Hair cell regeneration: winging our way towards a sound future

Olivia Bermingham-McDonogh* and Edwin W Rubel†

The discovery of hair cell regeneration in the inner ear of birds provides new optimism that there may be a treatment for hearing and balance disorders. In this review we describe the process of hair cell regeneration in birds; including restoration of function, recovery of perception and what is currently known about molecular events, such as growth factors and signalling systems. We examine some of the key recent findings in both birds and mammals.

Addresses
Virginia Merrill Bloedel Hearing Research Center and Department of Otolaryngology-HNS, University of Washington Medical School, Box 357923, Seattle, Washington 98195-7923, USA
*e-mail: oliviab@u.washington.edu
†e-mail: rubel@u.washington.edu

Current Opinion in Neurobiology 2003, **13**:119–126

This review comes from a themed issue on Development
Edited by Magdalena Götz and Samuel L Pfaff

0959-4388/03/$ – see front matter

DOI 10.1016/S0959-4388(03)00018-7

Abbreviations

FGFR3	fibroblast growth factor receptor 3
Hes	hairy and enhancer of split
MAPK	mitogen-activated protein kinase
Math1	mammalian atonal homolog 1
TGFα	transforming growth factor α
VOR	vestibulo-ocular reflex

Introduction

The discovery of hair cell regeneration in the inner ear of birds following exposure to ototoxic drugs or intense acoustic stimulation forever changed our views on how hearing and balance disorders may someday be treated (Figure 1). The majority of disorders causing permanent hearing impairment and many balance disorders are thought to be due to degeneration of the hair cells — the mechanosensory receptor cells — of the inner ear sensory epithelia. In mammals, when these cells are lost due to genetic mutation, disease, exposure to environmental toxins or aging, hearing and/or vestibular impairments are permanent. In 1987–88, five seminal papers [1–5] on hair cell regeneration were published, establishing: first, that experimental destruction of hair cells in the mature avian cochlea (basilar papilla) stimulates the proliferation of support cells and new hair cell production; second, in the undamaged avian cochlea, there is virtually no mitotic activity; third, in the mature avian vestibular epithelium, there is a low rate of cell cycle activity and ongoing production of new hair cells. These papers stimulated a new wave of research, the goals of which were, and still are: first, to understand hair cell regeneration in the inner ear of birds and other non-mammalian vertebrates; second, to examine the functional capabilities of the inner ear and neural pathways following regeneration; third, to stimulate replacement of lost or injured hair cells in the inner ear of mammals. In this review, we comment on recent progress in each of these areas.

Restoration of auditory and vestibular function

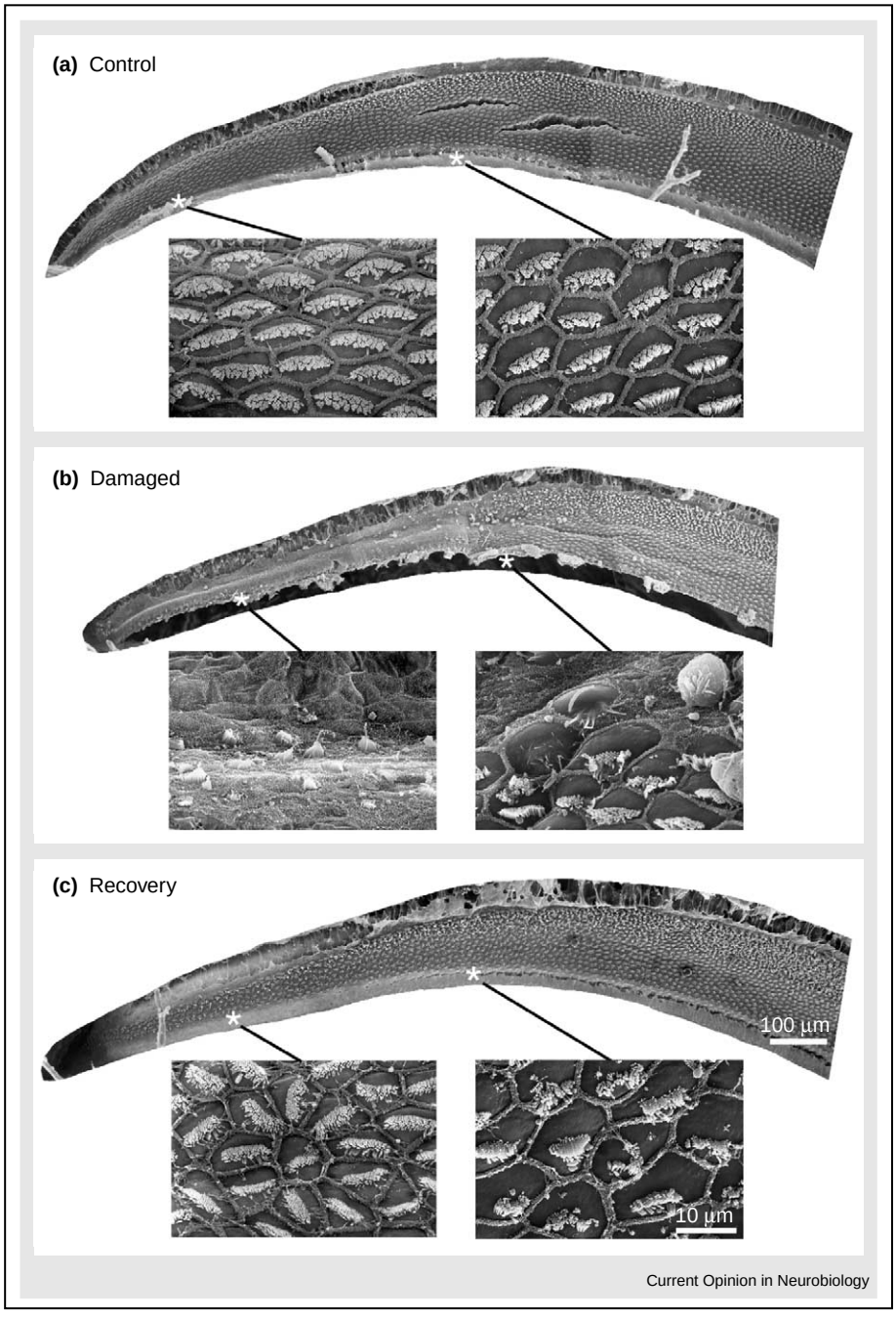
Once regeneration of hair cells in the inner ear of birds was confirmed, the question that immediately arose was: do the regenerated cells restore hearing and balance? To do this, new hair cells must develop appropriate ion channels for transduction and be re-innervated by the VIIIth nerve fibers that make appropriate connections in the CNS. Additionally, the animal must be able to access this information to make behaviorally meaningful responses. A thorough review of the literature up until 1999 on the recovery of auditory processing is available [6]. Table 1 categorizes this literature and provides additional references.

Recovery of sensory function: new cells or old cells with new life?

The earliest attempts to determine whether regenerated hair cells restore auditory function produced equivocal results. Studies from several laboratories showed recovery of electrical responses in the brain in response to acoustic stimulation and recovery of behavior following noise damage or aminoglycoside toxicity [7–10]. The problem was one of interpretation: is recovery of function mediated by the newly produced hair cells, or by recovery of hair cells and their associated structures that were injured, but not fatally so, by the intervention? When the time course of functional recovery precedes maturation of the new hair cells, interpretation leans toward recovery of damaged cells [11–14]. When functional recovery corresponds with the time course of new hair cell differentiation, or with the particular functional attributes of an area in which all the original hair cells were destroyed, interpretation favors regenerated hair cells as the transducers. It appears that both processes can mediate recovery under different circumstances.

Direct evidence for recovery mediated by the regenerated hair cells requires that all of the hair cells responsive to a particular sound attribute are destroyed, leading to a failure of the response (either physiological or

Figure 1



Scanning electron micrographs showing hair cell regeneration in the starling basilar papilla (BP) after aminoglycoside treatment. Each panel shows a low power scanning electron microscope image of the basal half of the BP, and two high power photomicrographs from the positions indicated. **(a)** shows the control untreated case; **(b)** shows a BP after drug treatment; and **(c)** shows a BP 20 weeks after drug treatment. Note that immediately after aminoglycoside treatment, almost all hair cells disappear from the basal end; apically, there is also a significant loss of hair cells. After 142 days (c), a normal complement of hair cells returns, but the mosaic is somewhat abnormal. The asterisks indicate the approximate location along the BP of the high power images shown below. These images came from the work of Marean *et al.* [18••].

behavioral), and that this is followed by recovery of the response with a time course consistent with the production and differentiation of new hair cells. This scenario was partially achieved by some early studies using recordings of responses to pure tone stimuli [9,15], and has been more definitively shown by several studies examining the responses of single VIIIth nerve axons [6,16,17••]. Formally, however, one could still argue that the response

Table 1

Studies evaluating the functional properties of regenerated hair cells

Level of analysis	Dependent variable	Type of damage	Recovery period studied	References
Inner ear	Ototacoustic emissions	Intense sound exposure Aminoglycosides	1 wk - 8 wks 1 wk - 22 wks+	[52–54]
	Endocochlear potential	Aminoglycosides Intense sound exposure	1 day - 4 wks 0 day - 12 day	[55–57]
	Vestibular hair cell physiology	Aminoglycosides		[58,59]
Eighth nerve	Compound evoked responses	Intense sound exposure Aminoglycosides	0 days - 30 days 2 days - 20 weeks	[7,60–68] [9,10,69–72]
	Single unit responses	Intense sound exposure Aminoglycosides	0 days - 4 mo 2 days - 20 wks	[66,73–77] [6,14,16,17••,21]
CNS	Single unit responses	Intense sound exposure	0 days & 12 days	[11,12]
	Metabolic influences	Intense sound exposure Aminoglycosides	2 days - 43 days	[78–81]
Behavioral studies	Basic psychoacoustics	Intense sound exposure Aminoglycosides	1 day - 4 mo	[8,18••,23,24,26,82,83]
	Complex behaviors	Aminoglycosides	5 days - 23 wks	[25••,84]
	Vestibular reflexes	Aminoglycosides		[20,29,85]

properties of the cochlea change following damage to a subset of hair cells, allowing a different subset to restore the response properties initially lost. Such changes are not unprecedented. For example, the frequency/place representation in the cochlea of birds and mammals changes dramatically during the early stages of hearing development, and small reversible changes in the tonotopic representation in the brainstem auditory nuclei of chicks are reported after hair cell damage [12].

One way to conduct a definitive test of the involvement of regenerated hair cells in recovery of function is to remove the regenerated cells after restoration of function and observe the specificity of the changes. This was achieved by behavioral studies examining the loss and recovery of high frequency sensitivity in starlings after aminoglycoside treatment. After recovery stabilized, a second aminoglycoside treatment, designed to kill hair cells only in the area of regeneration, reversed the recovery of high frequency sensitivity [18••]. This result argues strongly for the involvement of the regenerated hair cells in recovery of auditory function in birds. Although evidence for the participation of regenerated hair cells in functional recovery is now convincing, the recovery is not perfect. Most studies have reported residual long-term deficits, such as mild sensitivity impairments and mild to moderate tuning impairments (e.g. see [17••]).

In summary, both behavioral and physiological studies provide convincing evidence that regeneration of hair cells restores near normal vestibular reflexes and response properties in the vestibular afferents to the brain [19–21]. Does this same process occur in mammals to a limited extent, and has gone unnoticed? Alternatively, is the process started and then aborted before the differentiation of new hair cells? One report [22] suggests that extensive regeneration of mammalian vestibular hair cells can be induced by local application of growth factors and leads to recovery of vestibular reflexes. However, the lack of direct evidence for mitotic regeneration casts doubts on these claims.

Recovery of perceptual processing and behavioral plasticity

Although many reports have examined the role of hair cell regeneration in the recovery of sensory information processing, few have examined more complex properties of perceptual processing and behavioral plasticity. In general, studies have found that temporal and frequency resolution gradually return to normal or near normal in conjunction with the return of sensitivity [23,24,25••,26]. But what about the recognition and production of vocal signals that depend on hearing and are necessary for communication? And what about the stability of behavior, or its plasticity, as the receptor system and central pathways are experiencing such profound alterations in information flow? A few recent studies have examined these questions and provided new insights into, as well as new tools to investigate, old questions.

Dooling *et al.* [25••] used budgerigars (domesticated parakeets) to examine the perception of vocalizations. These birds are classical mimics who readily learn new vocalizations throughout life. Birds were trained to match their vocalizations to specific acoustic templates, and then subjected to a profound high frequency hearing loss by aminoglycoside injections. As their hearing recovered, the birds were retested with the same acoustic templates. The vocal mimicry initially declined, but was restored to pre-injection levels quite rapidly, before the full recovery of thresholds or discrimination abilities. This result suggests that relatively little acoustic feedback is necessary to guide previously learned vocal abilities, and that regeneration of only a few hair cells is sufficient to restore that feedback.

A second study examining complex communication behavior following hair cell regeneration was recently completed in our laboratory [27••]. Male Bengalese finches (*Lonchura striata domestica*) are songbirds that learn a single sequence of 'syllables' early in life from their father and reliably produce the same song throughout life, provided that hearing is normal. After recording each animal's song and verifying its stability, the birds were treated with a combination of low frequency noise exposure and aminoglycosides to cause a severe hearing loss that included both high and low frequencies. The songs rapidly deteriorated after the treatments, just as they do following surgical deafening [28]. As hearing was restored by hair cell regeneration, the song returned to its pre-exposure structure. Thus, restoration of hearing allows each bird to access a stored 'template' of its own learned vocalization and gradually match its new vocalizations to this stored memory. Remarkably, this period of hearing recovery also appeared to reinstate or unmask a capacity for behavioral plasticity not usually apparent in adult songbirds of this type. After the recovery of their normal (pre-deafening) song, some birds altered individual syllables so as to 'copy' portions of their cagemate's song. That is, as adults, they incorporated new elements from their acoustic environment into their otherwise stable song.

Hair cell regeneration in the avian vestibular system can also be used to study behavioral plasticity [29]. The vestibulo-ocular reflex (VOR) and the vestibulo-colic reflex normally disappear after hair cells in the crista of the semicircular canals are killed; these reflexes reappear as the hair cells regenerate (These reflexes involving the vestibular organs in the inner ear and the extraocular eye muscles (VOR) or the neck musculature (VCR) are essential for maintaining a steady gaze while the body is in motion) [19,20]. However, when an animal is subjected to an environment devoid of smoothly changing visual stimuli, by maintaining it in stroboscopic illumination, normal VOR recovery fails to occur. Apparently, by depriving the animal of retinal slip information, the central gain control system cannot be calibrated. Remarkably, however, just 48 h (and maybe even less) of normal visual environment is long enough to reinstate a normal VOR. This study and the one by Woolley and Rubel [27••] discussed above suggest that hair cell regeneration in birds may be a very useful new way to study how sensory information shapes neural structure and function.

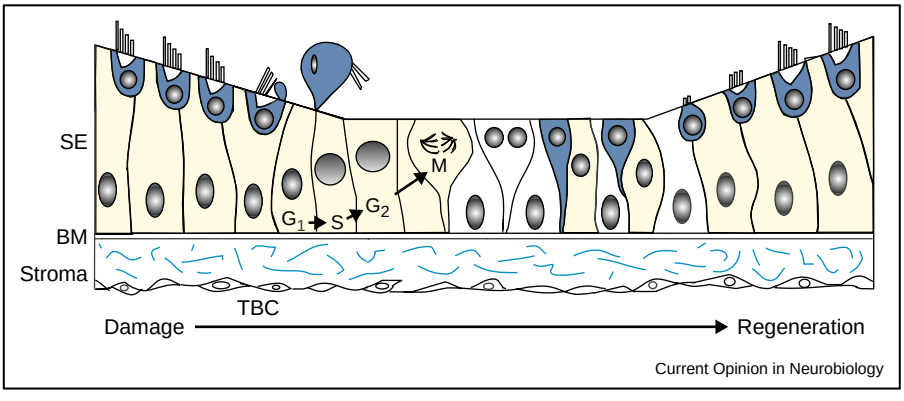
Molecular events leading to hair cell regeneration

The events leading up to the regeneration of the auditory and vestibular epithelia after damage in chicks have been the focus of much study; hopefully, understanding these phenomena will point the way to regeneration in the mammalian inner ear. To understand the molecular mechanisms of hair cell regeneration, three general approaches have been taken: the first looks at which proteins might be expressed or repressed during the regeneration process; the second examines the effects of exogenous signals such as growth factors on the regeneration process; and the third studies the intracellular signals that play a role in the progression of quiescent support cells into the G_1 and S phases of the cell cycle. Figure 2 provides a schematic of the steps involved in the regeneration of hair cells.

Proteins expressed during hair cell regeneration

Following the first approach, Lomax *et al.* [30] recently used differential display of expressed genes to show that a novel member of the ubiquitin ligase gene family is upregulated in response to noise-induced damage in the chick basilar papilla. In a directed screen for receptor

Figure 2



Schematic representation of hair cell regeneration after damage. Blue cells represent hair cells and yellow cells denote supporting cells. After damage, the hair cells are extruded to the lumen and some of the support cells are triggered to divide. The M phase of the cell cycle takes place at the lumenal surface of the sensory epithelium. After division, the new cells, shown in white, go on to differentiate into hair cells and supporting cells. SE, sensory epithelium composed of hair cells and support cells; BM, basement membrane; TBC, tympanic border cells. Drawing courtesy of Dr Jennifer S Stone.

tyrosine kinase genes expressed in support cells, we [31] found that the growth factor receptor fibroblast growth factor receptor 3 (FGFR3), which is highly expressed in the support cells of the auditory sensory epithelium, is rapidly downregulated after damage, and begins to be expressed again after the cells exit the cell cycle. These results suggest that FGFR3 plays a role in maintaining support cells in their quiescent state. Interestingly, this same gene is found to be upregulated after noise damage in the rat; thus, a system that does not regenerate appears to regulate this gene in the opposite direction [32]. An actin-interacting protein, WD40 repeat protein (WDR1), is also upregulated in the supporting cells of the chick basilar papilla after noise damage [33]. The expression of this gene is likely involved in actin turnover; the investigators propose that it might be important in the restoration of cytoskeletal integrity after damage. Many other developmentally important genes are likely to be upregulated in the regenerating regions of the basilar papilla. For example, Delta1 and Notch1 expression are upregulated during the process of regeneration, when new hair cell genesis is at its peak [34].

The role of signaling molecules and growth factors in hair cell regeneration

To identify factors that might promote regeneration, several investigators have tested defined signaling molecules and growth factors in assays of hair cell regeneration. Insulin-like growth factor 1 stimulates DNA synthesis in the chick vestibular sensory epithelium in a dose-dependent manner [35]. This factor has also been shown by PCR analysis to be upregulated after damage [36]. By contrast, FGF2 inhibits DNA synthesis in avian vestibular and auditory sensory epithelia [37]. IGFs and FGFs are important regulators of progenitor cell mitotic activity in other regions of the nervous system; thus, these results again demonstrate that regeneration is likely to be regulated by the same factors normally involved in embryonic development.

It has been known for some time that transforming growth factor α (TGFα) and epidermal growth factor in the presence of insulin stimulate cell proliferation in the cultured mature mammal vestibular epithelium [38,39]. Recent studies show that infusion of TGFα and insulin directly into the inner ear of adult rats stimulated DNA synthesis in the vestibular sensory receptor epithelium [40]. Corwin and colleagues [41–43] investigated various intracellular signal transduction pathways using *in vitro* cultures of utricular sensory epithelial sheets derived from both mature avian and neonatal mammalian inner ears. Although cell proliferation in both the avian and mammalian sensory epithelia was reduced by inhibitors of several key signaling intermediates, including phosphatidyl inositol 3′ kinase (PI3K), target of rapamycin (TOR), mitogen-activated protein kinase (MAPK), and protein kinase C [41–43], these investigators found that the MAPK pathway plays a more significant role in the avian cultures than in similar mammalian cultures.

Cell cycle regulation in hair cell regeneration

Once support cells in the sensory epithelia are enticed to enter and progress through the cell cycle, it is necessary for one or both daughter cells to receive the correct signals to differentiate into hair cells. Recently, mammalian atonal homolog 1 (Math1), a basic helix–loop–helix transcription factor, has been shown to be necessary for hair cell differentiation; mice deficient in this gene fail to develop hair cells in either the auditory or vestibular epithelia [44••]. Another class of related molecules, mammalian hairy and enhancer of split homologs (Hes1 and Hes5), act as negative regulators of hair cell differentiation, and deletion of Hes1 and Hes5 in mice leads to an overproduction of hair cells [45••]. The precise control of these activators and suppressors of hair cell fate leads to the patterned array of hair cells that is critical to the proper functioning of this system. The experimental manipulation of this system has led to a potential strategy for hair cell replacement. Results from two groups show that overexpression of Math1 in cultures of neonatal mouse inner ear leads to the production of extranumerary hair cells from the greater epithelia ridge in the case of the cochlea, and from the support cells in the case of utricles [46•,47]. This is an exciting finding because it suggests the potential of replacing lost hair cells using endogenous tissue. It is not clear, however, whether these new hair cells would make the correct functional connections with the spiral ganglion.

Another strategy for hair cell replacement in mammals has emerged from recent studies of cell cycle regulators in the inner ear [48,49]. The numbers and timing of hair cell and support cell production in the auditory and vestibular epithelium is highly regulated. For example, in mice deficient for the cell cycle inhibitor p27 (Kip1), hair cells are initially overproduced; later, a massive degeneration of hair cells occurs, particularly in the basal region of the organ of Corti, leading to hearing impairment. This study [49] does, however, demonstrate that production of hair cells can be extended into the postnatal period under the right conditions.

The finding that stem cells can be isolated from the CNS has lead to widespread speculation that they will lead to cures for many neurodegenerative diseases. Limited success has been achieved to date, but research in this arena is still in its formative stages. Recently Ito *et al.* transplanted neural stem cells into the mammalian inner ear [50••]. They showed that these cells survive and appear to integrate into various structures up to four weeks after transplant. They even saw some cells that appeared to take on the morphology of hair cells. Although these findings are encouraging, the extent to which such cells can develop into specialized sensory cells is unclear. For

example, stem cells transplanted into the eye integrated and took on the morphology of retinal neurons but failed to express any retinal-specific markers [51].

Conclusion and future directions

In summary, over 15 years of studies on hair cell regeneration in the inner ear of birds has taught us that a quick and easy 'cure' for sensory neural hearing loss is unrealistic. It is impossible to predict when these efforts will lead to a therapy for the hearing impaired. However, 15 years is but a short time in the history of science and great progress has been achieved in these years. The phenomenology of hair cell loss and regeneration has been well described in birds; new *in vitro* and *in vivo* preparations have been developed and cell lines are beginning to become available. Functional recovery due to regenerated hair cells has been confirmed and we are beginning to unravel some of the molecular signals that stimulate and inhibit regeneration in birds. Limited postnatal proliferation and ectopic hair cell expression has been achieved in mammals. Of most importance, hair cell regeneration has become a legitimate and exciting field of interest and exploration. That is the major change in the field that will someday lead to therapeutic interventions for the hearing impaired. The discovery of the structure of DNA was only half a century ago, yet we seem on the verge of promised cures for various conditions. It is exciting to speculate on the impact the next half-century of research will have on hair cell regeneration.

Acknowledgements

We thank Thomas Reh, Sarah Woolley and Elizabeth Oesterle for helpful suggestions on the manuscript, Jennifer Stone for creating Figure 2, Glen MacDonald and Dale Cunningham for help with Figure 1, and Laurie Johnson for assisting with preparation of the manuscript. The authors research was supported by The National Institute on Deafness and Other Communication Disorders grants DC02854, DC04661 and funds from the Oberkotter Foundation.

References and recommended reading

Papers of particular interest have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cruz RM, Lambert PR, Rubel EW: **Light microscopic evidence of hair cell regeneration after gentamicin toxicity in chick cochlea**. *Arch Otolaryngol Head Neck Surg* 1987, **113**:1058-1062.

2. Cotanche DA: **Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma**. *Hear Res* 1987, **30**:181-195.

3. Ryals BM, Rubel EW: **Hair cell regeneration after acoustic trauma in adult Coturnix quail**. *Science* 1988, **240**:1774-1776.

4. Corwin JT, Cotanche DA: **Regeneration of sensory hair cells after acoustic trauma**. *Science* 1988, **240**:1772-1774.

5. Jorgensen JM, Mathiesen C: **The avian inner ear. Continuous production of hair cells in vestibular sensory organs, but not in the auditory papilla**. *Naturwissenschaften* 1988, **75**:319-320.

6. Smolders JW: **Functional recovery in the avian ear after hair cell regeneration**. *Audiol Neurootol* 1999, **4**:286-302.

7. McFadden EA, Saunders JC: **Recovery of auditory function following intense sound exposure in the neonatal chick**. *Hear Res* 1989, **41**:205-215.

8. Hashino E, Sokabe M: **Kanamycin induced low-frequency hearing loss in the budgerigar (*Melopsittacus undulatus*)**. *J Acoust Soc Am* 1989, **85**:289-294.

9. Tucci DL, Rubel EW: **Physiologic status of regenerated hair cells in the avian inner ear following aminoglycoside ototoxicity**. *Otolaryngol Head Neck Surg* 1990, **103**:443-450.

10. Girod DA, Tucci DL, Rubel EW: **Anatomical correlates of functional recovery in the avian inner ear following aminoglycoside ototoxicity**. *Laryngoscope* 1991, **101**:1139-1149.

11. Cohen YE, Saunders JC: **The effects of sound overexposure on the spectral response patterns of nucleus magnocellularis in the neonatal chick**. *Exp Brain Res* 1993, **95**:202-212.

12. Cohen YE, Saunders JC: **The effect of acoustic overexposure on the tonotopic organization of the nucleus magnocellularis**. *Hear Res* 1994, **81**:11-21.

13. Adler HJ, Saunders JC: **Hair cell replacement in the avian inner ear following two exposures to intense sound**. *J Neurocytol* 1995, **24**:111-116.

14. Plontke SK, Lifshitz J, Saunders JC: **Distribution of rate-intensity function types in chick cochlear nerve after exposure to intense sound**. *Brain Res* 1999, **842**:262-274.

15. Girod DA, Rubel EW: **Hair cell regeneration in the avian cochlea: if it works in birds, why not in man?** *Ear Nose Throat J* 1991, **70**:343-350.

16. Salvi RJ, Saunders SS, Hashino E, Chen L: **Discharge patterns of chicken cochlear ganglion neurons following kanamycin-induced hair cell loss and regeneration**. *J Comp Physiol [A]* 1994, **174**:351-369.

17. •• Muller M, Smolders JW: **Responses of auditory nerve fibers innervating regenerated hair cells after local application of gentamicin at the round window of the cochlea in the pigeon**. *Hear Res* 1999, **131**:153-169.
In a thorough study of physiological responses to sound following aminoglycoside toxicity to the cochlea, the authors show a sustained deficit in responses to high frequency stimuli. This incomplete recovery may be caused by differences in the cellular physiology of regenerated versus original hair cells in the most basal regions of the cochlea.

18. •• Marean GC, Burt JM, Beecher MD, Rubel EW: **Hair cell regeneration in the European starling (*Sturnus vulgaris*): recovery of pure-tone detection thresholds**. *Hear Res* 1993, **71**:125-136.
Using well trained birds, we were able to carefully follow functional changes in hearing on a daily basis during the course of daily drug injections, both initially and after several months of recovery. In each case, the pattern of hearing loss followed high to lower frequency, consistent with the pattern of hair cell loss.

19. Carey JP, Fuchs AF, Rubel EW: **Hair cell regeneration and recovery of the vestibuloocular reflex in the avian vestibular system**. *J Neurophysiol* 1996, **76**:3301-3312.

20. Goode CT, Carey JP, Fuchs AF, Rubel EW: **Recovery of the vestibulocolic reflex after aminoglycoside ototoxicity in domestic chickens**. *J Neurophysiol* 1999, **81**:1025-1035.

21. Boyle R, Highstein SM, Carey JP, Xu J: **Functional recovery of anterior semicircular canal afferents following hair cell regeneration in birds**. *J Assoc Res Otolaryngol* 2002, **3**:149-166.

22. Kopke RD, Jackson RL, Li G, Rasmussen MD, Hoffer ME, Frenz DA, Costello M, Schultheiss P, Van De Water TR: **Growth factor treatment enhances vestibular hair cell renewal and results in improved vestibular function**. *Proc Natl Acad Sci USA* 2001, **98**:5886-5891.

23. Saunders SS, Salvi RJ, Miller KM: **Recovery of thresholds and temporal integration in adult chickens after high-level 525-Hz pure-tone exposure**. *J Acoust Soc Am* 1995, **97**:1150-1164.

24. Saunders SS, Salvi RJ: **Pure tone masking patterns in adult chickens before and after recovery from acoustic trauma**. *J Acoust Soc Am* 1995, **98**:1365-1371.

25. •• Dooling RJ, Ryals BM, Manabe K: **Recovery of hearing and vocal behavior after hair-cell regeneration**. *Proc Natl Acad Sci USA* 1997, **94**:14206-14210.

This is the only study to date that directly assesses perceptual behavior using behaviorally relevant stimuli. The rapid return of the remarkable ability to mimic indicates that matching a template is not a terribly difficult task. On the other hand, discriminations between synthetic and natural notes took months to recover, suggesting a relearning of central nervous system processes beyond merely regenerating hair cells.

26. Marean GC, Burt JM, Beecher MD, Rubel EW: **Auditory perception following hair cell regeneration in European starling (*Sturnus vulgaris*): frequency and temporal resolution**. *J Acoust Soc Am* 1998, **103**:3567-3580.

27. •• Woolley SM, Rubel EW: **Vocal memory and learning in adult Bengalese finches with regenerated hair cells**. *J Neurosci* 2002, **22**:7774-7787.

This is the first study to show preservation of a memory for the motor components of an individual song when feedback has been entirely removed and the motor components break down. Interestingly, the temporal and spectral properties show different rates of deterioration and recovery. It is important to recognize that the 'new' notes were subtle (but clear) modifications of existing elements as opposed to totally unique elements.

28. Woolley SM, Rubel EW: **Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for the maintenance of adult song**. *J Neurosci* 1997, **17**:6380-6390.

29. Goode CT, Maney DL, Rubel EW, Fuchs AF: **Visual influences on the development and recovery of the vestibuloocular reflex in the chicken**. *J Neurophysiol* 2001, **85**:1119-1128.

30. Lomax MI, Huang L, Cho Y, Gong TL, Altschuler RA: **Differential display and gene arrays to examine auditory plasticity**. *Hear Res* 2000, **147**:293-302.

31. Bermingham-McDonogh O, Stone JS, Reh TA, Rubel EW: **FGFR3 expression during development and regeneration of the chick inner ear sensory epithelia**. *Dev Biol* 2001, **238**:247-259.

32. Pirvola U, Cao Y, Oellig C, Suoqiang Z, Pettersson RF, Ylikoski J: **The site of action of neuronal acidic fibroblast growth factor is the organ of Corti of the rat cochlea**. *Proc Natl Acad Sci USA* 1995, **92**:9269-9273.

33. Oh SH, Adler HJ, Raphael Y, Lomax MI: **WDR1 colocalizes with ADF and actin in the normal and noise-damaged chick cochlea**. *J Comp Neurol* 2002, **448**:399-409.

34. Stone JS, Rubel EW: **Delta1 expression during avian hair cell regeneration**. *Development* 1999, **126**:961-973.

35. Oesterle EC, Tsue TT, Rubel EW: **Induction of cell proliferation in avian inner ear sensory epithelia by insulin-like growth factor-I and insulin**. *J Comp Neurol* 1997, **380**:262-274.

36. Saffer LD, Gu R, Corwin JT: **An RT-PCR analysis of mRNA for growth factor receptors in damaged and control sensory epithelia of rat utricles**. *Hear Res* 1996, **94**:14-23.

37. Oesterle EC, Bhave SA, Coltrera MD: **Basic fibroblast growth factor inhibits cell proliferation in cultured avian inner ear sensory epithelia**. *J Comp Neurol* 2000, **424**:307-326.

38. Lambert PR: **Inner ear hair cell regeneration in a mammal: identification of a triggering factor**. *Laryngoscope* 1994, **104**:701-718.

39. Yamashita H, Oesterle EC: **Induction of cell proliferation in mammalian inner-ear sensory epithelia by transforming growth factor alpha and epidermal growth factor**. *Proc Natl Acad Sci USA* 1995, **92**:3152-3155.

40. Kuntz AL, Oesterle EC: **Transforming growth factor alpha with insulin stimulates cell proliferation *in vivo* in adult rat vestibular sensory epithelium**. *J Comp Neurol* 1998, **399**:413-423.

41. Montcouquiol M, Corwin JT: **Intracellular signals that control cell proliferation in mammalian balance epithelia: key roles for phosphatidylinositol-3 kinase, mammalian target of rapamycin, and S6 kinases in preference to calcium, protein kinase C, and mitogen-activated protein kinase**. *J Neurosci* 2001, **21**:570-580.

42. Montcouquiol M, Corwin JT: **Brief treatments with forskolin enhance S-phase entry in balance epithelia from the ears of rats**. *J Neurosci* 2001, **21**:974-982.

43. Witte MC, Montcouquiol M, Corwin JT: **Regeneration in avian hair cell epithelia: identification of intracellular signals required for S-phase entry**. *Eur J Neurosci* 2001, **14**:829-838.

44. •• Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, Bellen HJ, Lysakowski A, Zoghbi HY: **Math1: an essential gene for the generation of inner ear hair cells**. *Science* 1999, **284**:1837-1841.

This paper demonstrates that Math1, a mouse homolog of the *Drosophila* proneural gene atonal, is required for the generation of both vestibular and cochlear hair cells. The null mutant mouse fails to develop any hair cells.

45. •• Zine A, Aubert A, Qiu J, Therianos S, Guillemot F, Kageyama R, de Ribaupierre F: **Hes1 and Hes5 activities are required for the normal development of the hair cells in the mammalian inner ear**. *J Neurosci* 2001, **21**:4712-4720.

This study demonstrates that while hair cells are dependent on Math1 for their differentiation, support cells are dependent on homologs of the *Drosophila melanogaster* enhancer of split complex the Hes's. Examination of cochleae from $Hes1^{-/-}$ mice showed a significant increase in the number of inner hair cells, whereas cochleae from $Hes5^{-/-}$ mice showed a significant increase in the number of outer hair cells. Supernumerary hair cells are also found in the saccule and utricle of these knockouts. These results suggest that the balance of the expression of Math1 and Hes 1 and 5 determine whether a progenitor becomes a hair cell or a support cell.

46. • Zheng JL, Gao WQ: **Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears**. *Nat Neurosci* 2000, **3**:580-586.

The authors of this paper were the first to show that certain cells in the mammalian organ of Corti can, at least in the young animals, be redirected towards a hair cell fate by the overexpression of Math1.

47. Hume CR, Oesterle EC: **Induction of hair cell phenotype in neonatal mouse inner ear by ectopic transcription factor expression**. *Assoc Res Otolaryngol Abstr* 2001:20841.

48. Lowenheim H, Furness DN, Kil J, Zinn C, Gultig K, Fero ML, Frost D, Gummer AW, Roberts JM, Rubel EW *et al.*: **Gene disruption of p27(Kip1) allows cell proliferation in the postnatal and adult organ of Corti**. *Proc Natl Acad Sci USA* 1999, **96**:4084-4088.

49. Chen P, Segil N: **p27(Kip1) links cell proliferation to morphogenesis in the developing organ of Corti**. *Development* 1999, **126**:1581-1590.

50. •• Ito J, Kojima K, Kawaguchi S: **Survival of neural stem cells in the cochlea**. *Acta Otolaryngol* 2001, **121**:140-142.

This study represents the first published paper on transplantation of stem cells into the ear. The transplanted cells survived at least four weeks and appeared to integrate into several structures including the organ of Corti.

51. Takahashi M, Palmer TD, Takahashi J, Gage FH: **Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina**. *Mol Cell Neurosci* 1998, **12**:340-348.

52. Trautwein P, Salvi RJ, Miller K, Shero M, Hashino E: **Incomplete recovery of chicken distortion product otoacoustic emissions following acoustic overstimulation**. *Audiol Neurootol* 1996, **1**:86-103.

53. Froymovich O, Rebala V, Salvi RJ, Rassael H: **Long-term effect of acoustic trauma on distortion product otoacoustic emissions in chickens**. *J Acoust Soc Am* 1995, **97**:3021-3029.

54. Norton SJ, Rubel EW (Eds): *Active and Passive ADP Components in Mammalian and Avian Ears.* New York: Springer-Verlag; 1990.

55. Chen L, Trautwein PG, Miller K, Salvi RJ: **Effects of kanamycin ototoxicity and hair cell regeneration on the DC endocochlear potential in adult chickens**. *Hear Res* 1995, **89**:28-34.

56. Poje CP, Sewell DA, Saunders JC: **The effects of exposure to intense sound on the DC endocochlear potential in the chick**. *Hear Res* 1995, **82**:197-204.

57. Trautwein PG, Chen L, Salvi RJ: **Steady state EP is not responsible for hearing loss in adult chickens following acoustic trauma**. *Hear Res* 1997, **110**:266-270.

58. Masetto S, Correia MJ: **Electrophysiological properties of vestibular sensory and supporting cells in the labyrinth slice before and during regeneration**. *J Neurophysiol* 1997, **78**:1913-1927.

59. Masetto S, Correia MJ: **Ionic currents in regenerating avian vestibular hair cells**. *Int J Dev Neurosci* 1997, **15**:387-399.

60. Saunders JC, Adler HJ, Pugliano FA: **The structural and functional aspects of hair cell regeneration in the chick as a result of exposure to intense sound**. *Exp Neurol* 1992, **115**:13-17.

61. Adler HJ, Kenealy JF, Dedio RM, Saunders JC: **Threshold shift, hair cell loss, and hair bundle stiffness following exposure to 120 and 125 dB pure tones in the neonatal chick**. *Acta Otolaryngol Stockh* 1992, **112**:444-454.

62. Umemoto M, Sakagami M, Ashida K, Fukazawa K, Matsunaga T, Senda T: **Regeneration of adult budgerigar's hair cells following acoustic trauma**. *ORL J Otorhinolaryngol Relat Spec* 1993, **55**:325-331.

63. Adler HJ, Poje CP, Saunders JC: **Recovery of auditory function and structure in the chick after two intense pure tone exposures**. *Hear Res* 1993, **71**:214-224.

64. Pugliano FA, Wilcox TO, Rossiter J, Saunders JC: **Recovery of auditory structure and function in neonatal chicks exposed to intense sound for 8 days**. *Neurosci Lett* 1993, **151**:214-218.

65. Pugliano FA, Pribitikin E, Adler HJ, Saunders JC: **Growth of evoked potential amplitude in neonatal chicks exposed to intense sound**. *Acta Otolaryngol* 1993, **113**:18-25.

66. Muller M, Smolders JW, Ding P-D, Klinke R: **Regeneration after tall hair cell damage following severe acoustic trauma in adult pigeons: correlation between cochlear morphology, compound action potential responses and single fiber properties in single animals**. *Hear Res* 1996, **102**:133-154.

67. Ding-Pfennigdorff D, Smolders JW, Muller M, Klinke R: **Hair cell loss and regeneration after severe acoustic overstimulation in the adult pigeon**. *Hear Res* 1998, **120**:109-120.

68. Woolley SM, Wissman AM, Rubel EW: **Hair cell regeneration and recovery of auditory thresholds following aminoglycoside ototoxicity in Bengalese finches**. *Hear Res* 2001, **153**:181-195.

69. Jones TA, Nelson RC: **Recovery of vestibular function following hair cell destruction by streptomycin**. *Hear Res* 1992, **62**:181-186.

70. Chen L, Salvi RJ, Hashino E: **Recovery of CAP threshold and amplitude in chickens following kanamycin ototoxicity**. *Hear Res* 1993, **69**:15-24.

71. Trautwein PG, Hashino E, Salvi RJ: **Regenerated hair cells become functional during continuous administration of kanamycin**. *Audiol Neurootol* 1998, **3**:229-239.

72. Muller M, Smolders JW: **Hair cell regeneration after local application of gentamicin at the round window of the cochlea in the pigeon**. *Hear Res* 1998, **120**:25-36.

73. Saunders JC, Doan DE, Poje CP, Fisher KA: **Cochlear nerve activity after intense sound exposure in neonatal chicks**. *J Neurophysiol* 1996, **76**:770-787.

74. Chen L, Trautwein PG, Shero M, Salvi RJ: **Tuning, spontaneous activity and tonotopic map in chicken cochlear ganglion neurons following sound-induced hair cell loss and regeneration**. *Hear Res* 1996, **98**:152-164.

75. Muller M, Smolders JW, Ding P-D, Klinke R: **Discharge properties of pigeon single auditory nerve fibers after recovery from severe acoustic trauma**. *Int J Dev Neurosci* 1997, **15**:401-416.

76. Chen L, Trautwein PG, Powers N, Salvi RJ: **Two-tone rate suppression boundaries of cochlear ganglion neurons in chickens following acoustic trauma**. *J Acoust Soc Am* 1997, **102**:2245-2254.

77. Salvi RJ, Chen L, Trautwein P, Powers N, Shero M: **Hair cell regeneration and recovery of function in the avian auditory system**. *Scand Audiol Suppl* 1998, **48**:7-14.

78. Lippe WR: **Reduction and recovery of neuronal size in the cochlear nucleus of the chicken following aminoglycoside intoxication**. *Hear Res* 1991, **51**:193-202.

79. Saunders JC, Adler HJ, Cohen YE, Smullen S, Kazahaya K: **Morphometric changes in the chick nucleus magnocellularis following acoustic overstimulation**. *J Comp Neurol* 1998, **390**:412-426.

80. Park DL, Girod DA, Durham D: **Tonotopic changes in 2-deoxyglucose activity in chick cochlear nucleus during hair cell loss and regeneration**. *Hear Res* 1999, **138**:45-55.

81. Park DL, Girod DA, Durham D: **Evidence for loss and recovery of chick brainstem auditory neurons during gentamicin-induced cochlear damage and regeneration**. *Hear Res* 1998, **126**:84-98.

82. Niemiec AJ, Raphael Y, Moody DB: **Return of auditory function following structural regeneration after acoustic trauma: behavioral measures from quail**. *Hear Res* 1994, **75**:209-224.

83. Ryals BM, Dooling RJ, Westbrook E, Dent ML, MacKenzie A, Larsen ON: **Avian species differences in susceptibility to noise exposure**. *Hear Res* 1999, **131**:71-88.

84. Woolley SM, Rubel EW: **High-frequency auditory feedback is not required for adult song maintenance in Bengalese finches**. *J Neurosci* 1999, **19**:358-371.

85. Carey JP, Fuchs AF, Rubel EW: **Hair cell regeneration and vestibulo-ocular reflex recovery**. *Ann New York Acad Sci* 1996, **781**:47-58.