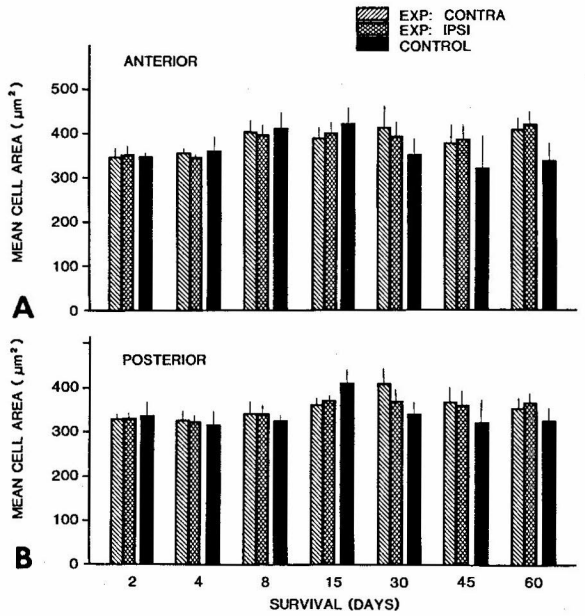

Fig 1. Mean cross-sectional cell area in nucleus magnocellularis (NM) as function of survival period following unilateral columella removal. Data shown are for NM cells ipsilateral (EXP:IPSI) and contralateral (EXP:CONTRA) to operated ear in experimental animals, and for two sides averaged for control animals (CONTROL). Lines indicate standard error of mean. Cell area measurements are from A) anterior and B) posterior portions of NM. (Reprinted with permission.[20])

Evoked potential thresholds were obtained for both air-conducted and bone-conducted signals following columella removal and oval window puncture. Thresholds were obtained at frequencies ranging from 500 to 3,000 Hz; a pure tone of 20-ms duration with a 4-ms rise-fall time was presented at a rate of 2/second. Stimulus presentation was by a speaker (Realistic Minimus-7) or by a bone vibrator (Radioear B-71) fixed to a dental cement base on the animal's head. Free field calibration was carried out at the normal position of the animal's head using a Knowles BL 1830 microphone and a Bruel and Kjaer narrow band spectrum analyzer (type 2031). The bone vibrator was calibrated using an artificial mastoid (Bruel and Kjaer type 4930). Evoked potential thresholds were recorded in anesthetized animals (ketamine hydrochloride, 80 mg/kg and Chloropent [Fort Dodge Labs; proprietary mixture of pentobarbital, chloral hydrate, magnesium sulfate, ethanol, and propylene glycol], 1.5 mg/kg or sodium pentobarbital [Nembutal], 0.5 mg/kg) 20 to 25 days after hatching. Grass pin electrodes were implanted through the intact skull into the right and left cerebellum at a level just above the brain stem auditory nuclei and in the thigh muscle (ground). Responses were amplified, filtered (20 to 2,000 Hz band pass),

digitized by an A to D converter at a rate of 10 kHz and then averaged over 200 stimulus presentations. Thresholds were defined to within 5 dB by identifying the lowest intensity for which the response was twice the amplitude of prestimulus baseline variations.

Air conduction thresholds were obtained from four animals following columella removal and three animals following oval window puncture. In all cases, hearing loss was determined by comparison with baseline thresholds in the intact ear of the same animal; all thresholds were measured immediately following the manipulation.

Bone conduction thresholds were recorded in two animals, following sequential columella removal and oval window puncture. In these animals, the contralateral cochlea had been removed either acutely or several days prior to testing in order to limit responses to the manipulated ear. Thresholds were also obtained from four columella removal animals and four oval window puncture animals 7 days following the surgical manipulation. In each case, baseline threshold measurements were made with one cochlea intact. The intact cochlea was subsequently removed to selectively test thresholds of the manipulated ear.

MULTINEURONAL SPIKE COUNTS

Eleven 3-week-old chickens were anesthetized as described for measurement of hearing loss, and secured in a specially designed headholder inside a double wall Industrial Acoustics Corporation chamber. Body temperature was maintained at 39°C. The skull over the cerebellum was removed to allow penetration of a glass-insulated tungsten recording electrode (1 to 2 MΩ resistance). Sound was delivered through a closed system via calibrated (Knowles BL 1830 microphone attached to a probe tube) ear tubes sealed at each auditory meatus.

Spontaneous and sound-driven multineuronal spike recordings were made from the second and third order nuclei, the NM and nucleus laminaris (NL), respectively, which were located by sound-evoked activity. A pulse height discriminator was set so that the trigger level was just above the electrical noise level when the electrode was in CSF above the brain. Spikes were counted in 1-second bins, and the average of 100 bins was calculated. The maintained spontaneous discharge from auditory brain stem nuclei was measured before, during, and after contralateral cochlea removal, ipsilateral tympanic membrane puncture, columella removal, and oval window puncture. In addition, sound-driven activity was monitored by obtaining rate intensity functions following each manipulation at the multineuronal best frequency. The stimulus used was a 1-second pure tone with 4-ms rise-fall times. The stimulus was presented at 2-second intervals.

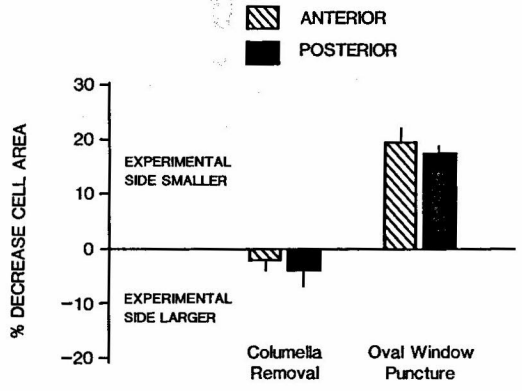

Fig 2. Percent decrease in cell area following columella removal and oval window puncture in animals that survived 60 days. Percent decrease in cell area is mean of 100 x (contralateral − area ipsilateral)/area contralateral; positive number indicates that ipsilateral cells are smaller than those contralateral to manipulated ear. Scores are given for anterior and posterior portions of NM. Lines indicate standard error of mean.

Changes in spontaneous spike rate were quantified by comparing the number of spikes/second before manipulations to the spike rate following each manipulation. The premanipulation baseline for the experimental ear was determined by averaging three samples, each of which was 100 seconds in duration. At least one of the premanipulation samples was taken after the contralateral cochlea was removed.

In order to determine whether changes in recorded neural activity occur as a result of long-term electrode placement, chronic recordings were made in one animal for 12 hours prior to any manipulation and in a second animal for 3 hours between columella removal and oval window puncture. In addition, movement of the electrode at the end of each experiment within a 100-μm region on either side of the original recording site confirmed that activity changes, when observed, were not limited to the original recording site. These maneuvers added confidence that observed changes in activity were not the result of tissue damage from the recording electrode.

At the end of each experiment, a small lesion was produced at the site of the recording electrode and the brains were processed for histology. Brains in which lesions were not located within the boundaries of the NM or NL were eliminated from the study. Microelectrode lesions were located in the NM or NL in eight of 11 experimental animals.

RESULTS

CELL AREA MEASUREMENTS

Mean cross-sectional cell area for anterior (30% of anterior-to-posterior extent) and posterior (70% of anterior-to-posterior extent) portions of the NM following columella removal are presented for each survival group in Fig 1. Examination of these data reveals no difference between experimental and

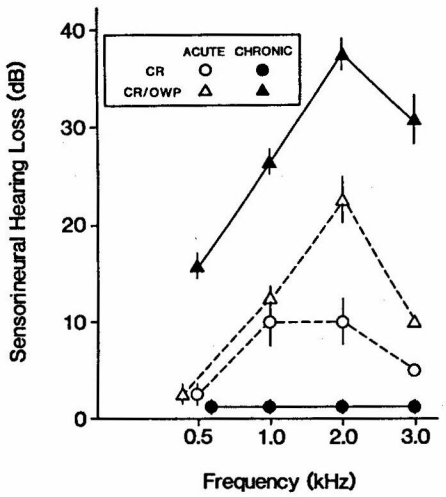

Fig 3. Sensorineural hearing loss measured in decibels as function of bone-conducted stimulus frequency after columella removal (CR) or columella removal and oval window puncture (CR/OWP). Lines indicate standard error of mean. (Reprinted with permission.[20])

control sides of the brain. A multiple dimension analysis of variance was used to test for statistically significant effects. Mean cell areas, difference scores (area left minus area right), and percent difference scores (difference scores divided by area left, multiplied by 100) were examined as a function of level in the nucleus and side of the brain (within subject variables) and by survival time and treatment condition (between subject variables). There was no main effect of side of the brain at either level and no interaction between side of the brain and survival time or level in the nucleus. There was a significant effect of level in the nucleus ($p<.001$), which reflects the fact that cells in the anterior NM are typically slightly larger than those in the posterior portion of the nucleus. Mean cell area data from the control animals were not statistically different from data obtained from the control or the experimental sides of the brain in experimental animals. Although there appears to be a consistent difference between mean cell area of control and experimental animals at the three longest survival times, this between animal difference does not approach statistical significance.

Mean cell areas for animals in the oval window puncture group are compared in Fig 2 with those for columella removal animals at a similar survival time. Cell area in the NM ipsilateral to the oval window puncture was reduced by 19.6% and 17.6% for anterior and posterior portions of the nucleus, respectively. An analysis of variance (cell area by position in nucleus and side of brain) confirmed the significant effect of this manipulation on cell area on the experimental side for both the anterior and the posterior positions ($p<.005$).

HEARING THRESHOLDS

In animals with the columella removed, evoked potential thresholds for free field stimuli revealed a 50- to 55-dB hearing loss across frequencies. Hearing loss following oval window puncture was greater than that following simple columella removal, but could not be measured exactly due to the output limitations of the sound delivery system.

Bone conduction thresholds are presented in Fig 3 for acute and chronic conditions. It is evident from these data that the hearing loss produced by columella removal is purely conductive in nature, while that associated with oval window puncture has a sensorineural as well as a conductive component. The 5- to 10-dB sensorineural hearing loss observed acutely following simple columella removal completely resolves over time. The initial difference

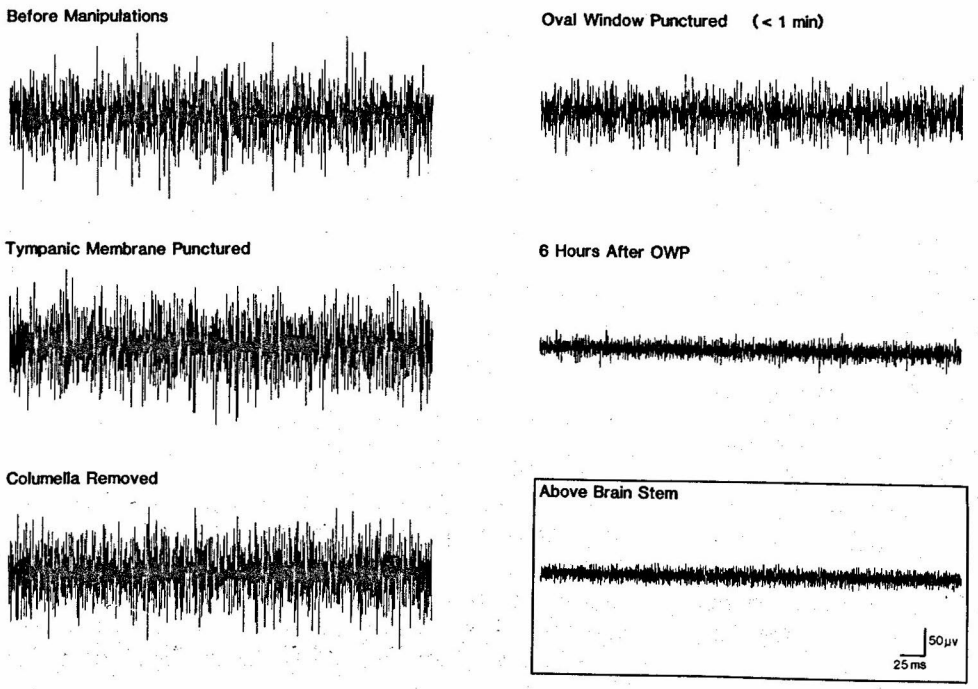

Fig 4. Maintained (spontaneous) multineuronal spike activity recorded from one animal before manipulation and after successive experimental manipulations. Activity just above brain stem (used to set discriminator level) is included for comparison.

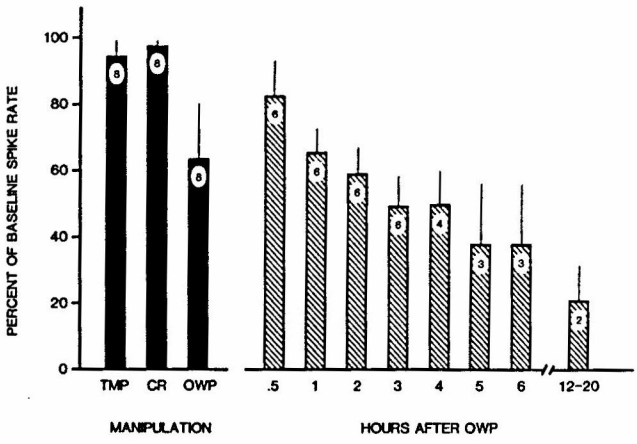

Fig 5. Averaged multineuronal spike rates presented as percentage of premanipulation activity. Number of animals averaged for each data point is displayed in histogram bars. Activity following each manipulation is shown by solid bars, and activity after successive periods following oval window puncture is shown by hatched bars. Lines indicate standard error of mean.

was probably due to mechanical trauma associated with removal of the ossicle. Conversely, the sensorineural hearing loss associated with oval window puncture increases with time, possibly due to a persistent perilymph fistula or altered perilymph-endolymph fluid relationships.

MULTINEURON SPIKE RECORDINGS

Spontaneous Activity. Spontaneous spike rates at the discriminator settings used ranged from 900 to 1,800 spikes/second prior to manipulations. Activity levels following contralateral cochlea removal were within 10% of the previously established baseline in five cases. In the remaining three animals, activity transiently increased following contralateral cochlea removal, by an average 25%. Figure 4 illustrates the changes in neural activity recorded from the NM in a representative animal before any manipulation, and then during successive manipulations: after tympanic membrane puncture, after columella removal, and following oval window puncture (immediate and 6 hours after the procedure). These illustrations of activity are compared with the electrical trace recorded with the electrode tip in CSF above the brain stem.

Averaged data from all eight animals are presented in Fig 5 as percent of premanipulation baseline activity levels. Recordings were taken from animals for variable amounts of time. Multineuronal activity rates remained relatively unchanged following manipulations that affected only the middle ear conductive apparatus. Average activity levels following tympanic membrane puncture and columella removal were 94% and 97% of baseline, respectively. However, once the integrity of the sensory organ was disrupted, as after the oval window puncture, activity levels quickly fell to an average 64% of baseline. Thirty minutes later we observed an unexpected partial recovery to 83% of baseline, which was followed by a continuous decline. Neural activity rates decreased to less than 50% by 3 hours after oval window puncture in six surviving animals. In the one animal from which we continued to take physiological data for over 20 hours after oval window puncture, the activity level fell to less than 20% of the original baseline level.

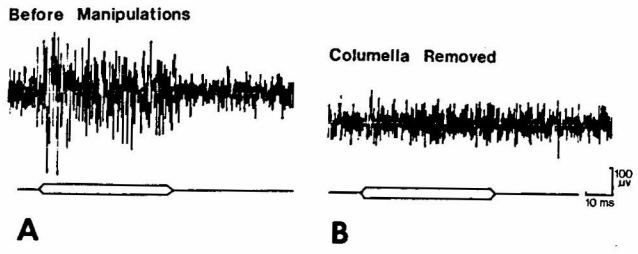

Fig 6. Acoustically driven activity for one animal A) before and B) after columella removal. Best frequency in this example was 2,485 Hz. Stimulus envelope is shown below each trace.

Results of long-term recordings revealed no effect of chronic electrode placement. For one animal in which activity was measured for 12 hours before any experimental manipulation was performed, activity was found to increase somewhat from 1,185 spikes/second to 1,243 spikes/second, with a mean activity rate during this period of 990 spikes/second. Likewise, activity was found to increase during the course of a 3-hour recording in one animal following columella removal, from 1,566 spikes/second to 1,628 spikes/second, with an average activity rate of 1,579 spikes/second during that period. Therefore the changes observed following oval window puncture cannot be attributed to trauma produced by the recording procedures.

Acoustically Driven Activity. Best frequency in the region of the recording electrode ranged from 650 to 2,350 Hz. An example of acoustically driven activity is shown in Fig 6. There is an obvious decrease following columella removal in the number of individual spikes and in the background activity in response to the pulsed tone, while baseline activity appears unaltered.

Rate-intensity functions were obtained at the best frequency of the recording site for all experimental conditions. A representative series of rate-intensity functions from one animal is shown in Fig 7. Auditory thresholds were determined from the rate-intensity functions as the point at which driven activity begins to increase with increasing stimulus intensity. In decibels sound pressure level (SPL), the average thresholds (±SD) were as follows: baseline 24 dB SPL (±2.6), tympanic membrane puncture 41 dB SPL ((±10), columella removal 72 dB SPL (±18), and oval window puncture >99 dB SPL. The hearing loss produced by columella removal (48 dB) correlates well with data obtained from our evoked potential measurements. Again, output limitations of the sound delivery system precluded observation of the entire rate-intensity function for animals following oval window puncture.

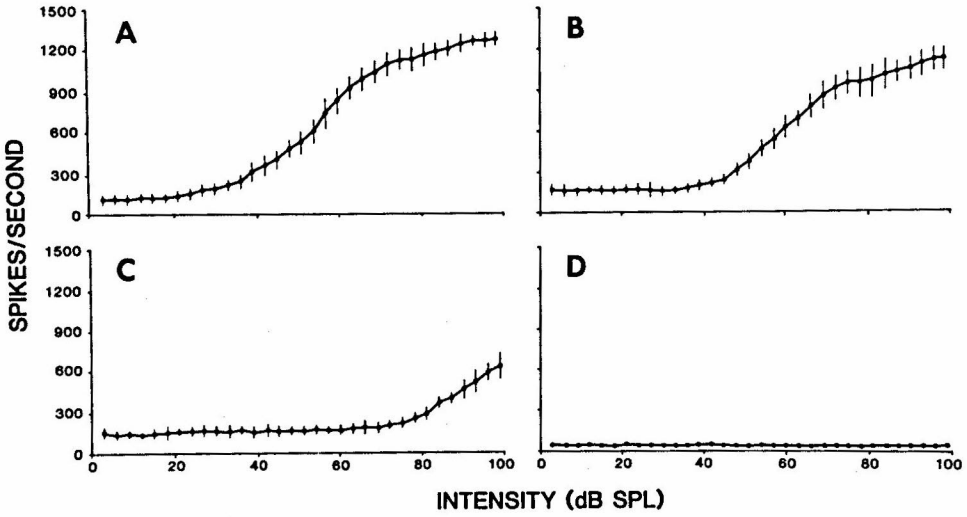

Fig 7. Unilateral rate-intensity functions for chick at consecutive stages of experiment: A) baseline (with contralateral basilar papilla removed), B) after tympanic membrane puncture, C) after columella removal, and D) after oval window puncture. Stimulus was 1-second pure tone at best frequency (1,298 Hz) and spikes/second were averaged over ten 100-ms bins. Lines indicate ±1 SD.

DISCUSSION

The "trophic" influence of afferent activity on the development and maintenance of postsynaptic target neurons has long been of interest. A relative decrease in activity is the factor often assumed responsible for transneuronal degenerative changes associated with both deafferentation and deprivation in various sensory systems.[17] However, until recently there has been no systematic quantitative examination of the effects of peripheral manipulation on both activity and morphology in a sensory system.

Kupperman and Kasamatsu[23] and Eysel and Wolfhard[24] studied the relationship between neural activity and cell area changes in the lateral geniculate nucleus (LGN) of cats; these studies yielded conflicting data. Tetrodotoxin (TTX), a sodium channel blocker, has been demonstrated to temporarily eliminate retinal ganglion cell action potentials and provides a means of selectively examining the influence of activity on postsynaptic neurons. Kupperman and Kasamatsu[23] found that successive TTX injections in 7-week-old kittens were associated with a 20% to 30% decrease in the LGN cell area after 1 week. However, in one adult cat, Eysel and Wolfhard[24] reported no greater decrease in LGN cell area after both TTX injection and a partial retinal lesion than after retinal lesion alone. They concluded that a loss of neural connections rather than a change in activity is the factor responsible for the observed LGN morphological changes.

The present study demonstrates that a columella removal, which produces a purely conductive hearing loss of a moderate degree, is not associated with cell area changes in second order neurons of the NM in the chicken. Conversely, an oval window puncture, which causes a severe mixed (conductive and sensorineural) hearing loss, is associated with a 20% reduction in cell area in the ipsilateral NM.

These initial findings of our study were quite unexpected in view of previous results.[7-10] Physiological measurements were performed with the hope of clarifying our anatomical data. Measurements of activity in second and third order auditory neurons revealed that spontaneous activity was not altered by those manipulations known to produce a purely conductive hearing loss, namely, tympanic membrane puncture and columella removal. However, once the integrity of the oval window was disrupted, producing a sensorineural hearing loss, activity decreased markedly. This decline in activity was variable over time, presumably because of the initial dynamic nature of the fistula. However, activity steadily decreased after 30 minutes, and after 12 hours it had dropped to 20% of baseline in the two surviving animals. These results imply that morphological changes in NM are related to changes in eighth nerve activity. Alterations in both neural activity and morphology are associated with manipulations that produce a sensorineural hearing loss (ie, oval window puncture) but not with columella removal, which produces a purely conductive hearing loss with little or no change in maintained activity.

The long-term nature of the fistula produced by oval window puncture has not been assessed. Therefore the morphological changes observed in brain stem auditory neurons could result from a variety of factors that were not completely assessed by absolute hair cell counts. For the purposes of clarity it is important to emphasize that we have distinguished conductive hearing loss from sensorineural hearing loss simply on the basis of the presence or absence of an air-bone gap on physiological testing. This does not define the actual mechanism responsible for the sensorineural hearing loss. In addition to the possibilities of cochlear injury related to formation of the fistula, chronic leakage of perilymph resulting in injury to the sensory epithelium or injury to the tegmentum vasculosum producing a loss of the endocochlear potential, a variety of alternative explanations should be

considered. These include impedance alterations due to changes in fluid dynamics in the middle or inner ear spaces, alterations in perilymph and associated CSF pressure changes, chronic changes in inner ear temperature, and inner ear infection not revealed by the histological analysis.

The activity changes observed in this study are consistent with data from several other investigators. Woolf et al[25] studied 2-deoxyglucose (2-DG) uptake in the gerbil in silence following unilateral tympanic membrane and malleus removal. Glucose metabolism, as reflected by 2-DG uptake, has been widely used as an indirect measure of functional activity in neural tissue. Labelled 2-DG uptake was at least as great (and reportedly slightly greater) in the auditory pathway innervated by the ear with the conductive disorder as in neural regions receiving input from the normal ear. Unilateral cochlea ablation, associated with a dramatic reduction in spontaneous neural activity,[26] produces a large decrease in 2-DG uptake.[25,27] In addition, Sachs et al[28] report that placement of an earplug in the external auditory canal of pigeons does not alter spontaneous activity in eighth nerve fibers. This manipulation increased auditory nerve discharge thresholds by 40 dB.

It is of interest that the anatomical results of the present study appear to differ from previous studies in the chick and mouse.[7,8,10] Possible explanations for these differences are discussed in more detail elsewhere.[20] It is unlikely that the manipulation used in this study was initiated after the termination of a critical period in development. Initiation of a conductive hearing loss at embryonic day 19, as in previous experiments,[10] also failed to produce changes in NM cell size.[20]

Two explanations of the dissimilarities between our data in the chick and those of Webster and Webster[7] and Webster[8] in the mouse seem most plausible. The first relates to differences in the spectrum of hearing in these laboratory animals. The range of frequencies to which the mouse is sensitive is considerably higher than the range for the chicken (1 to 60 kHz in the mouse compared with 0.1 to 5 kHz in the chicken). It is possible that a conductive hearing loss is more likely to affect high frequency neurons. The results of Smith et al[12] lend some support to this notion. The use of an animal that is sensitive to a broad frequency spectrum could permit the assessment of differences in the effects of a conductive loss on neurons responsive to high versus low frequencies. It will also be important to assess changes in spontaneous activity associated with a conductive deficit in both high and low frequency neurons. Another plausible explanation of the discrepancy is that experimental manipulations of the conductive pathways can lead to secondary inner ear changes that reduce spontaneous activity. While earlier reports seem to eliminate this possibility, Webster and Bobbin[29] have recently reported changes in electrical potentials recorded from the inner ear after reopening atretic ear canals in mice.

The findings of the present study show a relationship between neural activity and morphological changes in second order auditory neurons. These results demonstrate that auditory deprivation, as produced by a purely conductive hearing loss, is not simply a less severe case of deafferentation. In this study, a conductive hearing loss produced changes in the transmission of information from the environment to the brain, but no changes in overall level of spontaneous activity or atrophy of neurons in the second order brain stem auditory nucleus. Deafferentation, or for that matter, less severe damage to the inner ear produces at least acute changes in spontaneous activity as well as diminished information from the environment. This in turn resulted in chronic atrophic changes in these second order neurons. We suspect that these conclusions, on further testing, will prove valid across species. It is possible that otitis media could be associated with such changes only if a concomitant sensorineural hearing loss exists. A relationship between severe OME and sensorineural hearing loss has been proposed.[30] However, it is also possible that activity levels in other neural regions may be markedly altered by conductive deficits, and that other auditory capabilities such as binaural processing and cognitive development may be adversely affected by the hearing loss.[6] It is therefore premature to draw conclusions about the effects of uncomplicated otitis media on auditory development. It is clear that in future studies the type and degree of hearing loss associated with experimental manipulations must be carefully assessed so that the findings may be interpreted unambiguously.

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