The Role of Hair Cell Regeneration in an Avian Model of Inner Ear Injury and Repair from Acoustic Trauma

Abstract

The auditory system of young chicks (Gallus domesticus) is an important model for studying the structure and function of the inner ear. For over 20 years this model has gained interest because of the discovery that birds, and perhaps lower vertebrates in general, are capable of generating new hair cells to replace those lost to ototrauma, a capacity that is absent along the mammalian organ of Corti. Accompanying this remarkable capacity is the nearly complete restoration of auditory function to the chick peripheral ear. This article presents a review of findings on the toxic effect of exposure to extremely loud sound on the young chick ear, and the subsequent recovery from inner ear structural damage and accompanying recovery of auditory function. The evidence, surprisingly, suggests that the role of the regenerated hair cells in the latter may be minimal and that multiple other factors play more important roles. There is also a section on the unique problems encountered in using chicks as laboratory animal subjects in experiments designed to understand the consequences of acoustic trauma. The conclusion summarizes some of the issues that need to be addressed in future research.

Key Words: acoustic trauma; avian hearing; chick auditory system; cochlear nerve; Gallus domesticus; hair cell regeneration; inner ear structure and function

Introduction

Damage to the inner ear from exposure to intense sound is a major cause of sensorineural hearing loss. The most common pathology resulting from acoustic trauma is damage or destruction of hair cells on the sensory organ for hearing. This organ, called the organ of Corti in mammals, is in the cochlear portion of the inner ear labyrinth, which is located in the hardest bone of the body, the temporal bone, and is difficult to access in mammalian species. Postexposure hearing loss may be either temporary or permanent depending on whether the tissues of the inner ear are injured or destroyed. It is well established in mammals that once hair cells are destroyed they are incapable of being replaced, and whatever functional capacity they carried for hearing is lost with them (Davis et al. 1989).

Experimental studies of acoustic trauma are obviously limited in human listeners, so researchers have sought an ideal laboratory animal to investigate the phenomenon. Two factors have driven this search: (1) the need to identify an animal model whose auditory system mimics that of humans, and (2) the need to control subject-to-subject variability in the degree of acoustic injury. This variability, inherent in all mammalian species thus far tested, often requires the replication of experimental treatments in many subjects to achieve a statistically reliable characterization of the otoacoustic injury.

Nonhuman primate, canine, feline, rodent, and avian species have served as laboratory animals in these studies. Each presents a challenge in how well it models human hearing, the ease with which structure and function can be related, variability in the response to acoustic trauma, and the cost effectiveness of subject acquisition and maintenance. Nevertheless, all have contributed greatly to knowledge of the processes associated with acoustic trauma.

The utility of the chick model for auditory research became increasingly apparent as investigators recognized the ease of access to the chick’s inner ear. Although the morphology of the basilar papilla is different from the organ of Corti, the underlying physiological processes are nearly identical, and so the chick has become an important model for auditory research.

Throughout the 1970s a growing literature described many aspects of the avian auditory system, particularly at the level of the developing inner ear, responses of the auditory nerve, and avian auditory perception (e.g., Dooling and Saunders 1973; Rubel 1978; Sachs et al. 1974; Saunders et al. 1973; Saunders and Dooling 1974). Then a proliferation of papers in the 1980s reported on many biological aspects of the avian middle and inner ears (see summaries in Manley 1990; Saunders and Henry 1988).

Until the late 1980s it was doctrinaire belief that hair cells destroyed by genetic processes, developmental anomalies, viral or bacterial insult, vascular injury, ototoxic drug exposure, or acoustic overstimulation were not replaced. This point of view unraveled with the reports of hair cell regeneration on the chick basilar papilla. These reports described the relationship between intense pure tone sound...
exposure (0.9 kilohertz [kHz], 120 decibel [dB] sound pressure level [SPL]) for 48 hours) and the destruction and regeneration of hair cells in the inner ear of young chicks (Cotanche 1987a; Cotanche et al. 1986). Subsequent studies have shown that hair cell regeneration after acoustic trauma also occurs in quail, pigeons, and various songbirds (Dooling et al. 2008; Ryals and Rubel 1988; Smolders 1999).

In this article I discuss the important role of the avian peripheral ear in the study of acoustic trauma.

**Organization of the Avian Peripheral Ear**

It may be useful to review the main features of the peripheral auditory system of birds (for a detailed description see Dooling et al. 2000). The tympanic membrane, located at the end of a short ear canal, absorbs sound waves and transmits sound vibrations to the inner ear fluids through a single ossicle (the columella). A middle-ear muscle tugs on the tympanic membrane and, when this muscle is activated, attenuates the sound conducted to the labyrinth (Kühne and Lewis 1985). As in mammals, the bird middle ear is an impedance matching device that efficiently couples the energy in airborne sound to the fluid chambers of the labyrinth (Saunders et al. 1996a, b). The traveling wave produces a frequency-to-place translation in the location of maximum BM movement, and this forms the basis of frequency analysis. The THCs lie in an epithelial layer of the basilar papilla above the rather massive superior fibrocartilaginous plate (Figure 2). As far as is known, this epithelium and the plate do not move with sound stimulation (Saunders et al. 1996a; Saunders and Salvi 2008). There has been speculation about the mechanisms that stimulate THC hair bundles (Smolders et al. 1995; Saunders et al. 1996a, b); one possibility is that the up-down movements of the BM cause a radial stretching and relaxation of the tectorial membrane that mechanically stimulus the THC hair bundles (arrows in Figure 2B).

The cochlear nerve fibers pass along the underside of the papilla and project through the fibrocartilaginous plate to the cochlear ganglion (GC in Figure 2A). The THCs and SHCs are often likened to mammalian inner and outer hair cells, but, unlike the latter, a contractile mechanism in SHCs has yet to be shown (for summary see Saunders and Salvi 2008). The contractile property of outer hair cells is an essential mechanism in the exquisite frequency-resolving power of the mammalian ear. There is speculation that another cellular mechanism in avians may similarly serve to enhance frequency resolution. Hyaline cells (designated H in Figure 2A) at the inferior edge of the basilar membrane are innervated by efferent fibers (Osfie and Cotanche 1996; Zidanic 2002). The presence of contractile proteins in hyaline cells (Cotanche et al. 1992) raises the possibility that the up-down movements of the BM could lead to the contraction of these cells, pulling on the basilar membrane and thus altering its mechanical properties. Such an alteration might enhance the sharpness of tuning on the BM (see below), but this effect remains to be demonstrated.

Hair bundle displacement arises from the mechanics of the papilla (Figure 2B) and leads to the gating of transduction channels through tip links (TLs) that connect the tip of a shorter hair to the shaft of an adjacent taller hair (Kachar et al. 2000). Movement in the “excitatory” direction stretches the TLs and gates the channel open. The endolymphatic potential forces K⁺ ions into the open channel, depolarizing the hair cell membrane. Bundle movement in the opposite

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1Abbreviations used in this article: BM, basilar membrane; CF, characteristic frequency; EP, endocochlear potential; K⁺, potassium ions; Q₁₀dB, sharpness of tuning 10 dB above the CF threshold; SHC, short hair cell; SPL, sound pressure level; THC, tall hair cell; TL, tip link; TS, threshold shift

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inhbitory) direction closes the channel and, with membrane pumps removing K⁺ from the hair cell, the membrane hyperpolarizes. Membrane depolarization opens voltage-gated calcium ion (Ca++) channels in the basolateral region of the hair cell, and calcium influx through these channels releases neurotransmitter-containing vesicles tethered to synaptic ribbons (Spassova et al. 2001, 2004). Vesicle exocytosis secretes neurotransmitter (glutamate) into the synaptic cleft, where it diffuses across the cleft and binds with postsynaptic receptor sites in the cochlear nerve terminal, initiating neural discharges.

These processes are connected. For example, the louder the stimulus the greater the hair bundle displacement and the larger the number of transduction channels opened. A larger membrane depolarization results in more Ca++ entering the hair cell, greater vesicle exocytosis, and a higher discharge rate in the cochlear nerve fiber.

Acoustic Trauma and Recovery in the Chick Ear

In a 1974 study I presented evidence of noise-induced hearing loss in parakeets (Saunders and Dooling 1974) and reported the growth and recovery of behaviorally measured threshold shifts (TS). In the early 1980s tone burst–evoked responses were used to assess chick TS and recovery after exposure to intense narrow-band noise (Saunders and Tilney 1982), and in a subsequent study embryos and chicks were exposed to intense pure tones (Rubel and Ryals 1983). Because of the tonotopic organization of frequency along the basilar papilla, these pure tones damaged specific locations on the sensory surface, and an examination of these locations revealed that they shifted when older animals were similarly exposed. The use of acoustic injury on the basilar papilla thus demonstrated a developmental change in tonotopic organization.

The precursor study to the discovery of hair cell regeneration was a parametric investigation of chick acoustic trauma using groups of animals based on a matrix of posthatching age, pure tone exposure frequency, and exposure intensities (all exposures were 48 hours in duration) (Cotanche et al. 1987). Two consistent regions of papilla damage were identified: a “patch” lesion, at the tonotopic location of the exposure frequency, and a stripe lesion on the high-frequency (proximal) side of the patch (Figure 3A). The stripe lesion is poorly understood, but both areas of damage exhibited missing hair cells, and “wedges” in the patch.
indicated areas of missing SHCs (Figure 3B). The tectorial membrane over the patch was also destroyed (Figure 3B,C).

The scanning electron micrograph in Figure 4A shows the normal hair cell mosaic on the papilla surface. The same area shortly after removal from a 120 dB exposure for 48 hours appears in panel B; this picture of a wedge shows missing SHCs—about 32% of them were destroyed (Henry et al. 1988)—and enlarged surface areas of the supporting cells as they fill the void left by the lost hair cells. Panel D shows the same approximate papilla location 14 days later; the hair cell field was reconstituted but did not regain the organization evident in panel A (panel C is discussed below.)

Hair cells located outside the wedges, but within the patch lesion, on cursory examination appeared normal. However, at higher magnification, these surviving hair bundles exhibited abnormal features that suggested a loss in stiffness (Erulkar et al. 1996). Moreover, many TLs on surviving SHC hair bundles in the patch and on THCs immediately superior to the patch were broken (Kurian et al. 2003). It is widely understood that the loss of stereocilia stiffness and TLs compromises hair cell transduction.

The tegmentum vasculosum, whose dark cells secrete K⁺ into the scala media, was also damaged by overstimulation (Ryals et al. 1995), but a subsequent study reported the rapid recovery of these dark cells (Ramakrishna et al. 2004). Tegmental injury and repair may be related to the loss and recovery of the endocochlear potential (further discussed in the section below on Recovery of Auditory Function).

Cessation of the exposure triggered papilla repair almost immediately. The earliest structural repair appeared to be in the hair bundle TLs; Figure 5 shows the time course of TL loss and restoration (Kurian et al. 2003). Tip link breakage
from excessive hair bundle movements grew to a maximum (42% loss) during the first 24 hours of exposure, began to reverse during the next 24 hours of exposure, and by the time the sound was turned off only 33% of the links in the bundle were severed. The observation that TLs underwent repair during the exposure was unexpected, but eventually attributed to the destroyed tectorial membrane. This membrane, in the patch lesion, is completely removed after 24 hours of exposure (see Figure 3B). When it was intact, the membrane served as the mechanism by which the hair bundles were stimulated; when it was destroyed there was no longer a mechanical driving force capable of displacing them. With no tectorial membrane over the patch (see Figure 3C), the SHCs simply moved up and down with no lateral stress on the TLs and this permitted TL recovery to begin. After the exposure was discontinued the TL recovery continued and within 24 hours there was only a 19% loss. By 288 hours (12 days) after exposure TL recovery was complete (see Figure 5).

Recovery of the tectorial membrane honeycomb layer was also rapid (Cotanche 1987b). Figure 6A illustrates a papilla surface 12 days after exposure, and the repaired honeycomb in the lesion is apparent. Figure 6B, a cross-section drawing of the papilla, depicts the repaired lower layer of the tectorial membrane over the SHC field. This repair may restore the radial stretching to the tectorial membrane and reintroduce mechanical stimulation of the THC hair bundles. The damage to the upper fibrous layer was permanent.

Figure 4 Scanning electron micrographs of the chick (Gallus domesticus) basilar papilla surface. (A) Normal appearance of short hair cells (SHCs) on the papilla surface in the equivalent area of the patch in a 3-day-old chick. (B) Missing hair cells in a wedge zero days after exposure (3 days old). (C) Wedge area 5 days postexposure. New hair cells have short hair bundles and small surface areas; surviving hair cells are identifiable by their large surface areas. (D) Patch area 14 days postexposure. Regenerated hair cells are those with a smaller surface area. In each panel the calibration bar is 10 µm. Figure modified from Henry et al. (1988).

Figure 5 Loss of tip links during chicks’ exposure to 120 dB tone (between 0 and 48 hours) and recovery of tip links after cessation of the tone (between 0 and 300 hours of postexposure recovery). The two curves for short (SHCs) and tall hair cells (THCs) were normalized to the percent of broken tip links on control hair cells. A normal hair bundle has less than 100% of tip links, most likely because of preparation artifact. Figure modified from Kurian et al. (2003).

Figure 6 Papilla lesion 12 days after exposure. (A) Scanning electron micrograph of the patch lesion after 12 days of recovery. The honeycomb layer of the tectorial membrane (TM) has returned over the short hair cell (SHC) field. The TM is incompletely healed and the absence of the upper layer over the lesion is a permanent defect. (B) Cross-section drawing of the papilla in the region of the patch lesion. The repaired honeycomb over the SHCs may reconnect the basilar membrane (BM) movements (vertical arrow) to the tectorial membrane. This vertical movement may translate into lateral motion of the TM over the tall hair cells (THC; horizontal arrow), which could lead to the restoration of THC stereocilia stimulation. The repair of the SHC region of the basilar papilla in terms of the regenerated hair cells and honeycomb layer of the TM contrasts with the same area immediately after the exposure in Figure 3.
The loss of SHCs in the patch inevitably meant that the neuronal connections with these cells were broken (various aspects of neuron degeneration and synaptic restoration have been considered elsewhere; Saunders and Salvi 2008). Synaptic connections were restored to regenerating SHCs, but lagged behind the first appearance of the new hair cells (Ryals and Dooling 1996; Wang and Raphael 1996). Additionally, the initial SHC deafferentation in the patch had ramifications for auditory nuclei in the deeper central nervous system (CNS), and there was evidence of postexposure cellular changes in the nucleus magnocellularis (Saunders et al. 1998).

**Chick Hair Cell Regeneration**

As explained above, since the late 1980s studies have documented hair cell regeneration in chicks. For example, a scanning electron micrograph taken 5 days after exposure shows that the large hair cells, with an expanded surface area, were those that survived the exposure (Figure 4C) and that they contrasted greatly with the newly emerging hair cells, which were identified by their small hair bundle tufts and apical surface area 3 to 4 days after exposure (Cotanche 1987a; Henry et al. 1988). The appearance of new hair cells was complete 4 or 5 days after exposure, but there were still 10% fewer hair cells than in the original complement (Marsh et al. 1990). Yet despite the reduced number of SHCs in the lesion and the incomplete healing of the tectorial membrane, all other aspects of basilar papilla recovery were resolved by 12 days after exposure (Figure 4D); the new SHC hair bundles were now much longer, and the surviving hair cells were again identifiable by their larger surface area.

There has been much effort to identify the proliferating source of the regenerated hair cells. Mitotic marking, using the nuclear label bromodeoxyuridine (BrdU), has revealed cell divisions of supporting cells in the patch as the source of new hair cells (Corwin and Cotanche 1988; Raphael 1992; Ryals and Rubel 1988). This mitotic division resulted in two new “daughter” cells, one of which typically matured to a hair cell and the other to a supporting cell. It is important to recognize that this cellular proliferation was specific to the damaged papilla; age-matched control papilla were mitotically quiescent.

The mechanisms that trigger the supporting cell to reenter the cell cycle are not well understood, but were considered recently in detail (Meyers and Corwin 2008). Mitotic activity in damaged ears was largely localized to regions of lost hair cells. Hair cells express membrane proteins (cadherins) that communicate with surrounding cells and may suppress mature supporting cells from reentering the cell cycle. When hair cells are destroyed these contact molecules are broken, and this may reinitiate support cell proliferation (Meyers and Corwin 2008). Furthermore, new hair cells are sometimes seen slightly outside the lesion area, where no damage was observed, suggesting that a different molecular process may play a trigger role, perhaps in the form of some soluble factor. This and other information on the molecular aspects of hair cell regeneration is detailed elsewhere (Oesterle and Stone 2008).

**Recovery of Auditory Function**

In my laboratory we used tone burst–evoked field potentials, recorded from electrodes placed directly into the chick cochlear nuclei, to measure the sound pressure level at which a just-detectable threshold level of evoked activity was visible. The TS was measured as the threshold difference between age-matched nonexposed control and zero-day or 12-day postexposure animals. With zero days of recovery the TS was smallest, at 0.1 and 4.5 kHz, but grew to a maximum of 59 dB at 1.3 kHz. Figure 7 shows that substantial recovery occurred by 12 days postexposure. However, even with 12 days of recovery there remained a 4 to 5 dB permanent TS at 1.7 kHz (Saunders et al. 1996a).

The growth in TS during exposure was also examined with evoked response recordings (left panel of Figure 8). Growth of TS was derived from evoked response thresholds measured in chicks that experienced the 120 dB exposure for different durations as short as 1 hour or as long as 200 hours. The TS grew systematically with two plateaus: the first occurred around 25 dB after 5 hours of exposure, the second at 50 dB after 40 hours of exposure. The TS then remained constant with exposure durations longer than 40 hours (Saunders et al. 1993).

The observation of asymptotic threshold shift (ATS) after 40 hours is important. Although the underlying basis of ATS is not fully understood, it represents a balance between the destructive force of loud sound and the ability of the sensory epithelium to resist further damage. The results also show that TS variance was greatest in the growth phase and
The smallest at ATS levels. The variability in TS leading up to ATS represented the inner ear in an unstable state.

The two plots in the right-hand panel of Figure 8 show evoked response TS recovery after a 48-hour (McFadden and Saunders 1989) and after a 200-hour exposure (Pugliano et al. 1993b); the standard error of the mean data are reported only for the 48-hour exposure but were much the same for the 200-hour data. Both exposure durations show the same recovery profile. Using the maximum dB TS as a reference, 44% of threshold loss recovered within 24 hours after exposure, while at 48 and 120 hours the TS diminished by 66% and 90%, respectively. Figure 8 also shows a small 4 to 5 dB permanent TS 380 hours (16 days) after the exposure. This permanent loss may relate to the incomplete replacement of new hair cells or the failure of the tectorial membrane to fully recover. Other studies that have used evoked responses to explore aspects of acoustic trauma in chicks have also described the complete recovery of the measured parameter 12 days after exposure (Adler et al. 1993; Pugliano et al. 1993a).

A noninvasive index of basilar papilla health is the distortion product otoacoustic emission (DPOAE). The DPOAE reflects nonlinear mechanisms in the papilla, and intense sound compromises those mechanisms. This response, measured as an acoustic signal in the ear canal, was examined before and after exposure to the 120 dB pure tone. With the passage of postexposure time it also recovered (Ipakchi et al. 2005).

Postexposure changes in the endocochlear potential have also been the subject of study (Poje et al. 1995). Using control EP values as the reference, a 63% reduction was identified in exposed chicks at zero days of recovery. Rapid recovery ensued: by 3 days after exposure the EP was only 7% below normal and by 4 days recovery was complete. Because the EP, as noted above, is important to hair cell transduction, the restoration of this potential was deemed a significant contributor to the recovery of auditory function (Saunders et al. 1996a).

Another gauge of recovery involves in vitro examination of chick hair bundle micromechanics after overstimulation. Segments of the sensory epithelium were cut from the intact basilar papilla and maintained in an in vitro culture medium. The hair bundles were observed microscopically and, with a microwaterjet stimulus to “blow” them back and forth on individual hair cells, the amount of stimulation (in relative dB) necessary to just detect bundle movement was noted (the threshold was a displacement of ~30 µm peak to peak). The bundle was then overstimulated with a waterjet blast 20 to 25 dB more intense than the threshold (resulting in ~300 to 500 µm peak-to-peak displacement). Measurements of postexposure threshold levels over time revealed that it took fewer dB of waterjet stimulation to elicit the threshold level of movements, indicating that overstimulation reduced bundle stiffness. Within 15 to 20 minutes the TS recovered to preexposure levels (Szymko et al. 1995). Another in vitro experiment attributed the stiffness loss to broken TLs (Duncan and Saunders 2000). Other studies have reported the

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**Figure 8** Growth (left panel) and recovery (right panel) of evoked response thresholds. The two recovery curves were obtained after 48- and 200-hour exposures. The threshold shift data were averaged over three test frequencies: 0.7, 0.9, and 1.2 kilohertz (kHz). DB, decibel; TS, threshold shift. Plots summarized from Saunders et al. (1993), Saunders (1996a).
capacity of the stereocilia to regain stiffness by repairing their internal protein structure or to restore transduction capacity by repairing TLs (Schneider et al. 2002; Zhao et al. 1996).

Using a different strategy in 1996 I began recording from single units in the cochlear ganglion region of the chick cochlear nerve (see electrode tip location in Figure 2A), an approach that permitted greater specificity in describing the effects of acoustic trauma from different tonotopic locations along the papilla. Figure 9 shows tuning curves from three different cochlear nerve units, each with approximately the same characteristic frequency (CF). The CF threshold is the frequency at which the lowest SPL elicits a just-detectable sound-driven neuron discharge; tuning curves were determined in all units and provided insight into how well the ear could decompose complex sounds into separate spectral components (Saunders et al. 1996b for a description of how these tuning curves were obtained).

Figure 9A shows a control tuning curve in which the lowest sound-driven activity occurred at 26 dB SPL at 1.15 kHz. The sharpness of the tuning curve is an indication of its frequency selectivity and is described by the quality factor (Q; the sharpness of a filter) ratio (CF in Hz/bandwidth in Hz 10 dB above the CF threshold, or Q_{10dB}). The higher the value of Q, the more frequency selective the tuning curve, and since Q is a ratio it is possible to compare selectivity across CFs. The Q_{10dB} of the control example was 6.1. Panel B shows a tuning curve recorded from a cochlear nerve unit a few hours after removal from the exposure: the CF threshold was elevated to 79 dB SPL and had poorer selectivity (Q_{10dB} was 2.2). Panel C illustrates a unit recorded 12 days after exposure, when the CF threshold and Q_{10dB} (27.5 dB and 6.4 respectively) were the same as in the control unit, indicating considerable recovery.

Figure 10 presents results from a large sample of units. The three columns show control, zero-day, and 12-day-recovered observations, and the rows depict the CF threshold (dB SPL), the sharpness of tuning (Q_{10dB}), and unit spontaneous activity (SA in spikes per second). These parameters were clearly severely compromised in the zero-day recovery group (for details, Saunders and Salvi 2008), but the right-hand column of panels indicates that the recovery of cochlear nerve single-unit activity was complete 12 days after exposure.

Figure 11 presents rate-level (RL) functions in age-matched control (white circles) and 12-day-postexposure animals (black circles) averaged over five to eight units, each with a relatively similar CF and CF threshold (Plontke et al. 1999). These curves depict the growth of spike activity with increasing stimulus level. The RL curves at the lowest and highest CFs (panels A and C) were the same, indicating complete recovery. However, in units with CFs near 0.9 kHz, the discharge rate at 100 dB in exposed units was 300 spikes/sec compared to 250 spikes/sec in the control units. These results indicate that the coding of stimulus intensity remained abnormal 12 days after exposure for unit CFs near or at the exposure frequency, despite the fact that CF thresholds, Q_{10dB}, and spontaneous activity all returned to normal.

Other studies have reported measures of cochlear nerve single unit activity (Saunders and Salvi 2008) and described spatial tuning curves, neural adaptation, and phase locking.
Issues Related to the Use of Chicks as Experimental Subjects

There are several specific concerns that must be addressed when chicks serve as animal subjects in studies that use intense sound exposures. Protocol approval from the institutional animal care and use committee needs to justify the duration the animals are away from their home quarters (48 hours) and resolve the potential for pain and suffering from the intense sound exposure.

Removal from the home quarters for 48 hours arose from the need for an exposure duration of that length. This exposure duration was experimentally shown in Figure 8 as the amount of time needed to achieve the stability of asymptotic threshold shift (see text above). Permission to keep animals in the chamber for 48 hours depended on an exposure environment that allowed movement and provided the chicks with adequate food and water as well as proper heating, humidification, and air circulation. The chamber was inspected periodically during the exposure and each of these parameters recorded. The general condition of the chicks was also ascertained, and records of each inspection were kept.

The potential for pain and suffering was addressed in several ways, and included justification for the 120 dB exposure. The relatively low damage to the chick inner ear as a consequence of this exposure level was surprising, and initially suggested that the chick inner ear was resistant to acoustic injury. This proved not to be the case. Subsequent research revealed that the middle ear conductive system was immature at the time of hatching (Saunders et al. 2000), thus reducing the effective stimulus reaching the inner ear by 12 to 16 dB from that measured at the head. Accordingly, a louder sound had to be applied to the head to overcome the losses in middle-ear sound transmission.

Figure 12 demonstrates this point. Each drawing indicates the patch size following a 48-hour exposure, where the parameters are animal age and exposure intensity. The top drawing shows the typical lesion in 3-day-old animals after a 120 dB blast, and the middle shows the same exposure in roughly 70-day-old chickens. The mature conductive system transferred sound energy to the inner ear more effectively and the resulting lesion was substantially larger. A lesion similar in size to that at 3 days of age occurred when the exposure in the older animals was reduced to 105 dB, indicating that sound conduction in the older animals was 15 dB more effective, the same approximate amount by which the developing middle ear improved (Cohen et al. 1992; Saunders et al. 1986).

While a 120 dB stimulus in human listeners is uncomfortable, it probably does not give rise to pain sensations as the pain threshold for hearing in humans has been reported at 140 dB (von Békésy 1960). Furthermore, descriptions of the mammalian labyrinth over the last hundred years have yet to identify any pain receptors in its various tissue compartments. However, pain receptors (nociceptors) with fibers entering the trigeminal pain system do originate from the middle layer of the tympanic membrane. These nociceptors are mechanically activated by large membrane displacements, as might occur from outward bulging of the tympanic membrane during middle-ear infection. A 140 dB SPL acoustic stimulus also produces tympanic membrane displacements sufficient to activate these receptors. The 120 dB exposure, however, represented a pressure 10 times less than that at 140 dB, well below what might stimulate pain receptors.

The dilemma is whether the chick tympanic membrane has nociceptors, and if it does, is the pain threshold the same
Curves in Figure 8 showed a significant reduction in TS after exposure, the evoked response threshold recovery. Hair cells were first seen on the sensory surface 3 to 4 days after exposure (Köppl 2010). The rate of functional recovery and the emergence of hair cells destroyed by the exposure, and the 10% reduction in hair cell numbers in the patch lesion appeared to persist. Moreover, the upper layer of the tectorial membrane never recovered.

For the human ear, however, the 120 dB exposure is a powerful and dangerous stimulus that can produce hair cell “wipe-out” over one-third of the receptor organ within 10 to 15 minutes. Laboratory personnel must therefore take care to avoid exposure to this sound. In our studies the sound is always turned off before the door is opened for a chamber inspection. On the rare occasions when sound calibration was undertaken and a microphone had to be positioned in an active sound field, staff wore acoustically shielded earmuffs that provided adequate protection.

**Conclusions**

The avian acoustic trauma and hair cell regeneration literature yields some important overarching observations. In my laboratory we noted that the structures of the basilar papilla underwent a remarkable postexposure recovery, even to the extent that cochlear nerve fibers reconnected to the new hair cells and successfully sent afferent information into the auditory CNS. As remarkable as this recovery was, it did not restore the basilar papilla to its preexposure condition. The process of hair cell regeneration replaced 90% of the hair cells destroyed by the exposure, and the 10% reduction in hair cell numbers in the patch lesion appeared to persist. Moreover, the upper layer of the tectorial membrane never recovered.

The structural repair of the chick inner ear, despite the two exceptions noted above, resulted in almost all aspects of auditory function—the endocochlear potential, otoacoustic emissions, sound-evoked brainstem activity, and cochlear nerve single-unit activity all returned to normal capacity. The only exception yet identified was the abnormal rate-level functions at certain frequencies in 12-day-recovered units (Figure 11).

The most interesting aspect of these studies is the relation between the rate of functional recovery and the emergence of regenerated hair cells. Bearing in mind that new hair cells were first seen on the sensory surface 3 to 4 days after exposure, the evoked response threshold recovery curves in Figure 8 showed a significant reduction in TS within 4 days (96 hours). However, the time of first emergence does not mean that the new hair cells were functionally mature. It may take an additional day or so before they are working normally, and perhaps even longer before afferent fibers reconnect to the hair cells (Wang and Raphael 1996). The length of time for new hair cells to emerge and become functional stands in stark contrast with the results in Figure 8 that show substantial reduction in TS within the first 4 days after exposure. These data suggest that the new hair cells contribute minimally, if at all, to the recovery of auditory function. This is a controversial conclusion, but if correct it indicates that functional restoration is far more complicated than the reemergence of hair cells.

What then can account for the functional recovery? Most likely a combination of factors: restoration of the EP with tegumentum repair, restored transduction with replacement of tip links, rapid replacement of the lower-layer tectorial membrane (the honeycomb) with the restoration of mechanical input to the THCs, and the fact that the THCs come through the exposure largely unscathed. These factors dynamically interact with each other to restore peripheral capability. Furthermore, there is redundancy in the sensory epithelium of the avian auditory receptor organ. Repair or reactivation of the surviving hair cells may be sufficient to restore auditory function, and if so the arrival of new hair cells contributes little to an already redundant system.

Acoustic trauma research suffers from the fact that it is hard to unambiguously identify cause and effect—only correlations can be drawn between measurable tissue damage and repair and the loss and recovery of auditory function. The avian model of acoustic trauma, however, has provided perhaps the most compelling information about structure/function relationships—compelling to the point that they begin to look like cause and effect conditions. The comparative species approach (Köppl 2010), contrasting auditory processes in birds with those in mammals, offers the possibility of gaining new and useful insight to the process of acoustic trauma in both species.

**Acknowledgments**

Support for this research has been provided over the years by the National Organization for Hearing Research Foundation, the Pennsylvania Lions Hearing Research Foundation, and the National Institute on Deafness and Other Communication Disorders. The research efforts of many students over the years are gratefully acknowledged.

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