

Hair Cell Regeneration and Recovery of the Vestibuloocular Reflex in the Avian Vestibular System

JOHN P. CAREY, ALBERT F. FUCHS, AND EDWIN W. RUBEL

Department of Otolaryngology/Head and Neck Surgery, Virginia Merrill Bloedel Hearing Research Center, Regional Primate Research Center, and Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195

SUMMARY AND CONCLUSIONS

1. Although auditory and vestibular hair cells are known to regenerate after aminoglycoside intoxication in birds, there is only sparse evidence that the regenerated hair cells are functional. To address this issue, we examined the relation of hair cell regeneration to recovery of the vestibuloocular reflex (VOR), whose afferent signal originates at hair cells in the vestibular epithelium. Hair cell damage was produced by treating white Leghorn chicks (*Gallus domesticus*, 4–8 days posthatch) with streptomycin sulfate in normal saline ($1,200 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ im) for 5 days.

2. In the 1st wk after treatment, the VOR gain was essentially 0, and hair cell density as assessed by light microscopy was ~40% of normal. Between the 1st and 3rd wk after treatment, the VOR was present. Although VOR gain varied considerably from one chick to another, it increased, on average, between the 1st and 3rd wk, as did the average hair cell density. At the end of 8–9 wk, the gain and phase of the VOR had returned to normal values, as had the average density of hair cells.

3. Therefore, despite the catastrophic initial effect of hair cell loss on the VOR, recovered hair cells appeared to restore the VOR completely. Average hair cell density increased with average VOR gain. VOR gain correlated better with recovery of type I hair cells than with recovery of type II hair cells.

4. In contrast to hair cell density, the appearance of the vestibular epithelia as assessed by hair cell stereocilia in scanning electron micrographs was a poor indicator of VOR gain. In both treated and control birds, epithelia with the same appearance could have quite different VOR gains, suggesting a variation in the functional viability of the hair cells.

5. This observation suggests that several factors, such as the repair of stereocilia, the efficacy of hair cell synapses on afferent fibers, and the extent of compensation by central vestibular pathways, may affect the recovery of VOR gain. However, our data suggest that hair cell regeneration plays an important role in this recovery.

INTRODUCTION

A variety of studies has shown that hair cells in the avian ear can regenerate. For example, regeneration occurs after hair cell loss caused by noise (Corwin and Cotanche 1988; Cotanche 1987; Ryals and Rubel 1988) or administration of ototoxic aminoglycoside antibiotics (Cruz et al. 1987; Lippe et al. 1991; Weisleder and Rubel 1993). Not only is there regeneration after a toxic or environmental insult, but the vestibular epithelium of birds shows a continuous slow rate of hair cell turnover naturally (Jorgensen and Mathiesen 1988; Roberson et al. 1992). Moreover, afferent connections are established on regenerated hair cells in both the auditory

(Duckert and Rubel 1993; Ryals and Westbrook 1994; Ryals et al. 1992) and vestibular (Weisleder and Rubel 1993) epithelia. Thus it would seem that the anatomic elements necessary for sensory transduction in the avian ear are reestablished after hair cells regenerate. But do regenerated hair cells and the sensory systems they serve actually function?

Several studies have addressed this question as it relates to the auditory system. Norton and Rubel (1990) studied the effects of gentamycin on evoked otoacoustic emissions in chickens. These emissions, which are sounds that actually emanate from the ear in response to acoustic inputs, have passive and active components. The latter requires metabolically active hair cells. Gentamycin initially eliminates the active component, but the component recovers after hair cell regeneration and reorientation of the stereocilia. Froymovich et al. (1995) recently studied the effects of acoustic overstimulation on otoacoustic emissions. Less complete recovery of emissions was found, perhaps because of irreparable tectorial membrane damage. Thresholds of cochlear afferents to pure tones rise as a result of kanamycin-induced hair cell loss. As hair cells regenerate, thresholds recover to normal levels, even at frequencies corresponding to regions of the papilla where the initial hair cell loss was virtually complete (Salvi et al. 1994). In the brain stem, auditory evoked potentials recover from damage caused by noise (McFadden and Saunders 1989) and gentamycin (Girod et al. 1991; Tucci and Rubel 1990). Auditory perception returns after loss due to ototoxic aminoglycoside treatment. For example, adult budgerigars showed recovery of behavioral detection thresholds for pure tones following kanamycin-induced loss (Hashino and Sokabe 1989). European starlings showed similar recoveries of behavioral thresholds, and the extent of recovery paralleled the extent of hair cell regeneration (Marean et al. 1993). In noise-damaged quails, auditory behavioral thresholds returned to normal after only 8–10 days, even before complete structural regeneration had occurred (Niemiec et al. 1994).

To our knowledge, only one study has documented the recovery of avian vestibular function after ototoxic doses of an aminoglycoside. Jones and Nelson (1992) reported that compound action potentials from the vestibular nerves of chickens treated with streptomycin exhibited an immediate sixfold decrease in sensitivity to linear horizontal accelerations. Compound action potential thresholds returned to normal within 2 wk, but the amplitudes and latencies of the responses required 8 wk to return to normal. Jones and Nel-

son did not correlate these electrophysiological measurements of the vestibular deficit with hair cell loss, but by combining their results with findings by Weisleder and Rubel (1993), one might infer that there is no direct relation between hair cell density and vestibular function. Weisleder and Rubel treated chicks with the same streptomycin dose for one less day and found that in the utricle, the end organ stimulated by horizontal linear acceleration, 73% of the hair cells remained immediately after treatment, at which time Jones and Nelson had found no action potentials in response to linear acceleration. Moreover, even though Weisleder and Rubel found that the number of hair cells in the utricle had not increased significantly after 21 days, Jones and Nelson found vestibular nerve potentials with normal thresholds by that time. Thus there seem to be significant discrepancies between the level of afferent electrical activity recorded from the vestibular nerve and the anatomic status of hair cells in the sensory epithelium. Furthermore, whether these compound action potentials in the vestibular nerve convey behaviorally useful information to the brain stem vestibular nuclei remains an open question.

To determine whether there is a direct relation between hair cell regeneration and vestibular function, we tested the effect of hair cell regeneration in the avian horizontal crista (the canal sensory epithelium) on the horizontal vestibuloocular reflex (VOR). The VOR produces eye movements that compensate for movements of the head so that the visual scene does not slip across the retina. Consequently, the efficacy of the vestibular hair cells should be reflected in the size of the compensatory eye movement velocity (\dot{E}) in response to head movement velocity (\dot{H}), i.e., the VOR gain (\dot{E}/\dot{H}). By inducing a profound loss of hair cells and then testing the VOR throughout the course of hair cell regeneration, we were able to determine whether and when the regenerating crista generates adequate input signals to drive the VOR. We did indeed find that the loss and recovery of hair cells in the crista were reflected by changes in the gain of the VOR. We also obtained anatomic data that correlate hair cell loss and regeneration with the status of the VOR.

METHODS

Experimental subjects

Seventy white Leghorn chicks (*Gallus domesticus*) were given streptomycin sulfate (Sigma) in normal saline (1,200 mg · kg⁻¹ · day⁻¹ im) for 5 days beginning 4–8 days posthatching. Streptomycin, an aminoglycoside antibiotic, causes a loss of up to 83% of hair cells in the crista of the avian semicircular canal (Weisleder and Rubel 1993). All of the chicks became unconscious for several hours after receiving the drug, and 40% died despite careful husbandry. The 42 surviving chicks exhibited an intermittent tremor of the head for several days, but no other obvious motor abnormalities. Eight age-matched controls were given injections of similar volumes of normal saline. There were no detectable differences in the behavioral data obtained from saline-injected chicks and 28 noninjected control chicks. All chicks were housed in brooders that allowed for unrestrained walking with ambient light during the daylight hours. Injected chicks were allowed to recover for 1–4 days ($n = 8$), 1 wk (6–8 days, $n = 6$), 2 wk (13–15 days, $n = 8$), 3 wk (18–24 days, $n = 10$), 4 wk (27–30 days, $n = 6$), or 8–9 wk (54–64 days, $n = 4$) before rotational testing. Hereafter the experimental chicks and their age-matched controls will be referred to by their recovery periods, e.g., “1- to

4-day chicks,” “1-wk controls,” and so on. For example, a chick injected with streptomycin on days 5–10 posthatch and tested 7 days later would be considered a “1-wk treated” chick. Its chronological age would be 17 days and its data would be compared with data from a 17-day-old control chick, labeled a 1-wk control. In general, the chronological age equals the recovery period plus 10 days.

Surgical preparation

Eye movements were measured by means of an electromagnetic technique in which a coil of wire affixed to the eye intercepts stationary but alternating magnetic fields to generate a voltage proportional to eye position (Robinson 1963). The coil was attached to the eye via a post affixed to the surgically exposed superior pole of the globe in a manner similar to that described by Wallman et al. (1982). The chicks were sedated with ketamine (0.8 mg im per 10 g body wt), and subcutaneous 1% lidocaine with 1:100,000 epinephrine was injected at incision sites. An incision was made over the right orbit, and a window was created in the fascia and orbital bone. The window was made large enough that the post would not contact its edges throughout the range of eye movements. The sclera was kept moist with a pledget soaked in 0.5% lidocaine until just before VOR testing. Any adhesions of the globe to the orbital wound were lysed, and one end of the post (capillary tubing, 5 mm long × 1 mm diam) was affixed to the superior pole of the globe with n-butylcyanoacrylate adhesive (Vet Bond, 3M). A prefabricated search coil (9 mm diam, 3 turns of Teflon-coated AWG 38 wire with 15 strands per turn) was fastened to the other end of the post with hard wax in a “lollipop” configuration. A nylon post was attached to the skull so that the head could be fastened to the vestibular turntable (see below). A midline dorsal incision was made near the vertex, pericranium was cleared, and the post was glued to the skull with n-butylcyanoacrylate adhesive and dental cement. The chicks were allowed to recover until they could walk without difficulty, generally 2–4 h after anesthesia. Rotational testing generally required 15–60 min.

Rotational stimulation

The alert chick was put in a restraining cylinder, placed on a turntable, and subjected to passive whole-body horizontal (yaw) rotation. Bars fixed to the turntable held the beak and head post, thereby ensuring that the rotational stimuli were delivered directly to the head. Frequent observations confirmed that the head did not slip relative to the table during rotations, even at high frequencies. The head was positioned at the center of rotation with the beak pitched slightly down so that the horizontal semicircular canals were in the plane of rotation. The landmark for the horizontal canal plane, determined from preliminary dissections, was a line drawn from the midpupil to the most prominent point on the occiput. If any significant sinusoidal vertical eye movements occurred during horizontal rotation, the pitch of the head was altered so as to eliminate the vertical component and maximize the horizontal component.

All chicks were rotated sinusoidally at frequencies of 0.1, 0.3, 0.5, 0.8, 1.0, and 1.4 Hz; some also were rotated at 0.05 Hz. The frequencies were presented in a random order. The amplitude of the table excursion was fixed at $\pm 10^\circ$, which induced maximal peak rotational velocities of 88°/s (1.4 Hz). All testing was performed in the dark. At lower frequencies the combination of rocking motion and darkness sometimes caused the chicks to fall asleep. This tendency was countered by the use of occasional auditory stimuli, flashes of light between periods of rotation, and unexpected bursts of high-frequency rotation, which kept the chicks alert (i.e., their eyes were open and they vocalized frequently). All procedures were approved by the Animal Care and Use Committee at the University of Washington and conformed to the National Institutes

of Health's Guide for the Care and Use of Laboratory Animals (1985).

Eye movement measurement

An alternating magnetic field centered at the animal's head was induced by two orthogonal pairs of 14-in.-diam coils located 14 in. apart and built into the superstructure of the rotary table. The coils were driven sinusoidally at 35 kHz. Signals proportional to the horizontal and vertical components of eye position were extracted from the eye coil signal by phase-sensitive detectors, sent to analog amplifiers, filtered at 500 Hz, and stored on VCR tape with a Vetter 5000A PCM recorder (5-kHz sampling rate per channel). Turntable position was measured simultaneously by a potentiometer. The signals were digitized either simultaneously or subsequently on a Macintosh IIfx computer at a sampling rate of 1 kHz. The MIO16 Digitizer (National Instruments) was set at a rate dependent on the rotation frequency so as to give 600 data points per cycle at all frequencies.

Before and after attachment to the eye at each testing session, the eye coil was calibrated on a device that allowed precise angular displacements of the coil in the pitch and yaw planes. Measurements of angular displacement were accurate to within $\pm 0.5^\circ$ over a range of $\pm 30^\circ$. The entire system was also calibrated before each session. First the coil was fixed in space at the center of rotation and at the height of the chick's head, and then the table was rotated sinusoidally at 0.5 Hz about the fixed coil, a situation that simulated a perfectly compensatory VOR. Under these conditions, the analysis program yielded a VOR gain and phase relative to head velocity of $1.00 \pm 0.02^\circ$ (mean \pm SD) and $180.0 \pm 0.5^\circ$, respectively (see below).

VOR data analysis

An interactive analysis program for Macintosh computers displayed single cycles of sinusoidal turntable position (i.e., head position, because the head is held to the turntable) and the associated horizontal eye movement. The typical cycle shown in Fig. 1 contained two corrective saccades (\setminus), both of which could be clearly identified because of the 20- to 25-Hz oscillation characteristic of spontaneous eye movements in chickens (Wallman et al. 1982). These saccades were removed manually by selection of the involved portion of the cycle and deletion of those digitized points. The program then produced digital derivatives of the desaccaded eye position and of the head position (Fig. 1, *bottom 2 traces*) and fit each with a least-squares sine wave. Finally, the program calculated the gain of the VOR for each cycle as the ratio of the amplitude of the fitted horizontal eye velocity signal (\dot{E}) to the amplitude of the head velocity signal (\dot{H}). A perfectly compensatory VOR would have a gain of 1. The gain for the example in Fig. 1 was 0.65. The phase shift of the VOR was calculated as the difference in the phases of horizontal eye and head velocities. In the cycle shown in Fig. 1, peak leftward eye velocity occurred before peak rightward head velocity: the VOR had a phase lead relative to perfect compensation, i.e., $+180^\circ$. Alternatively, eye velocity can be viewed as lagging the ipsiversive head velocity by less than the compensatory 180° , in this case 165° . A phase between 0 and 180° indicates that the eye leads head movement; a phase between 180 and 360° indicates that it lags.

After ≥ 10 cycles were analyzed, average gains and phases were calculated from data in cycles that met two criteria: 1) $\geq 60\%$ of the data points remained in the cycle after desaccading and 2) the squared correlation coefficient (r^2) of the fit after desaccading exceeded 0.6. In other words, $\geq 60\%$ of the variance of the eye velocity from the cycle's DC level was sinusoidal. Cycles in which the calculated gain was < 0.1 did not meet these criteria. At such low levels of modulation the signal-to-noise ratio was usually so low that the regression variance did not exceed 0.2. Thus we refer to

gains of ≤ 0.1 as "negligible VOR," because we cannot be certain that such responses were significantly different from the accompanying noise. Furthermore, the calculated phases for these low-modulation cycles fluctuated widely (by more than $\pm 90^\circ$) and were therefore considered meaningless. Finally, the efficacy of the VOR for different ages and treatments is presented as the gains and phase shifts of the averages \pm their SDs as a function of stimulus frequency (i.e., Bode plots) (Melvill Jones and Milsum 1965).

Histologic preparation and analysis

Within 24 h of rotational testing, the chicks were killed with an intraperitoneal overdose of pentobarbital sodium. The labyrinthine tissues were fixed by transcardiac perfusion of heparinized saline followed by room-temperature fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 0.1 M sucrose, and 0.1 M phosphate buffer, pH 7.4). The left and then the right labyrinth were exposed and perfused directly with the same fixative. The entire head was then placed in fixative for 1–4 days at 4°C . Both horizontal canal cristae were then dissected for microscopic analysis. They were washed several times with phosphate-buffered saline, postfixed in 1% osmium tetroxide for 1 h, again washed several times with phosphate-buffered saline, and dehydrated in graded ethanol solutions.

One specimen (usually the left) from each chick was embedded in Spurr's resin for light microscopy (LM). The entire length of this crista was cut into $2\text{-}\mu\text{m}$ transverse serial sections with a diamond knife. Of every 25 sections, 3 were mounted, stained with Richardson's stain, coverslipped, and examined under a light microscope at $\times 400$ total magnification with Nomarski optics.

The other horizontal crista was prepared for scanning electron microscopy (SEM). The roof of the ampulla and the cupula were removed before critical-point drying and gold-palladium coating. They were viewed with a JEOL 63005 electron microscope at 15 kV accelerating voltage.

In the sections analyzed by LM, elements were counted as hair cells only if they contained a nucleus (with a visible nucleolus) situated above the row of supporting cell nuclei at the basement membrane, had a cell body that extended to the luminal surface of the sensory epithelium, and contacted underlying neural elements. Even though the cytoarchitecture of the crista is disrupted soon after streptomycin treatment, we found it possible to identify nuclei and nucleoli that appeared indistinguishable from those seen in control tissues (see Fig. 4B of Weisleder and Rubel 1993). A cell was typed on the basis of the morphology of its afferent ending (Wersäll 1956). An afferent with a complete calyx was considered to innervate a type I hair cell. An afferent ending that did not completely surround the hair cell was considered a bouton afferent, and the hair cell it innervated was considered a type II hair cell. The length of the basement membrane underlying the sensory epithelium was measured in each section by means of the National Institutes of Health Image morphometric program, Version 1.57 (Rasband), on a Macintosh IIfx computer. Hair cell density was defined as the number of hair cells per $100\text{-}\mu\text{m}$ length of basement membrane (Weisleder and Rubel 1993). The densities of type I, type II, and total hair cells were averaged from six to eight regularly spaced sections along the complete length of each crista. The selected sections in the control cristae spanned an average distance of $2,919\text{ }\mu\text{m}$, over which 83 ± 20 (SD) type I hair cells and 184 ± 30 type II hair cells were found. Table 1 details the number of specimens, mean number of hair cells, and the mean length of epithelium examined for each recovery period.

Redosing

Two treated chicks were not killed immediately after VOR testing. Rather, after they had demonstrated functional recovery of the VOR, their regenerated hair cells were destroyed by readministration of the same regimen of streptomycin 3 wk after completion

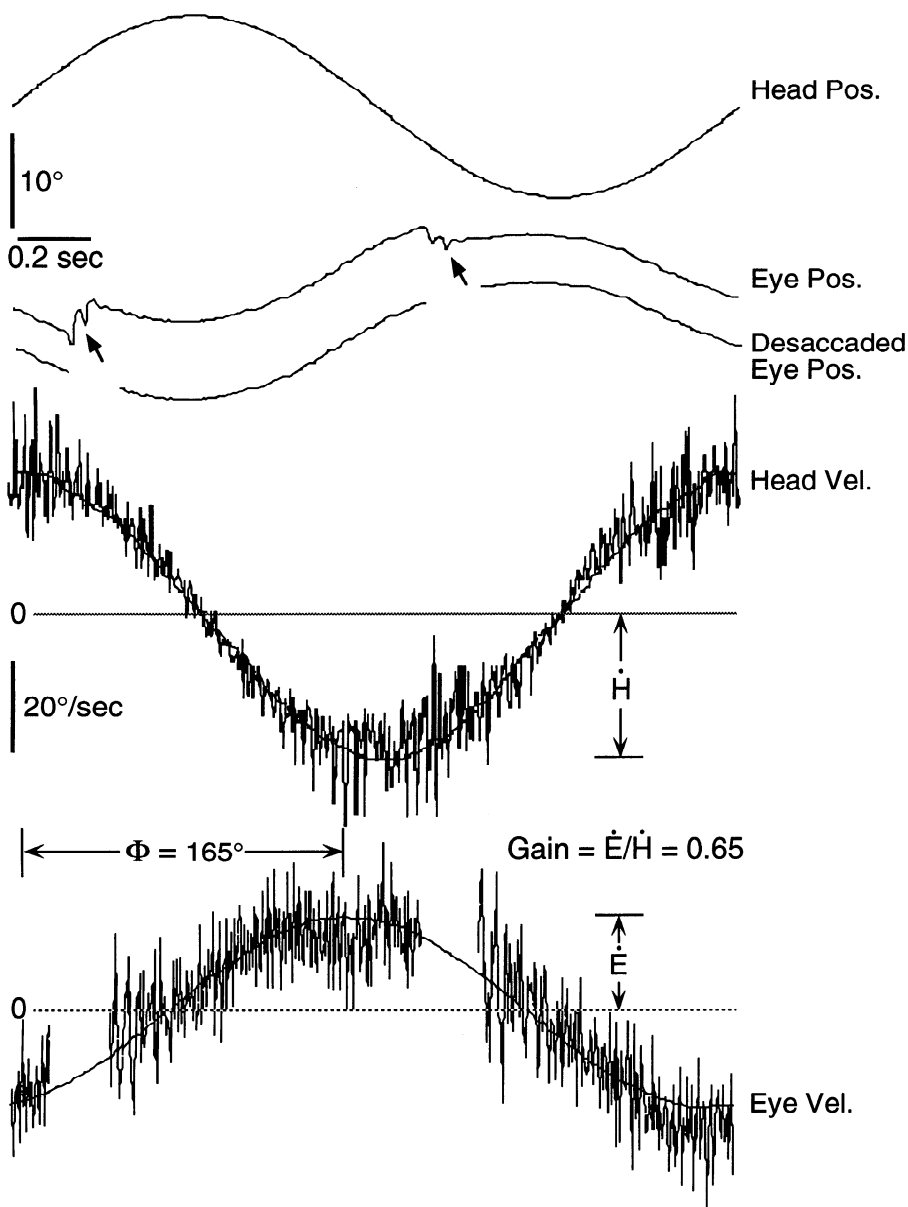


FIG. 1. Representative eye movement recordings of a normal 31-day-old chick (control for 3 wk poststreptomycin) to a single cycle of horizontal sinusoidal rotation at 0.5 Hz. *Top to bottom*: horizontal head position, horizontal eye position, horizontal eye position with saccades removed, horizontal head velocity (\dot{H}) and its least-squares fitted sine wave, and horizontal eye velocity (\dot{E}) and its least-squares fitted sine wave. Time calibration bar applies to all traces. The 10° calibration bar applies to the 3 position traces; the $20^\circ/\text{s}$ calibration bar applies to the 2 velocity traces. Upward deflection: rightward movement. Vestibuloocular reflex (VOR) gain, i.e., \dot{E}/\dot{H} , is 0.65 for this example. The phase of the response velocity relative to stimulus velocity (Φ) is 165° . Note the 2 oscillatory saccades (\setminus).

of the first antibiotic course. The VOR was measured before and after the second dose. Then the chicks were killed and their tissues processed as described above.

RESULTS

Functional recovery

VOR GAINS. The control chicks showed increasing VOR gain with age: mean gains increased from ~ 0.3 in 1- to 4-day chicks to 0.5 – 0.6 in ≥ 4 -wk chicks (Fig. 2, dashed lines). This developmental increase of VOR gain is consistent with an earlier report that chicks had maximum VOR gains of 0.1 – 0.2 at 1 Hz when newly hatched and ~ 0.6 at 4–6 wk of age (Wallman et al. 1982). (For comparison, our 1- to 4-day survivors were 11–14 days old, and 4-wk survivors were ~ 5.5 wk old.)

The VOR gain was quite variable among control subjects (Fig. 2, ranges are areas shaded by stripes). However, values

of ≤ 0.1 were encountered only in the youngest chicks at the lowest frequency measured, 0.1 Hz. At frequencies of ≥ 0.3 Hz, individual control chicks had measurable VOR gains ranging from a low of 0.12 at 1 wk to a high of 0.85 at 8–9 wk.

In contrast, chicks treated with streptomycin had severely reduced VOR gains immediately after treatment (Fig. 2, symbols). No VOR gain of > 0.1 could be measured in any of the 1- to 4-day or 1-wk chicks. Six of the eight 2-wk chicks had gains below the average control values, and three of these had gains of < 0.1 (indistinguishable from 0) across the entire frequency range. The remaining two had VOR gains in the control range at every frequency, suggesting complete recovery. The ten 3-wk chicks showed some recovery at all but the very lowest frequencies: two had normal VOR gains at frequencies of > 0.3 Hz and another three had VOR gains in the control range for three or four frequencies. Two of the six 4-wk chicks had essentially normal VOR gains, whereas the other four still showed gains below con-

TABLE 1. Morphological data obtained from light microscopy

Recovery Period, wk	Control Chicks				Treated Chicks			
	Number of cristae	Mean number of type I	Mean number of type II	Mean length examined, μm	Number of cristae	Mean number of type I	Mean number of type II	Mean length examined, μm
≤ 1	4	74	201	3,202	5	2	84	2,626
2	1	72*	178*	2,527*	3	7	139	2,807
3	2	87	179	3,071	2	13	171	3,845
8-9	3	100	178	2,878	4	58	153	2,627
All	10	83	184	2,919	14	20	137	2,976

For each recovery period the number of cristae examined and the mean number of each type of hair cell counted per crista are shown. Data are from evenly spaced ($2\text{-}\mu\text{m}$) transverse sections. Six to 8 such sections were examined per crista, and the length of the hair-cell-bearing surface of each section was measured. The average total length of epithelium per crista thus examined is given in the "Mean length examined" column. Note that this measurement is not the length of the crista. (* indicate individual measures, not means.)

control values, especially at frequencies between 0.5 and 1.4 Hz. Two of the 8- to 9-wk chicks showed normal VOR gains and two showed greater than normal values. One of these latter chicks (Fig. 2, \circ) had among the highest gains of any chick tested.

To summarize, the VOR was completely absent in the streptomycin-treated chicks from 1 day to 1 wk after treatment. A few chicks showed complete recovery of the VOR

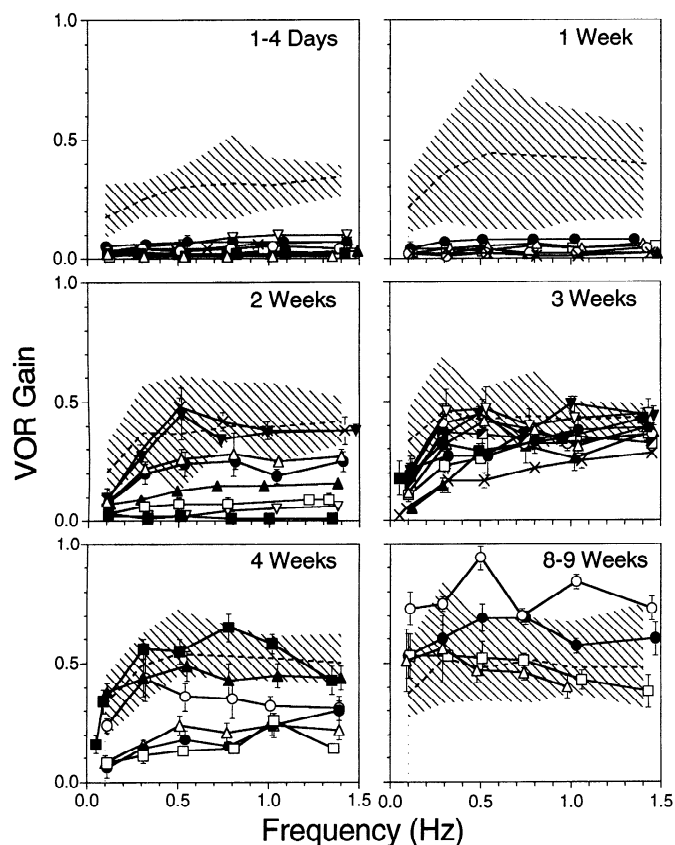


FIG. 2. VOR gain (\dot{E}/\dot{H}) as a function of the frequency of horizontal sinusoidal rotation for 36 control (striped area) and 42 treated (symbols connected by lines) chicks at 6 different recovery periods after streptomycin treatment. Actual chronological age ranges of chicks in days posthatching are: 10-12, 16-20, 21-27, 30-34, 36-42, and 63-74, respectively. Each symbol represents the mean gain of an individual chick derived from ≥ 10 cycles of sinusoidal rotation at that frequency. Error bars: SDs. Dashed lines: average gains of the control chicks. The areas shaded with stripes: range of control values.

after 2 wk, but most were still impaired. By 3 wk, all chicks had a clear VOR, which, on average, was still less than normal. By 8-9 wk after treatment, average VOR gains had returned to normal or exceeded normal levels. The time course of recovery of VOR gains at 0.1 and 1.0 Hz is summarized in Fig. 3. Although the average VOR gain of treated birds exceeded that of controls after 8-9 wk, this was not statistically significant (for 0.1 Hz, $P = 0.06$; for 1.0 Hz, $P = 0.66$; unpaired t -tests assuming unequal variances).

VOR PHASE SHIFTS. For all control chicks at all ages and frequencies, eye velocity led perfect compensation, i.e., 180° (Fig. 4, dashed lines and shading). The mean control phase lead was relatively constant ($13 \pm 3^\circ$, mean \pm SD) over the frequency range 0.3-1.4 Hz. At 0.1 Hz, controls showed a larger phase lead ($43 \pm 7^\circ$, mean \pm SD) for all ages.

For treated chicks at 2 and 4 wk postinjection, mean VOR phase leads at all frequencies were greater than mean control phase leads although the phase leads of some chicks fell within the normal range (Fig. 4). Like the control chicks,

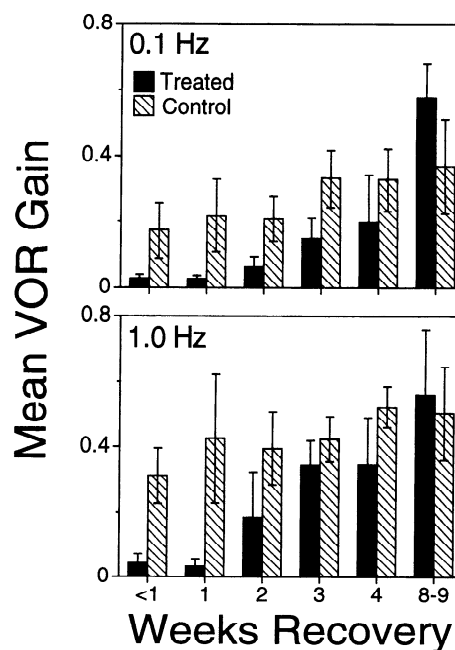


FIG. 3. Mean VOR gains at 0.1 and 1.0 Hz for treated chicks (filled bars) and controls (striped bars) as a function of the recovery time after treatment. Error bars: SDs. Each bar is the average from ≥ 4 chicks.

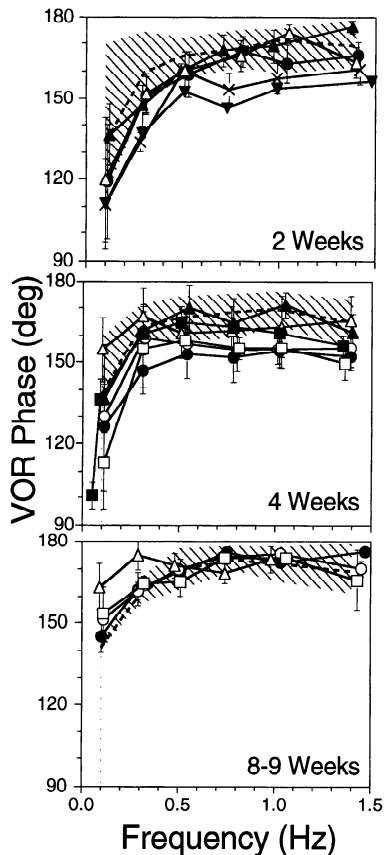


FIG. 4. VOR phase (eye velocity relative to head velocity) as a function of the frequency of horizontal sinusoidal rotation. Symbols: mean phases of individual treated chicks over ≥ 10 cycles of sinusoidal rotation. Individuals are represented by the same symbols used in Fig. 2. Error bars: SDs. Striped areas: range of control values. Dashed lines: mean control values. Data for periods earlier than 2 wk are not shown because gain values were too low to allow accurate phase measures (see METHODS).

these treated chicks had greater phase leads at lower frequencies than at higher frequencies. Phase data from earlier recovery periods and from several 2-wk chicks are not included here because the associated VOR gains were < 0.1 (see METHODS). As VOR gains increased during the 2–9 wk after streptomycin treatment, VOR phase leads and their variability both decreased. Both were at control values by 8–9 wk posttreatment.

Anatomic recovery

The density of hair cells in the crista of the horizontal semicircular canals was estimated by LM. The maturity, location, and configuration of the stereocilia were assessed with the aid of SEM.

LM ANALYSIS. Hair cell densities in the horizontal cristae were evaluated for two to five treated chicks and one to three age-matched control chicks for each recovery period; the results are summarized in Table 1 and in Fig. 5. The total number of all hair cells in both 1- to 4-day and 1-wk treated chicks (grouped together in Fig. 5, solid bars) was $\sim 40\%$ of control values (striped bars). These were predominantly (98%) type II hair cells. No attempt was made to determine whether these hair cells were new cells or residual cells not eradicated by the streptomycin treatment. The aver-

age density of all hair cells increased between 1 and 3 wk postinjection; the largest part of the increase was attributable to type II cells (Fig. 5). The average density of type II hair cells was 52% of their average control value within the 1st wk of recovery and increased to 88% of average control value by 3 wk posttreatment. In contrast, the density of type I hair cells in the 1st wk posttreatment was essentially 0 and did not recover significantly until after 8–9 wk, at which time type I cells were still less numerous than normal (64% of control values). Only a modest recovery of type I cells was seen in 2- and 3-wk subjects (7 and 17% of control values, respectively).

SEM ANALYSIS. In control chicks at all ages examined, the epithelium of the horizontal canal crista was covered with a dense mat of stereocilia. However, the appearance of the sensory epithelium was not necessarily reflected in VOR gain. For example, we could not distinguish the crista with the lowest gains from that of other control cristae on the basis of their SEM appearance.

In contrast to the control chicks, all chicks killed 1 day after streptomycin treatment had epithelia that were denuded of mature stereocilia over the entire surface of the crista except at its edges (Fig. 6). The apical aspect of the crista had no mature stereocilia when viewed with high magnification (Fig. 6, top left inset). Instead, its surface was formed by fusion of the apices of support cells occasionally interspersed

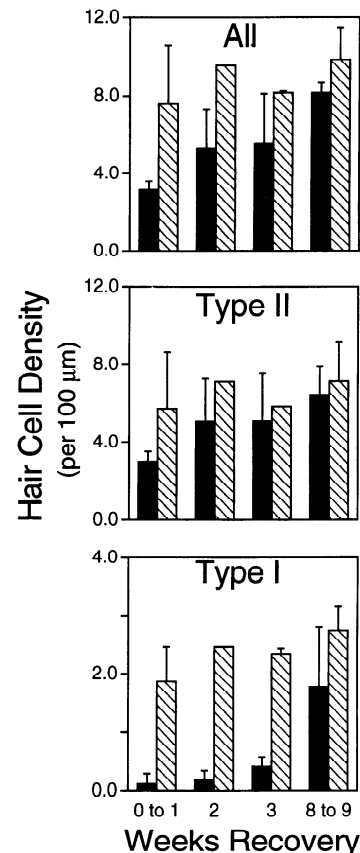


FIG. 5. Mean hair cell density (number of hair cells per 100- μm length of epithelium) as revealed by light microscopy (LM) of treated (filled bars) and control chicks (striped bars) as a function of recovery time from streptomycin intoxication. *Top*: all hair cells. *Middle*: type II only. *Bottom*: type I only. Error bars: SDs. The data used to construct these graphs are summarized in Table 1.

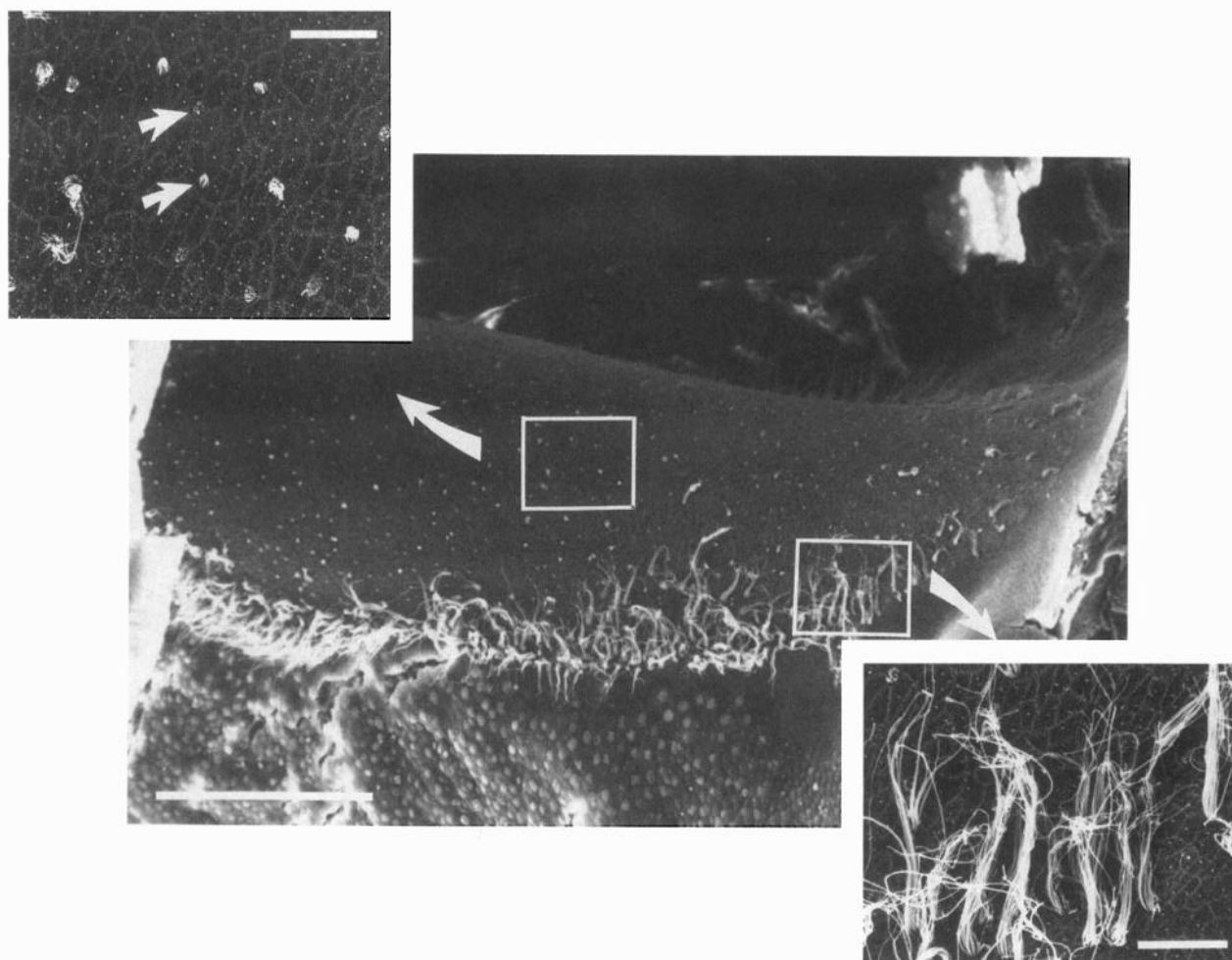


FIG. 6. Scanning electron microscopy (SEM) of the crista ampullaris of the horizontal semicircular canal of a chick 1 day after streptomycin treatment. The low-magnification view reveals that only hair cells at the edges of the epithelium survived. Hair cells at the edges (*bottom right inset*, high magnification) have long, mature processes, whereas cells at the apical region (*top left inset*, high magnification) appear to be fused support cells with only occasional short stereocilia (\rightarrow). The gain characteristic for this chick is shown in Fig. 2 (1–4 days, \square). Low-magnification calibration = 100 μm . *Inset* calibration = 10 μm .

with very immature stereociliary bundles (\rightarrow). Viewed at the same magnification, the utricular edge (Fig. 6, *bottom right inset*) revealed long stereociliary bundles resembling those seen in the control chicks. Like all other birds in the 1- to 4-day recovery group, the chick whose crista is shown in Fig. 6 had no measurable VOR (Fig. 2, 1–4 days, \square). Similar SEM was obtained from three other treated chicks in this group.

One week after streptomycin treatment the epithelia had a fairly uniform distribution of immature stereocilia (Fig. 7A). The utricular edge (Fig. 7D) did not have bundles of very long stereocilia; instead, intermediate-length bundles were interspersed with shorter ones. This suggests that the mature hair cells remaining at the edges of the epithelium immediately after treatment were lost and replaced by new hair cells in subsequent days. The apical region of this crista (Fig. 7B) had a relatively similar morphology, although stereocilia there were generally shorter than at the utricular edge. Several very short stereociliary bundles were present (Fig. 7B, arrows). Swelling and fusion of stereocilia, early signs of aminoglycoside damage (Wersäll et al. 1971), were still evident (Fig. 7C). Thus hair cell regeneration seemed

to be occurring even in the face of continued aminoglycoside damage. This chick had no VOR (Fig. 2, 1 wk, \square). Similar SEM was obtained from another 1-wk chick.

Hair cell recovery 2 wk after streptomycin treatment was quite variable (Fig. 8), being either scant (A), partial (B), or extensive (C). At this time after treatment, the extent of anatomic recovery seen in SEM corresponded well with the efficacy of the VOR. The chick with scant hair cell recovery (Fig. 8A) had no VOR (Fig. 2, 2 wk, \square); the chick with partial recovery (Fig. 8B) had a reduced VOR gain (Fig. 2, 2 wk, Δ); and the chick with extensive hair cell recovery (Fig. 8C) had a normal VOR (Fig. 2, 2 wk, \times). Unfortunately, adequate fixation was not obtained in the opposite horizontal crista in two of these three cases. Therefore quantitative statements based on hair cell counts cannot be made for these subjects. Similar correspondences between the appearance of stereocilia and the VOR responses were found for four other chicks in the 3-wk recovery group (not shown).

For the six 4-wk chicks, the correspondence between stereociliary density and the VOR response was less robust. Cristae from these subjects all appeared to have at least

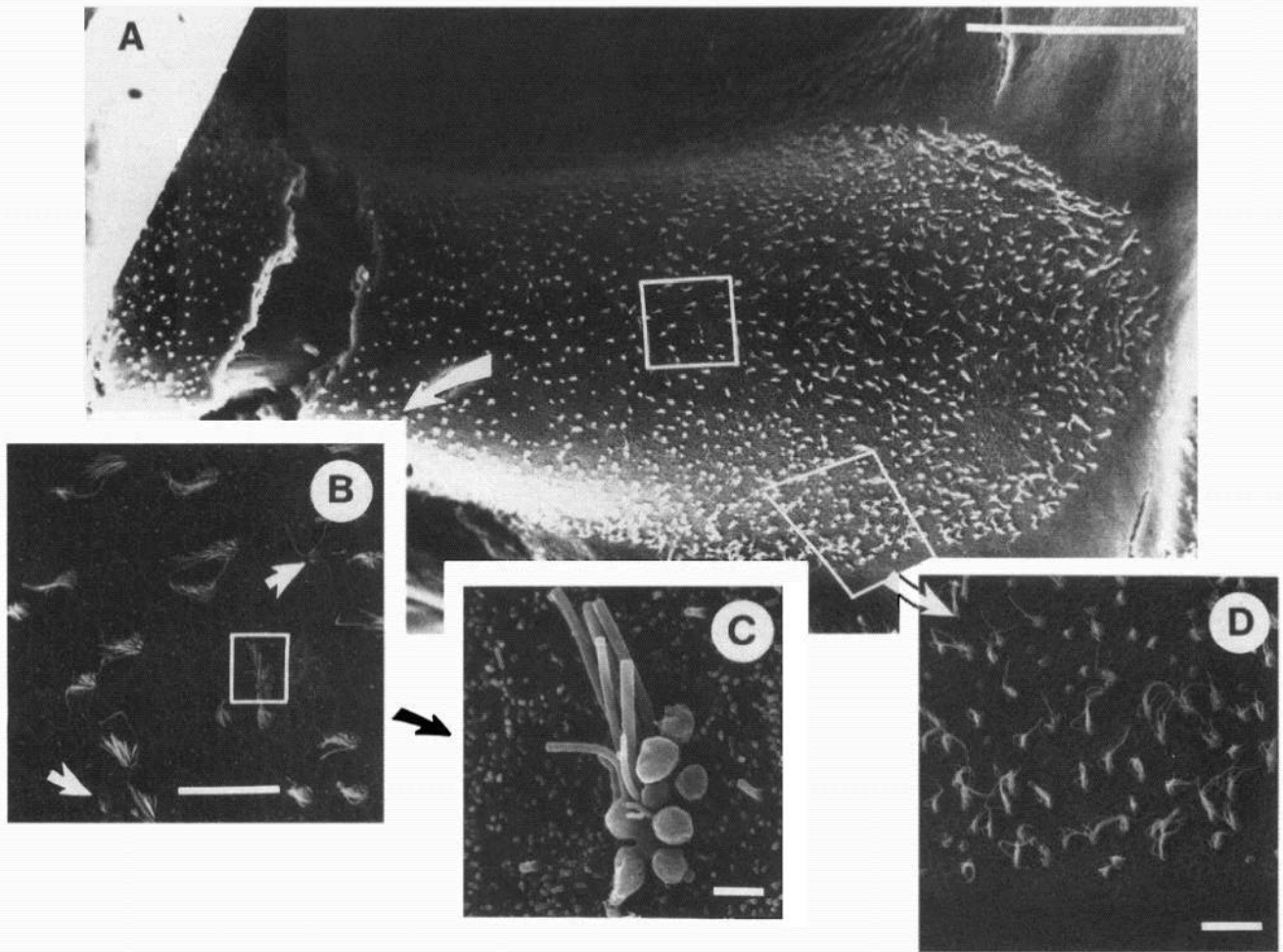


FIG. 7. SEM of the crista ampullaris of the horizontal semicircular canal of a chick 1 wk after streptomycin treatment. *A*: low-power view of the entire epithelium. Bar = 100 μm . *B*: high-power view of the apical region (boxed area in *A*). Note sparse, shorter stereocilia (\rightarrow). Bar = 10 μm . *C*: magnification of the boxed area in *B* shows swelling and fusion of stereocilia, signs of aminoglycoside damage. Bar = 1 μm . *D*: high-power view of the utricular edge outlined in *A*, showing the presence of intermediate-length stereocilia. Bar = 10 μm . The gain characteristic for this chick is shown in Fig. 2 (1 wk, \square).

the degree of stereociliary regeneration shown in Fig. 8C. However, their VOR gains (Fig. 2, 4 wk) ranged from slightly higher to much lower than those of the epithelium illustrated in Fig. 8C (Fig. 2, 2 wk, \times).

SEM images of the cristae from three subjects that recovered 8–9 wk after treatment (e.g., Fig. 9) were indistinguishable from each other and from those of controls. However, the range of gains in Fig. 2 at 8–9 wk is wide. Thus, in these fully recovered chicks, the difference in gains was not reflected in the appearance of their sensory epithelia.

To summarize the SEM data, the hair cell loss during the 1st wk after streptomycin treatment seemed to start at the apex of the crista and spread to the edge. One week after treatment, the longer stereocilia that characterized the pre-treatment crista were gone. Two weeks after treatment, the number of new stereocilia paralleled the return of VOR function. From then on there were no obvious anatomic differences among the cristae of the various chicks although their VOR gains could be quite dissimilar.

Retreatment with streptomycin

If VOR recovery is due to hair cell regeneration and not just central compensation, it might be expected that loss of

regenerated hair cells would again lead to loss of the recovered VOR. We tested this hypothesis by retreating two chicks that had recovered for 3 wk following the initial streptomycin treatment. Just before the second streptomycin treatment, their VOR gains had indeed recovered to the range of values typical of other 3-wk survivors (Fig. 2, 3 wk). After these chicks received a second course of streptomycin treatment, their gains again fell essentially to zero 3 days later.

The decrease of gain after retreatment also was associated with a loss of hair cells. SEM images of the cristae of the two retreated subjects appeared identical to those taken from chicks that had undergone only one streptomycin treatment (see Fig. 6). However, LM examination of the opposite horizontal cristae revealed that the average density of all hair cells was somewhat less (2.13 per 100 μm) than the average density seen in chicks immediately after a single treatment (3.10 per 100 μm). This reduction in overall hair cell density after two treatments rather than one was due both to fewer type II hair cells (2.08 vs. 3.00 per 100 μm) and fewer type I hair cells (0.05 vs. 0.11 per 100 μm).

DISCUSSION

The major finding of this study is that the regenerated avian vestibular epithelium is functional: the VOR, whose

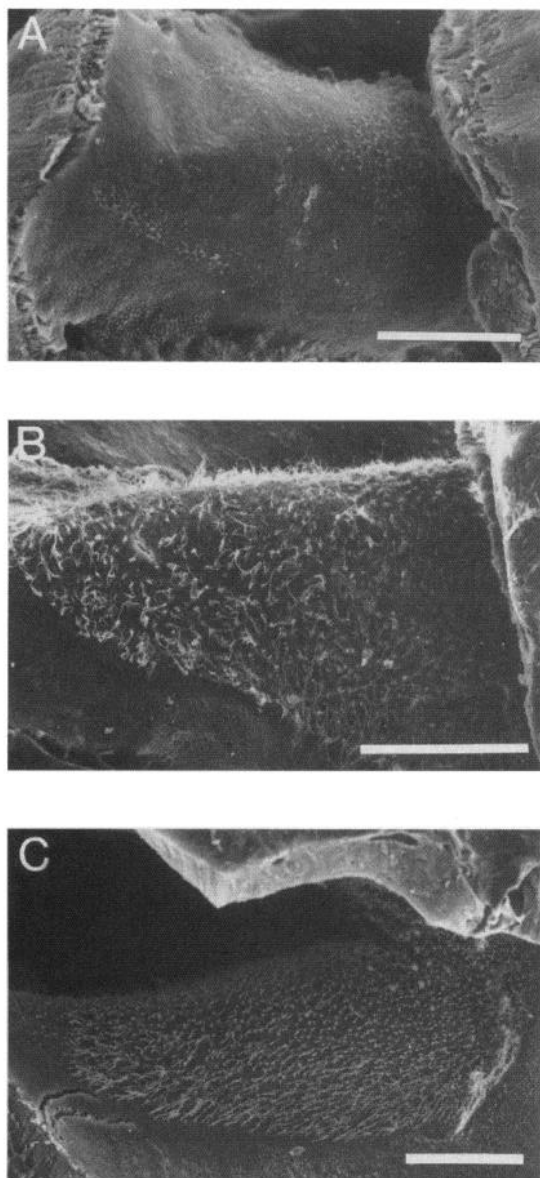


FIG. 8. Low-power SEM of the crista of the horizontal semicircular canals of 3 chicks 2 wk after streptomycin treatment. At this age, the epithelium can appear very denuded (A), partially recovered (B), or extensively recovered (C). The VOR gain characteristics for the chicks in A–C are shown in Fig. 2 (2 wk, □, △, and ×, respectively). Bars = 100 μ m.

input arises from the hair cells on the crista ampullaris of the semicircular canals, had normal characteristics after the streptomycin-damaged hair cells had recovered or been replaced. Our conclusion that VOR integrity depends on hair cell recovery is supported by two major correlations. First, a streptomycin-induced reduction of $\geq 60\%$ in the total number of hair cells in the crista ampullaris of the lateral semicircular canal was associated with virtual abolition of the horizontal VOR. Second, after the vestibular epithelium had returned to its normal appearance, as revealed by the number of its hair cells and the morphology of their stereocilia, there was a parallel return of the VOR to its normal gain and phase.

Even these conservative conclusions depend on an accurate measurement of the VOR and on the criteria employed

in our morphometric analyses. Therefore we begin our discussion by exploring possible limitations in these techniques. We then evaluate whether recovery of the VOR depends on hair cell density and whether type I or type II hair cells are preferentially involved. Finally, we consider the limitations associated with drawing conclusions about the functionality of hair cells on the basis of their appearance.

Technical considerations

ASSESSMENT OF THE VOR. The apparent loss of the VOR immediately after streptomycin treatment was not due to inadequate stimulation, the effects of anesthesia, or subject somnolence. To optimize our chances of detecting the existence of even a very small VOR, we used frequencies of rotation at which the control VOR gain was maximal, i.e., ~ 0.5 – 1.0 Hz. Rotational testing of both control and treated chicks was conducted only after the birds were completely recovered from the ketamine anesthesia used during the brief procedure to attach the search coil and post to the eye; no testing was performed until chicks were able to stand and walk normally. Finally, the alertness of the chicks was monitored by on-line inspection of the VOR. If rotational stimulation produced no VOR, we resorted to flashes of light, noise, manual stimulation, and bursts of high-frequency rotation in attempts to alert the subject. Although one might presume that noise would be less effective in treated chicks because of attendant damage to auditory hair cells, both groups of chicks could be alerted with these stimuli.

ESTIMATION OF HAIR CELL NUMBERS. How reliable is our determination of the density of hair cells in the crista? We estimated that streptomycin treatment caused a loss of $\geq 60\%$ of the hair cells in the crista of the horizontal canal. This percentage was based on counts of hair cells by LM immediately after treatment. A cellular profile was counted as a hair cell only if it contained a nucleolus. Although it is true that this stringent criterion may have caused us to underestimate the actual density of hair cells present, this concern would have obtained for both treated and control cristae. Thus our measurement would still give a valid means for comparing the two groups, but might not provide an accurate measure of the absolute density of hair cells.

Our estimates of total hair cells per 100 μ m of sensory

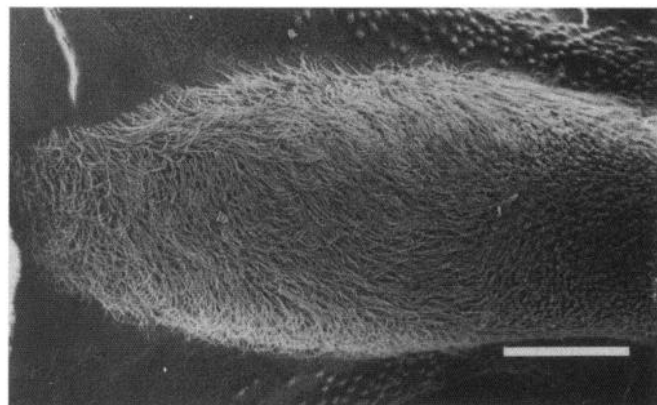


FIG. 9. Low-power SEM of the crista ampullaris of the horizontal semicircular canal of a chick 60 days post-treatment. Its VOR gain characteristics are shown in Fig. 2 (8–9 wk, □). Bar = 100 μ m.

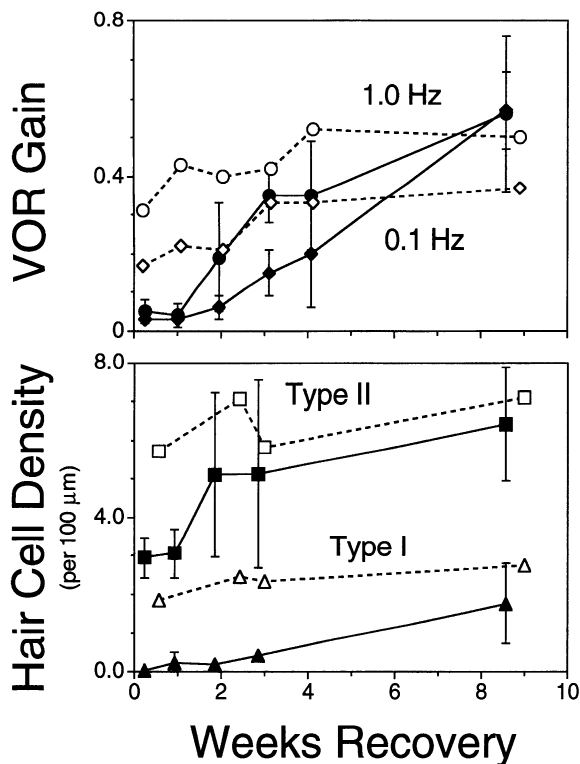


FIG. 10. Comparison of VOR gain recovery and hair cell density recovery. *Top*: mean VOR gain as a function of recovery time from streptomycin intoxication at rotational frequencies of 0.1 Hz (diamonds) and 1.0 Hz (circles). *Bottom*: mean density of type I (triangles) and type II (squares) hair cells as a function of recovery time. Solid symbols and solid lines: treated chicks. Open symbols and dashed lines: age-matched control chicks. Error bars: SDs.

epithelium in the horizontal canal crista 1 wk after streptomycin treatment differed substantially from estimates in the superior canal cristae reported by Weisleder and Rubel (1993, their Table 1): our numbers were higher for treated chicks (3.1 vs. 1.97) and lower for control chicks (7.6 vs. 11.6). The disparate estimates may reflect differences in the susceptibility of the horizontal and superior canals to streptomycin damage, differences caused by variation in damage over the length of the crista (Weisleder and Rubel counted only the lateral 30% of the epithelium), or differences in the quality of tissue fixation between the studies.

On the basis of the SEM in Fig. 6, it might be questioned whether the LM-based estimate of 40% hair cell survival is accurate. Stereocilia occupy a smaller percentage of the crista surface area shown, only ~25% by planimetry. However, the densely populated medial (neural) end of the crista (Fig. 6, *left*) is not shown. This region usually was obscured by collapse of the medial ampulla. LM sections showed that many hair cells were present in this region 1–4 days posttreatment. For example, LM sections from the crista contralateral to that shown in Fig. 6 revealed that stereocilia were present over 27% of its flat portion (like the portion shown in Fig. 6) but over 43% of its entire surface, in better agreement with the hair cell counts. Thus, when all parts of the crista are considered, the LM estimates of hair cell density are accurate.

Correlation of hair cell density and VOR gain

CONSIDERATION OF ALL CELLS. If hair cell regeneration were the chief determinant of VOR recovery, VOR gain

should rise with hair cell density. However, we did not find a strict correlation between hair cell density and the gain of the VOR. First, immediately after intoxication, the VORs in all treated chicks had gains of <0.1 , even though ~40% of the normal number of hair cell nuclei were still present (Fig. 5). The absence of the VOR despite the presence of so many hair cells suggests that these hair cells were not functional, perhaps because they remained intoxicated immediately after streptomycin treatment. Alternatively, these may have been new hair cells that were not yet capable of signal transduction. Our data do not allow us to decide between these possibilities.

Second, although hair cell density and VOR gain increased in parallel until 2 wk after intoxication, this relationship disappeared at 3 and 4 wk. For example, chicks with a 2.5-fold difference in average gain (Fig. 2, 4 wk, \blacktriangle vs. \bullet) had cristae with similar appearances (not shown). Even nontreated, older chicks with apparently identical epithelia exhibited large variations in VOR gain. Thus, even in normal chicks, VOR gain does not reliably reflect the appearance of the epithelium or its hair cell density.

Nevertheless, there are some consistent correlations between a chick's VOR and the condition of its vestibular epithelium. All of our control chicks had measurable VOR gains of >0.1 at almost every frequency. In contrast, none of the treated chicks had a measurable VOR up to 1 wk after intoxication, at which time hair cell density was considerably reduced. In time, all of the treated chicks acquired some VOR, which eventually returned to normal when the complement of hair cells had returned to control values. Therefore the fact that the VOR recovered at all supports the hypothesis that the regenerated and/or repaired vestibular hair cells were functional and that they contributed to the recovery.

SEPARATION OF HAIR CELLS ACCORDING TO TYPE. The rather poor correlation between VOR gain and cell density in intoxicated chicks might be improved if hair cells were divided according to whether they had type I or type II morphology. In mammals, a variety of evidence converges on the suggestion that regularly discharging vestibular affer-

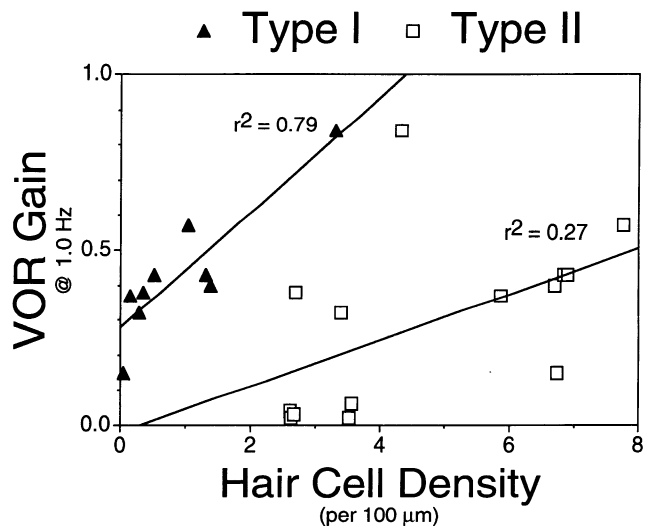


FIG. 11. VOR gain at 1.0 Hz vs. hair cell density for type I hair cells (\blacktriangle) and type II hair cells (\square). Each symbol represents data from an individual treated chick. Data are from all recovery times. Least-squares lines fit to the data are shown with their squared correlation coefficients.

ents and their prominent inputs from type II hair cells are largely responsible for the VOR (Highstein et al. 1987; Minor and Goldberg 1991). If this situation also applies to birds, recovery of type II hair cells should have a greater effect on the recovery of VOR gain than the recovery of type I hair cells. We did not find this to be the case.

During the 1st wk after treatment, when the VOR gain was essentially 0 (Fig. 10, *top*), the density of type II hair cells was 52% of normal and the density of type I hair cells was only 8% of normal (Fig. 10, *bottom*). This observation suggests that the absence of type I hair cells might account for the absence of the VOR. Indeed, after 2 wk the VOR gains were still considerably less than normal (Fig. 10, *top*), despite the return of type II hair cell density to 72% of control value (Fig. 10, *bottom*). Type I hair cell density remained low (8% of control) at this point. Furthermore, even 4 wk after treatment, the VOR gains were still less than normal (Fig. 10, *top*) despite type II hair cell repletion by 3 wk (Fig. 10, *bottom*). On the basis of these data, the number of type II hair cells is not always indicative of VOR gain.

Indeed, VOR gain seems better correlated with the relative percentage of type I cells (Fig. 11). Our finding that the earliest cell loss occurred at the apex of the crista (Fig. 6), where we and others (Weisleder and Rubel 1993) have observed a majority of type I cells, helps explain these observations. Also, the early loss of apical hair cells is consistent with the known preferential toxicity of streptomycin for type I hair cells (Duval and Wersäll 1964; Wersäll and Hawkins 1962). Perhaps the type II cells are responsible for early recovery and the type I cells are required for the complete recovery at 8–9 wk.

PROBLEMS WITH HAIR CELL/VOR CORRELATIONS. The major caveat in attempting to correlate the number of hair cells with VOR gain is the underlying assumption that all counted hair cells are functional. We already have reason to suspect this assumption, because immediately after intoxication VOR gain was 0 even though 40% of the normal complement of hair cells could be counted. Our SEM suggests that there was considerable turnover of hair cells in the 2 wk following treatment. Initial damage stripped the stereocilia from the apex of the crista but left a band of long stereocilia at the edges (Fig. 6). Within 1 wk after streptomycin treatment, there was a complete disappearance of mature stereocilia. During this time, numerous short stereocilia appeared throughout the epithelium (Fig. 7), indicating that many new hair cells were already being generated. Thus, although hair cells were never completely absent in this preparation, there are compelling SEM data to suggest that the initial complement of hair cells was lost and replaced. Although we counted hair cell nuclei in significant numbers throughout the 1st wk, many of these cells may have had cilia too short to extend into the cupula or afferent terminals that were disconnected from their basilar synapses.

Also, it is possible that recovery of the VOR is due not to regenerated hair cells but rather to repaired hair cells that have survived aminoglycoside treatment (Baird et al. 1993). In this scenario, the 40% of hair cells remaining simply lose their stereocilia and cuticular plates but not their afferent contacts. A gradual regrowth/repair of these hair cell components would underlie VOR recovery, and new hair cells

would assume less importance. Recent *in vitro* observations in the developing mouse suggest that such hair cell repair occurs after mechanical damage of the organ of Corti (Sobkowicz et al. 1992). If repair of surviving hair cells rather than the generation of new cells indeed is the basis of VOR recovery, there would be a very poor correlation of VOR gain with cell number.

Last, it is possible that VOR recovery might have a large central component. In this scenario, the input signal generated by either regenerated or repaired hair cells would be adequate to drive the VOR only if the signal were amplified by compensation in central pathways. Again, VOR gain could increase without an associated increase in hair cells if a constant number of surviving hair cells provided an afferent signal, which was made more effective by a gradual increase in efficacy of central synapses. Admittedly, our experiments of repositioning regenerated hair cells did not directly address the relative roles of central and peripheral compensation in the recovery process. Even if reorganization of central pathways had occurred during the first recovery period, it would be impossible to reveal it without an input signal to drive the VOR. Future experiments should allow VOR recovery and then follow the subsequent recovery by a second treatment to study the rate of VOR improvement. If central pathways play an important role, the second recovery might be faster.

The activity in the vestibular nerve of streptomycin-treated chickens supports the hypothesis that hair cell regeneration underlies functional recovery (Boyle et al. 1994). In chickens treated with the same streptomycin regimen used in this study, mechanical stimulation of the anterior semicircular canal elicited no responses in afferents tested soon after treatment. Thereafter, responses gradually returned, and the depth of modulation approached control values 70 days after treatment. These preliminary results are consistent with the hypothesis that functional vestibular recovery parallels hair cell regeneration.

In conclusion, VOR recovery probably involves a number of events, including replacement of hair cells by regeneration, the attendant growth of stereocilia on new and surviving cells, the establishment of new afferent contacts, and compensation by brain stem circuits for changes in afferent signals. Although we cannot sort out the relative contributions of each of these components, they clearly are very effective because they can restore an initially absent VOR to its normal characteristics within 8–9 wk. Our data suggest that hair cell regeneration plays an important role in this process.

We gratefully acknowledge E. Grossman and R. Cent for writing the analysis program, R. Schor for programming assistance, S. Kreig and D. Cunningham for histology, and K. Elias for artful editing. We are grateful for the comments of Y. Iwamoto, C. R. S. Kaneko, L. Ling, S. Newlands, J. O. Phillips, M. Pong, F. R. Robinson, R. Soetedjo, and A. Straube on the initial version of this manuscript.

This study was supported by National Institutes of Health Grants EY-00745 and RR-00166, Training Grant DC-00018, and Grant DC-00395, and funds from National Organization for Hearing Research and from the Virginia Merrill Bloedel Hearing Research Center.

Address for reprint requests: A. F. Fuchs, Regional Primate Research Center, Box 357330, University of Washington, Seattle, WA 98195-7330. Received 18 December 1995; accepted in final form 9 July 1996.

REFERENCES

- BAIRD, R. A., TORRES, M. A., AND SCHUFF, N. R. Hair cell regeneration in the bullfrog vestibular otolith organs following aminoglycoside toxicity. *Hear. Res.* 65: 164–174, 1993.

- BOYLE, R., CAREY, J. P., HIGHSTEIN, S. M., RUBEL, E. W., AND RABBITT, R. D. Recovery of anterior canal afferent spontaneous activity and responses to mechanical stimulation following aminoglycoside-induced hair cell loss in chicks. *Soc. Neurosci. Abstr.* 20: 1191, 1994.
- CORWIN, J. T. AND COTANCHE, D. A. Regeneration of sensory hair cells after acoustic trauma. *Science Wash. DC* 240: 1772–1774, 1988.
- COTANCHE, D. A. Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma. *Hear. Res.* 30: 181–195, 1987.
- CRUZ, R. M., LAMBERT, P. R., AND RUBEL, E. W. Light microscopic evidence of hair cell regeneration after gentamycin toxicity in chick cochlea. *Arch. Otolaryngol. Head Neck Surg.* 113: 1058–1062, 1987.
- DUCKERT, L. G. AND RUBEL, E. W. Morphological correlates of functional recovery in the chicken inner ear after gentamycin treatment. *J. Comp. Neurol.* 331: 75–96, 1993.
- DUVAL, A. J. AND WERSÄLL, J. The site of action of streptomycin on inner ear sensory cells. *Acta Otolaryngol.* 57: 581–598, 1964.
- FROYMOVICH, O., REBALA, V., SALVI, R. J., AND RASSAEL, H. Long-term effect of acoustic trauma on distortion product otoacoustic emissions in chickens. *J. Acoust. Soc. Am.* 97: 3021–3029, 1995.
- GIROD, D. A., TUCCI, D. L., AND RUBEL, E. W. Anatomical correlates of functional recovery in the avian inner ear following aminoglycoside ototoxicity. *Laryngoscope* 101: 1139–1149, 1991.
- HASHINO, E. AND SOKABE, M. Kanamycin induced low-frequency hearing loss in the budgerigar (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.* 85: 289–294, 1989.
- HIGHSTEIN, S. M., GOLDBERG, J. M., MOSCHOVAKIS, A. K., AND FENANDEZ, C. Inputs from regularly and irregularly discharging vestibular nerve afferents to secondary neurons in the vestibular nuclei of the squirrel monkey. II. Correlation with output pathways of secondary neurons. *J. Neurophysiol.* 58: 719–738, 1987.
- JONES, T. A. AND NELSON, R. C. Recovery of vestibular function following hair cell destruction by streptomycin. *Hear. Res.* 62: 181–186, 1992.
- JORGENSEN, J. M. AND MATHIESEN, C. The avian inner ear. Continuous production of hair cells in vestibular sensory organs, but not in the auditory papilla. *Naturwissenschaften* 75: 319–320, 1988.
- LIPPE, W. R., WESTBROOK, E. W., AND RYALS, B. M. Hair cell regeneration in the chicken cochlea following aminoglycoside toxicity. *Hear. Res.* 56: 203–210, 1991.
- MAREAN, G. C., BURT, J. M., BEECHER, M. D., AND RUBEL, E. W. Hair cell regeneration in the European starling (*Sturnus vulgaris*): recovery of pure-tone detection thresholds. *Hear. Res.* 71: 126–136, 1993.
- McFADDEN, E. A. AND SAUNDERS, J. C. Recovery of auditory function following intense sound exposure in the neonatal chick. *Hear. Res.* 41: 205–215, 1989.
- MELVILL JONES, G. AND MILSUM, J. H. Spatial and dynamic aspects of visual fixation. *IEEE Trans. Biomed. Eng.* 12: 54–62, 1965.
- MINOR, L. B. AND GOLDBERG, J. M. Vestibular-nerve inputs to the vestibulo-ocular reflex: a functional-ablation study in the squirrel monkey. *J. Neurosci.* 11: 1636–1648, 1991.
- NIEMIEC, A. J., RAPHAEL, Y., AND MOODY, D. B. Return of auditory function following structural regeneration after acoustic trauma: behavioral measures from quail. *Hear. Res.* 75: 209–224, 1994.
- NORTON, S. J. AND RUBEL, E. W. Active and passive ADP components in mammalian and avian ears. In: *The Mechanics and Biophysics of Hearing*, edited by P. J. Dallos, C. D. Geisler, J. W. Matthews, M. A. Ruggero, and C. R. Steele. New York: Springer-Verlag, 1990, p. 219–226.
- RASBAND, W. *NIH Image Program*. Developed at the U.S. National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from the National Technical Information Service, Springfield, VA, part number PB95-500195GEI.
- ROBERSON, D. W., WEISLEDER, P., BOHRER, P. S., AND RUBEL, E. W. Ongoing production of sensory cells in the vestibular epithelium of the chick. *Hear. Res.* 57: 166–174, 1992.
- ROBINSON, D. A. A method of measuring eye movements using a scleral search coil in a magnetic field. *IEEE Trans. Biomed. Eng.* 10: 137–145, 1963.
- RYALS, B. M. AND RUBEL, E. W. Hair cell regeneration after acoustic trauma in adult Coturnix quail. *Science Wash. DC* 240: 1774–1776, 1988.
- RYALS, B. M. AND WESTBROOK, E. W. TEM analysis of neural terminals on autoradiographically identified regenerated hair cells. *Hear. Res.* 72: 81–88, 1994.
- RYALS, B. M., WESTBROOK, E. W., STOOFS, S., AND SPENCER, R. F. Changes in the acoustic nerve after hair cell regeneration. *Exp. Neurol.* 115: 18–22, 1992.
- SALVI, R. J., SAUNDERS, S. S., HASHINO, E., AND CHEN, L. Discharge patterns of chicken cochlear ganglion neurons following kanamycin-induced hair cell loss and regeneration. *J. Comp. Physiol.* 174: 351–369, 1994.
- SOBKOWICZ, H. M., AUGUST, B. K., AND SLAPNICK, S. M. Epithelial repair following mechanical injury of the developing organ of Corti in culture: an electron microscopic and autoradiographic study. *Exp. Neurol.* 115: 44–49, 1992.
- TUCCI, D. L. AND RUBEL, E. W. Physiologic status of regenerated hair cells in the avian inner ear following aminoglycoside ototoxicity. *Otolaryngol. Head Neck Surg.* 103: 443–450, 1990.
- WALLMAN, J., VELEZ, J., WEINSTEIN, B., AND GREEN, A. E. Avian vestibulo-ocular reflex: adaptive plasticity and developmental changes. *J. Neurophysiol.* 48: 952–967, 1982.
- WEISLEDER, P. AND RUBEL, E. W. Hair cell regeneration after streptomycin toxicity in the avian vestibular epithelium. *J. Comp. Neurol.* 331: 97–110, 1993.
- WERSÄLL, J. Studies on the structure and innervation of the sensory epithelium of the cristae ampullaris in the guinea pig. A light and electron microscopic investigation. *Acta Otolaryngol. Suppl.* 126: 1–85, 1956.
- WERSÄLL, J., BJÖRCKROTH, B., FLOCK, Å., AND LUNDQUIST, P. Sensory hair fusion in vestibular sensory cells after gentamycin exposure: a transmission and scanning electron microscope study. *Arch. Klin. Exp. Ohren. Nasen. Kehlkopfheilkde.* 200: 1–14, 1971.
- WERSÄLL, J. AND HAWKINS, J. E. The vestibular sensory epithelia in the cat labyrinth and their reaction in chronic streptomycin intoxication. *Acta Otolaryngol.* 54: 1–23, 1962.