

# Afferent Influences on Brain Stem Auditory Nuclei of the Chicken: Effects of Conductive and Sensorineural Hearing Loss on N. Magnocellularis

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## ABSTRACT

Nucleus magnocellularis is the avian homologue of the spherical cell region of the mammalian anteroventral cochlear nucleus. Its primary excitatory synaptic input is from large end bulbs of Held from the eighth nerve ganglion cells. We have examined the effects of three peripheral manipulations—middle ear ossicle (columella) removal (monaural and binaural), columella removal and oval window puncture (monaural), and monaural earplug—on cross-sectional cell area ("cell size") of second-order auditory neurons in n. magnocellularis of the chicken. Manipulations were performed between embryonic day 19 and posthatch day 4. Survival time was varied from 2 to 60 days. Air conduction and bone conduction thresholds were determined to assess for conductive and sensorineural hearing loss associated with each of these manipulations. Hair cell counts were made from basilar papillae of each experimental group.

We found that a columella removal alone, which produced a 50–55-dB purely conductive hearing loss, was not associated with changes in cell size of n. magnocellularis neurons. Similarly, chronic monaural earplugging did not affect the cross-sectional area of these second-order auditory neurons. Conversely, a combined columella removal and oval window puncture, which produced a mixed hearing loss with a 15–40-dB sensorineural component was associated with an 18–20% reduction in n. magnocellularis cell area. Hair cell counts for experimental ears were not significantly different from control ears. These results, in conjunction with measurements of multiunit activity recorded in n. magnocellularis, suggest that manipulations which markedly attenuate extrinsic auditory stimulation, but do not result in chronic change in the average activity levels, also do not influence the size of n. magnocellularis cell bodies. On the other hand, a manipulation which influences overall activity levels, but does not result in degeneration of receptor cells, resulted in marked changes in n. magnocellularis cell size.

**Key words:** deprivation, development, cochlear nucleus

Experimental auditory "deprivation" refers to a manipulation that reduces the normal amplitude of sound pressure to the peripheral auditory end organ. This is achieved either by reduction or modification of environmental exposure to sound, or by production of a purely conductive (theoretically completely reversible) hearing loss. In the latter case, the peripheral end organ presumably remains intact. If this is true, then changes in auditory neurons that may occur secondary to functional deprivation are not confounded by

transneuronal degenerative changes resulting from partial deafferentation. Efforts to describe the effects of deprivation on the developing auditory system have used anatom-

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ical (see below), physiological (Clopton and Silverman, '77; Silverman and Clopton, '77; Clopton, '80; Moore and Irvine, '81; Sanes and Constantine-Paton, '85), and behavioral (Tees, '67; Gottlieb, '76, '78; Clements and Kelly, '78; Kerr et al., '79; Knudsen et al., '84) methods of investigation.

Anatomical studies have examined deprivation effects on cross-sectional cell areas in various auditory nuclei (Webster and Webster, '77, '79; Webster, '83a-c; Coleman and O'Connor, '79; Coleman et al., '83; Blatchley et al., '83; Conlee and Parks, '81), as well as on dendritic morphology in these nuclei (Feng and Rogowski, '80; Gray et al., '82; Smith et al., '83; Conlee and Parks, '83). Anatomical investigations in mouse, rat, and chicken have shown that manipulations which are intended to produce functional auditory deprivation result in changes in first- through fourth-order peripheral and brain stem auditory neurons. Investigators have addressed the following questions in an effort to describe these anatomical alterations: (1) do "critical" periods exist that define the developmental time during which experimental manipulations are most likely to affect auditory neurons? (2) If a deprivation effect does exist, is it dependent upon the interactions between active and quiescent terminals at a postsynaptic target? Such a competitive mechanism has been described for the developing visual system (Guillery and Stelzner, '70; Guillery, '72; Sherman et al., '74; Sherman and Spear, '82).

One issue which has not been adequately addressed is that of the type and degree of hearing loss effected by the various experimental procedures. For example, are degenerative changes associated with manipulations shown experimentally to produce a purely conductive hearing loss? Is it possible that the degree of morphologic change seen is dependent upon the amount of conductive hearing loss present during the period of deprivation? A proper assessment requires that the magnitude of both conductive and sensorineural hearing loss be experimentally determined.

The chicken is an excellent subject for investigation of the effects of peripheral manipulations on the developing auditory system. The anatomy of brain stem nuclei has been well characterized (Boord and Rasmussen, '63; Boord, '69; Rubel and Parks, '75; Parks and Rubel, '75; Parks and Rubel, '78; Smith and Rubel, '79; Parks, '81; Jhaveri and Morest, '82a; Young and Rubel, '83). In the avian, the second-order neurons of nucleus magnocellularis (NM) receive monaural input from the spiral ganglion cells via ipsilateral eighth nerve projections. Nucleus magnocellularis in the adult bird receives no projections from the contralateral peripheral end organ. This nucleus is considered homologous to the spherical cells of the mammalian anteroventral cochlear nucleus (AVCN, Boord, '69). Nucleus magnocellularis projects to ipsilateral and contralateral third-order neurons in nucleus laminaris (NL). Cells in NM and NL receive tonotopically organized input; for NL, to spatially segregated dendritic fields from NM on both sides of the brain stem. In addition, anatomical and functional development of the chick auditory system has been studied extensively (Saunders et al., '73; Rubel and Rosenthal, '75; Rubel et al., '76; Jackson and Rubel, '78; Kerr et al., '79; Gray and Rubel, '81; Rebillard and Rubel, '81; Smith, '81; Jackson et al., '82; Jackson and Parks, '82; Jhaveri and Morest, '82b; Rubel and Ryals, '83; Lippe and Rubel, '83, '85; Parks and Jackson, '84).

In the present study, young hatchling or prehatch chickens were used to determine the effects of three experimental manipulations—simple columella (ossicle) removal,

columella removal followed by oval window puncture, and earplug placement—on several anatomical and physiological parameters. Cross-sectional areas of second-order neurons in nucleus magnocellularis (NM) were measured. Auditory thresholds were obtained to an air-conducted stimulus for all three experimental conditions, and to a bone-conducted stimulus for the simple columella removal and oval window puncture groups. Cochlear integrity was assessed by hair cell counts in the basilar papilla, which is the avian equivalent of the mammalian cochlea. We found that a manipulation that produced a purely conductive hearing loss of up to 55 dB had no effect on the cross-sectional area of second-order neurons in NM. In contrast, a manipulation that produced a mixed conductive-sensorineural hearing loss led to a significant decrease in the area of these neurons. Cochlear integrity, as measured by hair cell counts in the basilar papilla, was not affected by any of these experimental manipulations.

## MATERIALS AND METHODS

Broiler stock chickens served as the subjects of these experiments. Fertilized eggs were obtained from a local supplier (Heatwole Hatcheries; Harrisonberg, MD), hatched in the laboratory, and housed communally with free access to food and water.

### Surgical manipulations (Table 1)

**Columella removal.** Four-day-old chickens were used. Animals were anesthetized with ketamine hydrochloride (80 mg/kg i.m.) and Chloropent (1.5 mL/kg i.p., Fort Dodge Laboratories, Inc.). Atropine (0.01 mg/kg i.m.) was given to inhibit tracheal secretions. The chicken has one middle ear ossicle, the columella. Using jeweler's forceps, a small hole was created in the right tympanic membrane just anterior and superior to the attachment of the columella. The shaft of the columella was visualized through the hole, grasped with the forceps, and removed; the ossicle was inspected to insure that it was removed intact. Tympanic membranes were noted to be completely healed within 4 days. In an additional group of four animals, the right columella was removed at 1 day after hatching.

Bilateral columella removal was performed in one group of nine animals. These animals were raised until 8 or 30 days of age, and the tissue was processed with a group of control animals hatched on the same day.

**Oval window puncture.** The above procedure was followed. After columella removal, the closed forcep tip (#5 Dumond) was inserted through the oval window, to a depth of 1–2 mm. Care was taken not to come into contact with bony structures. Although we can not be precisely certain what this manipulation does in the intact animal, observ-

TABLE 1. Number of Subjects for Each Experimental Group

Experimental groups	Survival (days postsurgery)						
	2	4	8	15	30	45	60
Columella removal							
Unilateral, 4 days posthatch	6	4	4	5	6	6	5
Unilateral, 1 day posthatch						4	
Controls for unilateral removal	2	3	2	3	6	2	3
Bilateral, 4 days posthatch			4		5		
Controls for bilateral removal			4		3		
Oval window puncture							
Unilateral, 4 days posthatch							7
Earplug							
Unilateral, embryonic day 19							5

ing the effect on fresh tissue suggests that it ruptures the thin oval window membrane, allowing leakage of perilymph from the labyrinth. As we have no indication of direct damage to hair cells (see below), this puncture probably results in a perilymph fistula rather than in free mixing of endolymph and perilymph (Duvall and Rhodes, '67; Kimura et al., '77; Kimura, '84).

**Earplug.** This procedure is described in detail in Kerr et al. ('79). The chicken normally hatches on embryonic day 21. At embryonic day 19, prior to emergence of the chicken's head into the egg air space ("tenting" stage), the shell over the air space was removed, the chorioallantoic membrane incised, and the chicken's head positioned above the membrane. After allowing fluid to drain from the right ear canal, the canal was gently filled with True-mold impression compound (Scientific Plastics, New York, New York). The right external auditory meatus was then sutured closed with 7.0 Ethilon suture (Ethicon). Chickens were allowed to hatch together in a separate hatching incubator. Earplugs and sutures were replaced on a weekly basis, using progressively thicker nylon or silk suture. When removed, the earplugs were found to always completely occlude the canal, but never to extend to the tympanic membrane (TM). Impressions of the TM and/or columella were not found on the earplugs. The TM was carefully examined each time the earplugs were changed and at the end of the 60-day survival period; at no time was an infection apparent. Epithelial debris was found in the external canal beginning in the fifth week.

#### Tissue preparation

After survival times as shown in Table 1, animals were deeply anesthetized with ketamine hydrochloride and Chloroform, and transcardially perfused with either Heidenhain's fixative without mercuric chloride (Lillie and Fullmer, '76) or with a mixture of 2% glutaraldehyde and 2% formaldehyde in  $PO_4$  buffer. Brains fixed with Heidenhain's solution were postfixed for 12 hours and transferred to 10% buffered formalin. The mixed aldehyde-fixed brains were placed directly in 10% buffered formalin. Brains were not removed from the skull for at least 2 days after perfusion, unless cochleas were also perfused (see below), in which case they were removed after cochlea fixation. After at least 1 week in buffered formalin, brains were dehydrated, embedded in paraffin, and coronal sections were cut through the entire brain stem at 10  $\mu$ m. A one-in-four series was mounted on slides and stained with thionin. In addition, a one-in-four series was prepared for two brains in the oval window puncture group and stained by a modification of Bodian's protargol method (Bodian, '36).

#### Analysis of NM neuron size

Eighth nerve fibers project from the ipsilateral basilar papilla (cochlea) providing monaural input to second-order neurons in NM. In the posthatch chicken no contralateral input from primary, secondary, or tertiary auditory fibers has been described. For both experimental and control animals cross-sectional areas of cells in NM were measured on both sides of the brain in three consecutive sections centered at 30% and 70% of the anterior-to-posterior extent of the nucleus. The tonotopic organization of NM is known; these positions correspond roughly to the 2,000- and 500-Hz regions, respectively (Rubel and Parks, '75). Cell measurements were made with a Zeiss Videoplan image analysis system using a 100 $\times$  planapochromatic oil-immersion ob-

jective (N.A. 1.3). All measurements were made by one experimenter. Cells with a visible nucleus and nucleolus were outlined in their largest diameter. Twenty to 90 (average 70) cells were measured in the three sections at each location on each side of the brain. Data from 23,174 cells are included in this study. In addition, ten brains were remeasured by the same experimenter to assess reliability. Six brains (three from the oval window puncture group and three from the earplug group) were coded and then remeasured by a student helper who was naive as to the manipulation, side, or purpose of the experiment. These measurements were compared to those originally made by the experimenter. Repeats of cell measurements made by the same experimenter and by the naive observer varied less than 5% from the original measurements.

Data from columella removal animals and their controls were analyzed using a multiple-dimension analysis of variance (MANOVA). 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) by survival times and treatment conditions (between subject variables). An analysis of variance (ANOVA) was used to analyze data from the earplug and oval window puncture treatment groups. Area, difference scores, and percent difference scores were examined as a function of level of the nucleus and side of brain for each group. All analyses were carried out on a Control Data Corporation Cyber 730 computer using SPSS routines (Nie et al., '75).

#### Hair cell counts

The number of hair cells along the basilar membrane was analyzed in nine animals in three treatment groups (four oval window puncture, three columella removal, and two earplug). All animals survived 60 days post-treatment. Following 10 minutes of transcardial perfusion with a mixture of 2% glutaraldehyde 2% formaldehyde fixative, animals were decapitated and direct intralabyrinthine perfusions were performed bilaterally with 5 cc of the same aldehyde fixative. Brains were removed and heads stored in cold fixative at least overnight. Basilar papillae were removed from the skull within 3 days and processed by the procedure described by Rubel and Ryals ('82). After a phosphate buffer wash, basilar papillae were post-fixed in 2% buffered osmium tetroxide for 2 hours, dehydrated, and embedded in Epon. The embedded papillae were then sectioned transverse to the long axis, from base to apex. Three or four 3- $\mu$ m sections were collected at each 100- $\mu$ m interval. Sections were mounted on slides, stained with toluidine blue and coverslipped.

Hair cell counts were determined by one experimenter on coded, randomly ordered slides from each set of papillae. Sections were examined under a 40 $\times$  planapochromatic oil-immersion objective (N.A. 1.0). Criteria for counting a hair cell included the presence of a cell body, at least a portion of the nucleus, a cuticular plate, and intact cilia. The counts of the nucleus, a cuticular plate, and intact cilia. The counts for the three sections at each 100- $\mu$ m interval were averaged. Counts of long-and short-type hair cells were combined. The averages were then plotted at 5% intervals through the length of each papilla, and the scores averaged across papillae. This provided an estimate of the number of hair cells across the basilar membrane throughout the length of the basilar papilla in experimental and control ears.



### Physiological measurements

Evoked potential auditory thresholds were obtained for animals following columella removal, oval window puncture, and earplug insertion. Chickens used for these experiments ranged from 20 to 25 days posthatch and had undergone unilateral cochlea removal of the nonexperimental ear either acutely or 2–5 days prior to the experiment. Evoked potential thresholds were obtained to either a free field sound- or a bone-conducted stimulus. Thresholds to air-conducted stimuli allow determination of total hearing impairment, originating in the middle ear as well as the inner ear. Bone conduction thresholds permit measurement of the sensorineural (inner ear) component of the hearing loss independent of the conductive (middle ear) component. Measurement of these two types of thresholds is routinely done in clinical settings to assess the conductive and sensorineural components of a hearing loss. Animals were initially anesthetized with ketamine hydrochloride (80 mg/kg i.m.) and Chloropent (1.5 mL/kg i.p.) or Nembutal (0.5 mL/kg i.p.) and given atropine (.01 mg/kg i.m.) to reduce tracheal secretions. Chloropent or Nembutal was given as needed to maintain anesthesia throughout the experiment.

Animals were prepared for recording in the following manner. Skin covering the skull was cleared and two skull screws were fixed into the skull. The animal was secured with wire and dental acrylic in a specially designed holder. When a bone vibrator was used it was securely fastened to the animal's skull on a bed of dental acrylic.

Stimulus frequency ranged from 500 to 3,000 Hz. Pulsed pure tones were presented with a 4-msec rise/fall time, 20-msec duration, and an interstimulus interval of 200–300 msec. The stimulus was delivered by a speaker (Realistic Minimus-7) positioned at the animal's ear or by a bone vibrator (Radioear B-71). Free field calibration was carried out at the entrance to the ear canal using a Knowles BL1830 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 responses were recorded using Grass pin electrodes implanted through the intact skull into the right and left cerebellum at a level just above the cochlear nuclei (active and reference) and in the thigh muscle (ground). Responses from the brain stem were amplified, filtered 20–2,000 Hz, displayed on an oscilloscope, and digitized by an A to D converter at a rate of 10 KHz. Responses to stimuli were determined from averages of 200 twenty-msec sweeps. Criterion for threshold was the lowest intensity at which the response was at least twice the amplitude of prestimulus baseline variations. Thresholds were determined to within 5 dB.

**Air conduction thresholds.** Air conduction thresholds were obtained from four animals following columella removal and three animals following oval window puncture. In two additional animals, thresholds were measured following placement of two different sizes of earplugs. Initially, an earplug was placed which was occlusive but did not completely fill the ear canal. The first earplug was removed after testing, and a second earplug was inserted which completely filled the canal, and on which an imprint of the tympanic membrane was noted after removal from the canal. In all cases hearing loss was determined by comparison with baseline thresholds in an intact ear in the same animal; all threshold measurements were made immediately following the manipulation.

**Bone conduction thresholds.** Bone conduction thresholds were obtained in two animals following sequential

columella removal and oval window puncture (acute condition). In these animals the contralateral cochlea had been removed. Thresholds were obtained in four columella removal and four oval window puncture animals 7 days following these manipulations (chronic condition). In each case, baseline threshold measurements were made with one cochlea intact. The intact cochlea was then removed to selectively test the manipulated ear.

## RESULTS

### Cell area measurements, NM

**Columella removal.** Mean cross-sectional cell area for anterior (30% of the anterior-to-posterior extent) and posterior (70% of the anterior-to-posterior extent) sections of NM following unilateral columella removal at 4 days posthatch is presented for each survival group in Figure 1. The nucleus on the side contralateral to the columella removal served as a within-subjects control, as it receives input only from the unoperated ear. However, because it has been suggested that a compensatory increase in the size of contralateral neurons may occur with unilateral deprivation (Coleman and O'Connor, '79), averaged data from the two sides of the brain in control animals were included for comparison with data from each side of the brain in the experimental subjects. Examination of the data shown in Figure 1 revealed that there was no effect of columella removal on mean cell area on either side of the brain in experimental animals. Even at the 30-day survival period, where the largest differences exist between groups, there was considerable overlap between the distributions of cell areas, as indicated by the standard error bars.

In four animals, the right ear columella was removed at 1 day posthatch. All of these animals were sacrificed at day 45. Data from this group are similar to those from the 45-day survival group in which the columella was removed at 4 days posthatch. Percent decrease in cell area (area left – area right/area left × 100) was 1.09% for the anterior portion of the nucleus and 3.02% for the posterior portion of NM. These results revealed that there was no effect when the columella removal was performed at this younger age.

Mean NM cell areas following bilateral columella removal on day 4 posthatch are given in Table 2 along with data from their control animals. No significant differences existed between the experimental and control populations (see below).

In summary, columella removal alone had no discernable effect on cell area in NM of the chicken. A multiple-dimension analysis of variance was used to test for statistically significant effects. This analysis confirmed that there was no reliable effect of side of brain at either the anterior or posterior positions. There were no reliable interactions between side of the brain and survival time or side of brain and level. In addition, there was no main effect of treatment, indicating that the contralateral NM did not change as a result of columella removal. There was a significant effect of level of the nucleus ( $P < .001$ ), which reflects the fact that cells in the anterior portion of the nucleus are typically larger than those in the posterior portion.

**Oval window puncture.** Effects of oval window puncture following columella removal were evident on visual inspection of this tissue. Tissue shown in Figure 2 is from the anterior portion of NM on both sides of the brain from one animal. In the ipsilateral nucleus (Fig. 2B) cells appeared more closely packed than on the contralateral side (Fig. 2A). Certain changes were more evident at a higher mag-



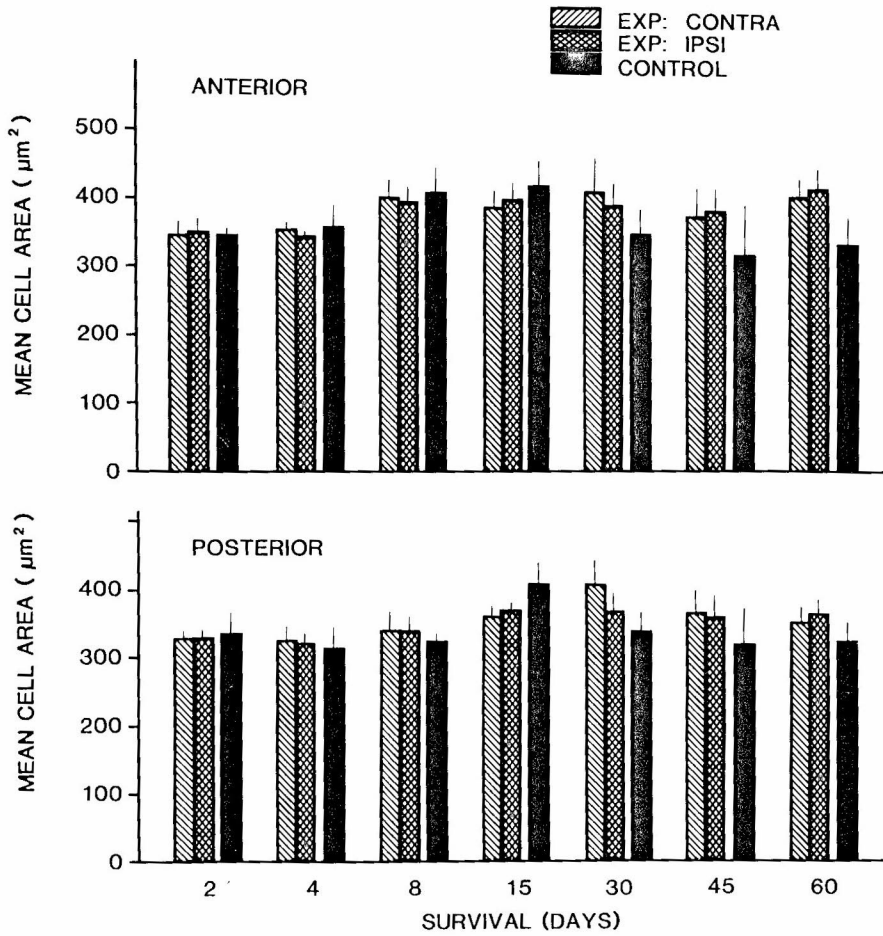


Fig. 1. Mean cross-sectional cell areas in n. magnocellularis (NM) as a function of survival time following unilateral columella removal (CR). Data shown are for NM cells ipsilateral (EXP: IPSI) and contralateral (EXP: CONTRA) to the operated ear in experimental animals, and for the two sides averaged in control animals. Bars indicate standard error of the mean. Cell area measurements are from the anterior and posterior portions of NM.

TABLE 2. Mean Cell Area  $\pm$  Standard Deviation in Anterior and Posterior NM Following Bilateral Columella Removal

	Survival (days)			
	Anterior		Posterior	
	8	30	8	30
<b>Unoperated control</b>				
Right	339.3 $\pm$ 56.65	312.8 $\pm$ 24.88	310.5 $\pm$ 48.09	286.2 $\pm$ 24.18
Left	330.2 $\pm$ 40.86	316.8 $\pm$ 17.91	312.0 $\pm$ 38.17	286.7 $\pm$ 48.63
<b>Operated</b>				
Right	323.9 $\pm$ 33.33	390.7 $\pm$ 81.64	297.6 $\pm$ 26.11	353.3 $\pm$ 82.06
Left	292.7 $\pm$ 10.88	369.9 $\pm$ 91.82	309.1 $\pm$ 26.44	352.2 $\pm$ 100.8

nification. As illustrated, cells from the ipsilateral nucleus (Fig. 2D) were also smaller in size than cells on the control side (Fig. 2C), and there appeared to be a condensation of Nissl substance at the perimeter of the cells. These changes were always apparent in tissue from animals in the oval

window puncture group, and were never seen after a simple columella removal. Examination of Bodian-stained tissue from two animals suggested a decrease in the quantity of nerve fibers in portions of the ipsilateral nucleus. This finding may account for the greater density of the ipsilateral NM cells, although the report by Conlee and Parks ('83) suggests that dendritic loss may also be involved.

Mean cell areas in NM for animals in the oval window puncture group are shown in Table 3. In Figure 3, the percent decrease in cell area is compared to that seen following a simple columella removal; the marked effect of oval window puncture is evident. 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 ANOVA (cell area by level of nucleus and side of brain) confirmed the significant effect of the manipulation on cell area in the ipsilateral nucleus for both the anterior and posterior positions ( $P$ 's  $<$  .005).

**Earplug.** Mean cell areas in NM following unilateral earplug placement and suturing of the ear canal on embry-

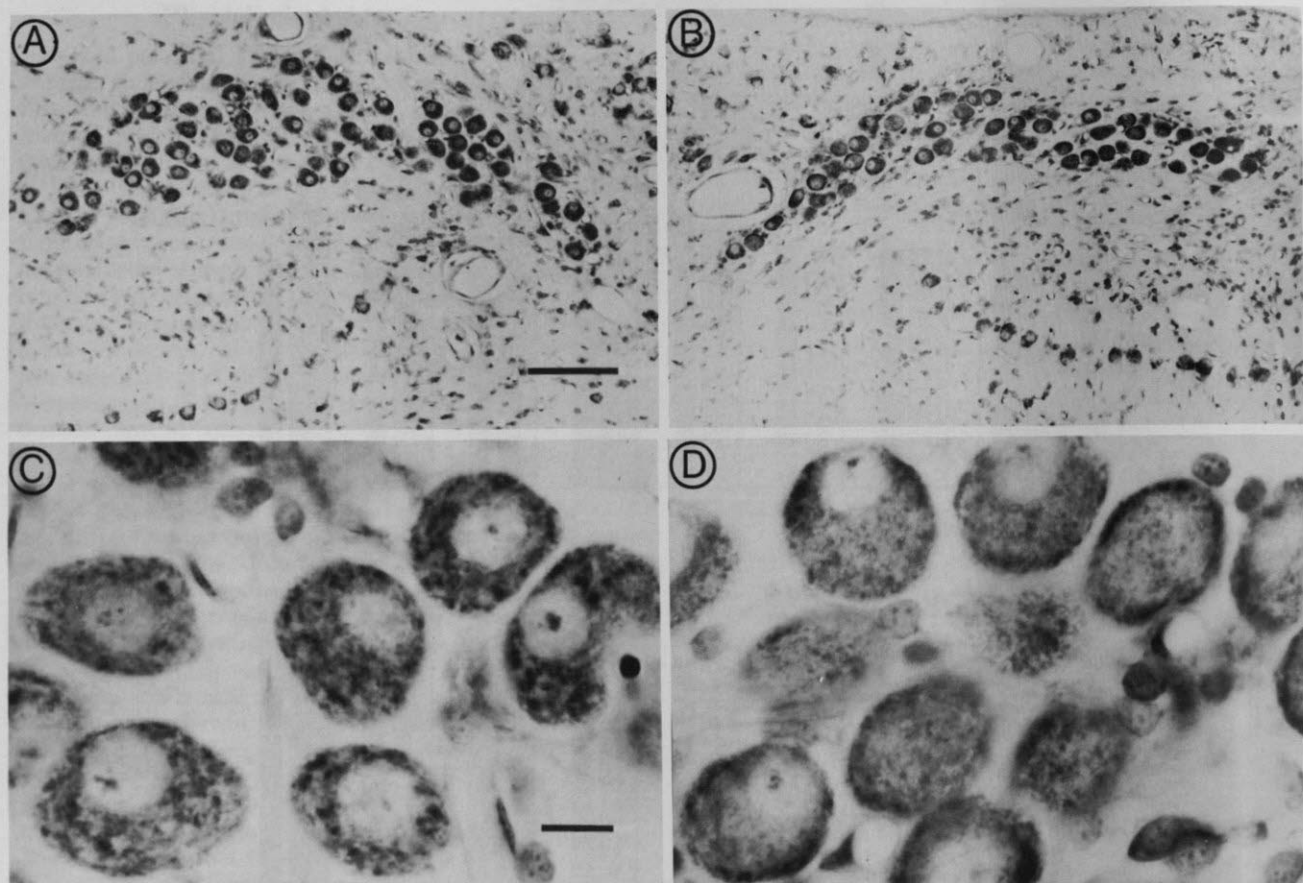


Fig. 2. High- and low-power photomicrographs of NM following right CR and oval window puncture (OWP). Tissue shown is from NM contralateral (A, C) and ipsilateral (B, D) to the operated ear. Thionin stain. Scale bars: low power = 100  $\mu$ m, high power = 10  $\mu$ m.

TABLE 3. Mean Cell Area  $\pm$  Standard Deviation in NM Following Oval Window Puncture

	Anterior	Posterior
Ipsilateral	232.6 $\pm$ 24.33*	233.7 $\pm$ 34.58*
Contralateral	289.4 $\pm$ 37.16	283.5 $\pm$ 38.77

\*Difference between cell area measurements ipsilateral and contralateral to oval window puncture is significant ( $P < .005$ ).

TABLE 4. Mean Cell Area  $\pm$  Standard Deviation in NM Following Earplug Placement

	Anterior	Posterior
Ipsilateral	357.4 $\pm$ 42.56	357.0 $\pm$ 30.87
Contralateral	363.7 $\pm$ 19.19	350.0 $\pm$ 20.04

onic day 19 are shown in Table 4. As is evident from these data and the percent changes (Fig. 3), there was no effect of a chronic earplug on NM cell size. An ANOVA (area  $\times$  level of nucleus  $\times$  side of brain) confirmed that there was no reliable effect of chronic earplug on cell area measurements.

#### Hair cell counts

Figure 4 illustrates hair cell counts as a function of position along the basilar papilla for four ears following oval window puncture (60-day survival) and for nine control ears. These functions have a very similar configuration. Hair cell counts from ears following simple columella removal and earplug placement are not shown, but also are not significantly different from the control data.

#### Physiological measurements

**Air conduction thresholds.** Hearing thresholds were measured for all experimental manipulations, and results are reported in Table 5 as amount of hearing loss in deci-

bel. Columella removal produced a relatively flat 50–55-dB hearing loss across the frequencies tested. While the hearing loss that followed an oval window puncture was greater than that which followed columella removal, the exact magnitude of the loss could not be quantified at all frequencies due to output limitations of the sound delivery system. Thresholds could be measured both pre- and post-puncture only to the 1,000-Hz stimulus. At this frequency, the mean hearing loss measured 58 dB. An occlusive earplug produced a 25–35-dB hearing impairment, unless it was large enough to acquire an imprint of the tympanic membrane. In the latter case, the magnitude of the hearing loss was increased to approximately 35 dB at most frequencies.

**Bone conduction thresholds.** In order to assess the sensorineural component of the measured hearing loss, bone conduction thresholds were obtained from normal animals, from animals after columella removal, and from animals that had sustained an oval window puncture. Hearing loss

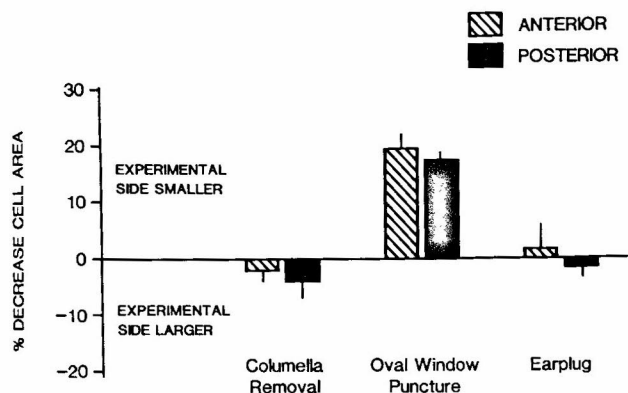


Fig. 3. Percent decrease in cell area as a function of treatment in 60-day-survival animals. Percent decrease in cell area is the mean of  $[(\text{area contralateral} - \text{area ipsilateral}) / \text{area contralateral}] \times 100$ , so that a positive number indicates that ipsilateral cells are smaller than those contralateral to the manipulated ear. Scores are given for anterior and posterior portions of NM. Bars indicate standard error of the mean.

was measured either immediately following these manipulations (acute condition), or 1 week following surgery (chronic condition). Results are presented as amount of hearing loss in decibels (Fig. 5). A sensorineural hearing loss was present for both experimental groups immediately following the surgical manipulation. For columella removal animals this hearing loss did not exceed 10 dB and was temporary. Impairment was not evident when the animals were tested 7 days following the manipulation, as indicated by the filled circles in Figure 5. We believe that the transient sensorineural hearing loss which followed columella removal was due to the surgical trauma which accompanies manipulation and extraction of the columella. It is interesting to note that, of the four animals tested 1 week following columella removal, there was no sensorineural hearing loss to any of the stimuli in the two animals in which there appeared to be no trauma at the time of surgery. On the other hand, in the other two animals we noted a small amount of bleeding at the oval window following the procedure, and in these subjects there was a slight sensorineural hearing loss (5 dB). The hearing loss which immediately followed an oval window puncture was greater than that which followed the simple columella removal, especially at 2,000 Hz, where an average 22-dB impairment occurred. Moreover, in the chronic situation, impairment increased substantially during the 7 days following oval window puncture (Fig. 5, filled triangles). Interestingly, the shape of the hearing loss curve across frequency was maintained, with the greatest loss being 37 dB at 2,000 Hz. It is in this region along the cochlea that the columella inserts and the oval window puncture was made.

These results support the conclusion that the chronic effect of a columella removal is a purely conductive hearing loss, whereas a columella removal followed by an oval window puncture results in a mixed conductive-sensorineural hearing loss.

#### Summary of results

A columella removal, which was shown to produce a consistent 50–55-dB conductive hearing loss, had no effect on

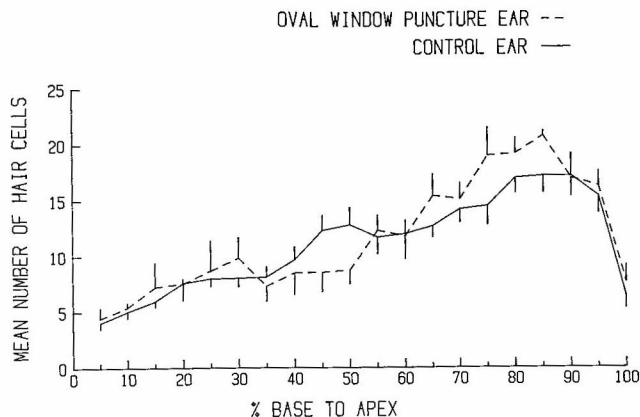


Fig. 4. Averaged hair cell counts as a function of position along the basilar papilla for four ears 60 days post CR/OWP and for nine control ears at the same age. Bars indicate standard error of the mean.

the size of second-order neurons in NM in the chicken. A columella removal with an accompanying oval window puncture produced a severe mixed conductive-sensorineural hearing loss, and was associated with a consistent 18–20% decrease in cell size in the ipsilateral NM. An earplug which was occlusive but which did not contact the tympanic membrane was found to produce a 25–35-dB hearing loss, and was not associated with a decrease in NM cell size. None of these manipulations was found to alter the number of hair cells in the chicken basilar papilla.

#### DISCUSSION

In a previous study (Smith, et al., '83) we examined the influence of monaural acoustic attenuation (earplugs) on the size of dendrites in nucleus laminaris (NL) of the chick. It was found that dendrites that received input from the deprived ear were slightly shorter (6–7%) than those that were innervated by the control ear in the high-frequency region of NL. However, quite unexpectedly, dendrites receiving input from the low-frequency region of the deprived ear were considerably longer than dendrites receiving input from the control ear. One hypothesis advanced to explain this surprising finding was that the earplug may have produced a "mass loading" of the external auditory system, thus enhancing low-frequency bone-conducted sounds and internal sounds.

Removal of the columella was devised as a method of external sound attenuation that would not be associated with peripheral mass loading. We were concerned, however, that columella removal could be associated with basilar papilla damage. For this reason hair cell counts and bone and air conduction thresholds were obtained. The results of this study were that columella removal produced a moderate, purely conductive, hearing loss in the chicken. However, in contrast to other experiments (Webster and Webster, '77, '79; Coleman and O'Connor, '79; Conley and Parks, '81; Coleman et al., '82; Webster '83a,b; Blatchley et al. '83) we found no effect on cell size of the second-order auditory neurons. We will discuss this finding in relation to the



since sensitivity to peripheral destruction continues at least through 6 weeks posthatch (Born and Rubel, '85; Durham and Rubel, '85; Steward and Rubel, '85). There are, of course, many other variables related to species specializations which could be relevant. For example, unknown differences between precocial and altricial species may render the latter more susceptible to environmental modulation of sensory system development. This and other possibilities await examination in other species.

Finally the issue of competitive interactions should be addressed (Guillery and Stelzner, '70; Guillery, '72; Sherman et al., '74). Several investigators have drawn the analogy between changes in the cochlear nucleus following monaural ear occlusion and changes in the lateral geniculate nucleus (LGN) following monocular eye occlusion (Coleman and O'Connor, '79; Feng and Rogowski, '80). Coleman and O'Connor ('79) report that binaural auditory deprivation, unlike monaural deprivation, has no effect on cell area in the rat AVCN. In contrast, Webster and Webster ('79) found similar reduction in cochlear nucleus cell areas following either monaural or binaural deprivation. In the visual system there is considerable evidence for two classes of effects in the LGN. The first is a "pure deprivation effect," most clearly observed after long survival times in all LGN cells. The second is an effect which appears to result from a competitive disadvantage of the deprived cell when it comes to making appropriate synaptic connections with its cortical target neurons; it is most evident in binaural segments of the LGN laminae (Guillery, '72; Guillery and Stelzner, '70). The situation is quite different in NM or the mammalian AVCN. While these neurons project bilaterally, they terminate primarily on separate dendritic surfaces in the ipsilateral and contralateral NL or medial superior olive, respectively. At least in the chick, this spatial segregation is evident and complete from the time axons arrive at the target neurons (Young and Rubel, '84). Thus competition for postsynaptic membrane space does not seem to exist. Indeed, one would not expect to see the effects of competition in NM.

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