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Noise-Induced Hearing Loss: New Animal Models

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Abstract

Purpose of the review—This article presents research findings from two invertebrate model systems with potential to advance both the understanding of noise-induced hearing loss mechanisms and the development of putative therapies to reduce human noise damage.

Recent findings—Work on sea anemone hair bundles, which resemble auditory hair cells, has revealed secretions that exhibit astonishing healing properties not only for damaged hair bundles, but also for vertebrate lateral line neuromasts. We present progress on identifying functional components of the secretions, and their mechanisms of repair. The second model, the Johnston's organ in *Drosophila*, is also genetically homologous to hair cells and shows noise-induced hearing loss similar to vertebrates. *Drosophila* offers genetic and molecular insight into noise sensitivity and pathways that can be manipulated to reduce stress and damage from noise.

Summary—Using the comparative approach is a productive avenue to understanding basic mechanisms, in this case cellular responses to noise trauma. Expanding study of these systems may accelerate identification of strategies to reduce or prevent noise damage in the human ear.

Keywords

sea anemone hair bundle; *Drosophila* chordotonal organ; insect hearing; noise-induced hearing loss; repair proteins

Introduction

For humans, noise-induced hearing loss has become a vast problem for many reasons. We have generated noisy environments to live, work and play in, and because we are living longer than ever before, the noise effects accumulate over longer time spans. As mammals, we have benefitted from some of the most important studies of noise-induced hearing loss that have been carried out in other mammalian species, including cats, guinea pigs, rats, mice and chinchillas. Nevertheless, great understanding has been derived from comparative studies, especially in birds and lizards, which unlike mammals, have the ability to regenerate their auditory hair cells lost through noise damage or other ototoxic insults. In this review, we look beyond vertebrates at more distant species that can provide value insights into the mechanisms of noise-induced hearing loss. Though not a comprehensive review, we will explore interesting findings in two emerging invertebrate models, namely sea anemones and

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Conflicts of Interest

The authors declare no conflicts of interest.

fruit flies. Each of these species has unique properties that will enable novel perspectives on noise-induced hearing loss and potential therapies. Sea anemones have structurally recognizable hair cells that clearly have a common ancestor with mammalian hair cells, and have amazing repair mechanisms that could inform the development of novel therapies for human noise-induced hearing loss. In the fruit fly, *Drosophila*, which has served as a genetic workhorse for understanding the genetic basis of biological processes for over a century, the auditory cells are structurally divergent from the hair cell format, yet they are derived through the activation of the same conserved developmental cascade of transcription factors that include *atoh1* and *pax2*. Because these cells can recover from levels over overstimulation that would easily kill mammalian hair cells, this model is well suited to understanding signal pathways and repair mechanisms that protect against permanent noise-induced hearing loss.

Sea Anemone Hair Bundles

Sea anemones are sessile invertebrates in the phylum *Cnidaria*, which contain arrays of stinging cells (cnidocytes) on their tentacles used to subdue prey. Cnidocytes contain evertible stinging organelles (nematocysts) that penetrate prey animal integuments and inject neurotoxins. Cnidarians are also the most basal phylum possessing hair bundle mechanoreceptor organs. Hair bundles lie adjacent to the cnidocytes, and detect prey movements. Activation of the hair bundles *sensitizes* specific subtypes of nematocysts (microbasic p-mastigophore nematocyst) to fire, such that a test probe vibrating at the ‘best frequency’ of hair bundles will induce about twice the number of nematocyst discharges upon contact as a non-vibrating probe [1]. Hair bundles are ‘tuned’ in the sense that prey-induced vibrations at certain frequencies will most effectively sensitize nematocyst discharge [1]. Like cochlear outer hair cells, anemone hair bundles actively change length, involving dynamic reorganization of actin [2-4]. Unlike hair cells, anemone stereocilia change length in the presence of prey-derived chemicals, including N-acetylated sugars (such as N-acetylneuraminic acid (NANA)), and specific amino acids, such as proline or glycine [2-4]. The result of these morphodynamic changes shifts the sensitivity of the hair bundles, sensitizing nematocyst discharge to lower frequencies and amplitudes [3, 4].

Unlike vertebrate hair cells, hair bundles are a multicellular complex composed of a single sensory neuron and 2-4 supporting cells. (Fig. 1a) [3-5]. A single non-motile kinocilium and 5-7 large-diameter stereocilia extend from the apical surface of the neuron, while the apical surface of each supporting cell supports 100-300 small-diameter stereocilia [5, 7]. While the small-diameter stereocilia resemble hair cell stereocilia, with ordered parallel arrays of cross-linked actin filaments [3, 4], the large-diameter stereocilia have less ordered actin structure, thought to be related to their ability to elongate or shorten in the presence of chemical signals from prey [3, 4]. As in vertebrate hair cells, the stereocilia of anemone hair bundles (particularly the small-diameter stereocilia from support cells) are connected with a variety of linkages including basal, distal, and tip-links, matching in size those seen in hair cells [3, 4]. Recent models of vertebrate tip-link structure contain two homodimers each of two members of the cadherin family, a cadherin 23 (CDH23) and a protocadherin 15 (PCDH15), interacting in trans [8]. Using the zebrafish CDH23 sequence, Watson et al. [9] identified a cadherin 23-like peptide in the model anemone *Nematostella vectensis* genome.

The peptide localizes to hair bundles and tip-links, and incubation with antibodies against the peptide eliminated vibration-sensitivity after 15 min, reduced hair bundle numbers, and altered hair bundle morphology in a manner consistent with the disruption of stereocilia linkages [9, 10]. This suggests that stereocilia linkages, including tip-links in anemone hair bundles are organized similarly to vertebrate hair cells in composition with homologs of at least one of the two major cadherin constituent proteins. While the connections between hair bundle stereocilia resemble vertebrates in structure and composition, the multicellular nature and stereocilia connectivity results in hair bundles having a radially symmetric structure and force sensitivity compared with the linear arrangement seen in hair cells (Fig. 1a,b).

Studies support the idea that it is the small-diameter stereocilia-bearing support cells that perform the majority of mechanotransduction, and these cells have been the main focus for electrophysiological recording, primarily in *Haliplanella luciae* [3, 5]. Despite the structural differences and phylogenetic distance from vertebrates, anemone hair cells share a number of physiological and functional properties. Both show graded, directionally selective responses to stimuli varying in strength with stimulus magnitude, ultimately saturating [3, 5]. Responses may be either depolarizing or hyperpolarizing depending on the direction of the stimulus with respect to stereocilia orientation, with depolarization thought to correspond with the tension-induced opening of mechanotransduction channels (positive deflection of stereocilia), and showing larger amplitudes and reduced saturation compared to hyperpolarizing stimuli [3, 5]. Hair bundle responses also show near-complete adaptation, unlike vertebrate hair cells [5]. Overall anemone hair bundles show a reduced sensitivity to displacement compared with hair cells, with displacements $<1\text{ }\mu\text{m}$ rarely eliciting a response, while most of the hair cell response range involves displacements $<0.5\text{ }\mu\text{m}$ [3, 5, 11]. This may be an adaptation due to the greater amplitudes of ‘environmental noise’ seen in the marine environment.

Damage in Hair Cells and Anemone Hair Bundles

In both vertebrate hair cells and anemone hair bundles, mechanotransduction can be reduced or eliminated through a variety of treatments. Exposure to low concentrations of aminoglycoside antibiotics reversibly inhibits mechanotransduction in the bullfrog sacculus [12]. Similarly, hair bundle electrophysiological responses to mechanical deflection and vibration-enhanced nematocyst discharge are abolished by incubation in aminoglycoside-containing seawater, with the effects rapidly reversible after washing animals with fresh seawater [3-6]. Due to the similar kinetics, this rapid and reversible effect occurs ostensibly through blockage of mechanotransduction ion channels [12], and is separate from the cytotoxic effects of aminoglycosides, thought to involve ROS generation and mitochondrial interactions [13].

Like hair cells, mechanotransduction in anemone hair bundles relies on the coupling of tip-links to force-sensitive ion channels [3, 5], and treatments known to disrupt stereocilia linkages affect both structure and function. Exposure of guinea pigs and anemones to elastase abolishes mechanoelectric transduction and vibration-induced nematocyst discharge, respectively [3, 14, 15]. Brief exposure (from ~10 seconds up to 15 minutes) to calcium-depleted solutions or calcium-chelators (BAPTA, EGTA, etc.) eliminates tip-links

and transduction currents in vertebrate hair cells [16-18], while in hair bundles, vibration-induced discharge sensitivity and stereocilia linkages, including tip-links, disappear after exposure to Ca^{2+} -free seawater [3, 4, 15]. Current models suggest that Ca^{2+} ions are necessary to maintain integrity of tip-link cadherin proteins, partially by stabilizing cadherin repeat domains, affecting protein stiffness and protein-protein interactions [19-21]. As anemone hair bundle linkages contain cadherin homologs [9, 10], the mechanism of low Ca^{2+} induced loss of linkages and mechanotransduction are most likely similar. Like exposure to low Ca^{2+} media, noise-induced traumatic changes in hair cell morphology also include loss of linkages [22-24]. As exposure to both Ca^{2+} -free environments and noise-trauma result in a similar morphological and physiological phenotype, the former treatment may closely mimic the latter on multiple levels [16, 18], elevating the anemone hair bundle's potential as a model for noise-induced trauma. Indeed, immersion of anemone hair bundles in Ca^{2+} free seawater mimics similar damage to vertebrate stereocilia, as well as moderate 'sub-lethal' trauma, such as brief exposure to traumatic noise or mechanical overstimulation [25, 26].

Repair of Anemone Hair Bundles

The unique phenomenon that gives the sea anemone particular distinction as a promising model is its extraordinary repair secretions, as will be outlined in this section. After 1-hour exposure to Ca^{2+} -free seawater, anemones not only display a loss of vibration-sensitive nematocyst discharge but appear to lose most, if not all inter-stereocilia linkages, resulting in disorganized hair bundles [3, 15]. Reducing exposure to 30 min. maintained some bundle structure, which still exhibited a widened, 'splayed' appearance, suggesting partial loss of linkages [15]. After 4 hours in normal Ca^{2+} water, normal vibration-sensitivity and morphology are recovered, and this process can be delayed using the protein-synthesis inhibitor cycloheximide [15]. A longer time-course is seen in avian hair cells, which require up to 12 hours of recovery after 10-15 min. exposure to Ca^{2+} free buffers [18, 27]. Interestingly, recovery in vertebrate hair cells does not require protein synthesis, and is cycloheximide-insensitive [3].

Amazingly, repair of hair bundles can be accelerated by placement of animals in conditioned seawater in which animals had previously undergone Ca^{2+} -free exposure/recovery cycles. Incubation in this 'repair seawater' (RSW) reduced recovery of vibration-sensitivity from ~4 hours to ~15 minutes [15]. Further enhancement was effected by the concentration and filtration of RSW ('concentrated repair seawater', 'CRSW'), allowing recovery times of only 8 minutes [15]. After a 30 min. immersion in Ca^{2+} -free seawater followed by CRSW, stereocilia linkages reappeared over the course of 3-25 min. in a base-to-tip direction, suggesting a 'zipping-up' of stereocilia as linkages are progressively re-incorporated into the bundle [3, 15]. Splayed hair bundles show no electrophysiological responses to deflections, only showing responses after ~5 min. of CRSW application [3, 15].

As boiling RSW or co-incubation of anemones with RSW and trypsin increased recovery time back to ~4 hours [15], the authors concluded that secreted proteins ('repair proteins', RP) re-establish stereocilia linkages, including tip-links. After performing chromatographic fractionation, a particular 2000 kD protein fraction – termed fraction β – had an efficacy in

restoring vibration sensitivity comparable to the whole protein compliment [3, 15]. Like CRSW, fraction β repair activity was delayed by cycloheximide, and multiple repair cycles without replacement of additional protein eventually 'exhaust' some portion of fraction β , eliminating the repair bioactivity [15]. Negative staining of fraction β showed filamentous structures approximating the dimensions of stereocilia linkages, as well as globular structures [15]. Incubation with RP and polyclonal antibodies against fraction β delayed repair of hair bundles past 5 hours (compared to 7-8 *minutes* for controls) [28]. Incubation of non-traumatized anemones in anti-fraction β resulted in a gradual loss of vibration-sensitivity over the course of 15-45 minutes, suggesting direct interaction with stereocilia linkages, and this was confirmed by immunoelectron microscopy [28]. The authors conclude that fraction β consists of both replacement linkages (of multiple types) and some enzymatic/accessory compliment to facilitate functional incorporation into stereocilia [3, 15, 28]. A detailed study of the time-course of fraction β -mediated structural and functional recovery after 1 hour in Ca^{2+} -free seawater showed very rapid recovery of morphology. After near-total elimination of recognizable hair bundles by Ca^{2+} -free treatment, identifiable hair bundles began to appear 1 minute after initial exposure to fraction β , with increasing organization until ~42 min, when bundle dimensions reached those of un-traumatized controls [26]. Electrophysiological recovery was delayed compared to that of hair bundle structure. While, no evoked potentials could be recorded from hair bundles until ~13 min after initial application of fraction β , responses increased over time, reaching control levels after ~43 min [26].

In addition to elimination of stereocilia linkages, sub-lethal noise-trauma in vertebrate hair cells induces transient 'softening' of stereocilia bundles thought to arise from actin reorganization [29-31]. To see if changes in actin dynamics were involved in fraction- β enhanced hair bundle repair, actin depolymerization or polymerization was blocked via incubation with phalloidin or cytochalasin D, respectively. This resulted in a significant reduction in vibration-sensitive enhancement of nematocyst discharge 20 min after repair initiation, suggesting delayed or inhibited repair [26]. *In vivo* monitoring of both F- and G-actin levels during exposure to Ca^{2+} -free buffer followed by fraction β revealed a decrease in polymerized (F-) actin after removal from the low Ca^{2+} treatment, followed by an sustained increase over the course of 40 minutes after initial application of fraction β , when levels were indistinguishable from non-traumatized controls. [26]. Levels of depolymerized (G-) actin increased after Ca^{2+} -free trauma, and then only marginally after 10 min of fraction β incubation [26]. These results suggest that, like vertebrate hair cell repair after noise trauma, anemone hair bundles undergo active reorganization of the actin cytoskeleton in stereocilia during repair, potentially to facilitate re-integration of linkages by reducing the rigidity and 'splay' of the stereocilia [26].

Further studies showed that hair bundle repair requires ATP or related adenosine-phosphatases. Adding exogenous ATP or ADP (μM range) to RP significantly decreased recovery time of vibration-sensitivity from ~8 to ~2 min [32]. Chemical analysis showed that ATP was secreted and consumed by anemones after hair bundle disruption, and that RP displayed ATPase activity, although the precise function of ATP in the repair process is not clear [32]. Currently, the possible role of ATP in vertebrate hair cell repair after sub-lethal

trauma is also unknown, although ATP is present in the endolymph after noise trauma [33] and has known neurohumoral and apoptotic signaling functions [34].

Fraction β contains a number of protein components comprising linkages and accessory proteins, but only a few have been significantly characterized. The anemone homolog to Arl-5b (ADP-ribosylation factor-like protein-5b) was isolated from a cDNA expression library generated to traumatized anemones screened with polyclonal antibodies to fraction β [35]. Anemone Arl-5b shows a relatively high sequence similarity with vertebrate homologs [35]. Antibodies to the Arl-5b peptide label some, but not all hair bundles, with a significant increase in labeling after 1 hour Ca^{2+} -free treatment [35]. Incubation with Arl-5b antiserum failed to recover vibration-sensitivity after trauma after 7 hours (compared with 4 hours in control animals) [35]. Microscopy showed that hair bundles in traumatized, antiserum-treated animals had splaying of the large-diameter stereocilia, and little convergence of small-diameter stereocilia onto them. Immuno-EM gold-particle labeling was observed on the stereocilia of healthy non-traumatized hair bundles, especially distally at the putative tip-link region, suggesting that Arl-5b may interact with linkages, particularly tip-links, and might be a co-factor in the linkage maintenance or in the replacement process [35]. While the expression and function (if any) of the vertebrate Arl-5b or similar genes in the cochlea is currently unclear, ADP-ribosylation factors often activate ADP-ribosyltransferases, which are involved in ribosylation of purinoceptors and integrins in models of myocyte injury [36].

Recent research has also begun to examine the composition and role of other components of RP, including fraction γ . Like fraction β , fraction γ directly binds to hair bundles [7], but fraction γ has a different time course of activity. Incubation with fraction γ after 2 min of low- Ca^{2+} induced trauma resulted in recovery of vibration-sensitivity after 6 minutes, and was cycloheximide-insensitive, indicating that fraction γ alone is sufficient to induce repair [7] after short trauma. However, after 1 h low- Ca^{2+} , recovery of hair bundle function was not reduced in animals treated with fraction γ , compared with controls [7]. Compared to fraction β , γ is of lower complexity and mass, approximately 60 kDa, consisting of Hsp60-like proteins, and treatment with anti-coral Hsp60 antibodies inhibits vibration-sensitivity and hair bundle repair after Ca^{2+} -free exposure [7]. Although molecular partners or targets of fraction γ are unknown, the fact that fraction γ is effective in repair after only mild trauma suggests that its activity may not be associated with replacing missing stereocilia linkages, but in repairing destabilized linkages. This chaperone function is consistent with heat shock protein function in general, as is the fact that addition of exogenous ATP enhances fraction γ -mediated recovery [7].

There is much homology and conservation between anemone hair bundles and vertebrate hair cells at the level of protein structure, function, and response to low- Ca^{2+} mediated trauma. Most intriguingly, there appears to be significant conservation of repair factor functions, even between taxa separated by millions of years of evolutionary divergence. Blind cave fish, *Astynax hubbsi*, rely on their lateral line organs for orienting in the water and detecting and responding to fluid flow and pressure. The hair bundle mechanoreceptors in the superficial neuromasts of the lateral line system are critical for mechanosensory function, as well as mediating reflexive responses to fluid mechanical stimuli, such as the startle reflex and rheotaxis to current flow. After exposing fish to Ca^{2+} -free water for 15 s,

both startle responses and rheotaxis ability were inhibited for several days, and neuromast hair bundles displayed signs of anatomical disruption, including increased area - indicative of 'splaying' [25, 37]. Spontaneous recovery of neuromast-mediated behaviors took 5-11 days [25, 37]. When incubated with anemone RP or fraction β for 1 hour post Ca^{2+} -free trauma, fish showed evidence of recovery of hair bundle function. Animals treated with RP showed gradual increases in startle responses over 5 d after initial trauma compared to untreated controls [37]. Incubation with purified fraction β produced a particularly rapid improvement in normal rheotactic orientation – fish recovered to control levels within 1.3 h of the start of fraction β treatment - compared with > 8d in animals not exposed to fraction β [25]. Although the exact mechanism of RP/fraction β mediated recovery of neuromast function in blind cave fish is unknown, it is probable that – like in the anemone – linkage replacement and stereocilia actin reorganization are involved [25, 37]. Perhaps the most unexpected finding is the apparent conservation of repair mechanisms – the fact that proteins from cnidarians can functionally repair sensory cells in teleosts despite hundreds of millions of years of evolutionary divergence. These findings are harbingers of hope for therapeutic interventions against human noise-induced hearing loss designed with the insights of anemone hair bundle repair biology.

Drosophila auditory mechanoreceptors

A second emerging invertebrate model for noise-induced hearing loss is the fruit fly, *Drosophila*. The auditory systems of insects, and *Drosophila* in particular, have been studied intensively in recent years, revealing exciting mechanisms of mechanotransduction that appear to reflect homologies and analogies to mammalian hearing. Insect auditory organs are of the chordotonal type [38], whether they innervate tympanal organs in long-distance calling insects, or antennal organs (Johnston's organs) in insects that specialize in near-field hearing. While morphologically divergent from the hair cell format, chordotonal organs share developmental genetic pathways with vertebrate auditory hair cells, including specification and differentiation transcription factors Pax2, Atoh1, spalt and others (reviewed recently by [39]). *Drosophila* hearing, which is used to detect species-specific courtship sounds produced by male wing vibration, has been shown to display high temporal resolution of auditory stimuli [40], frequency tuning [41], rapid adaptation [40, 42], wide dynamic range facilitated by nonlinear amplification mechanisms [43], active mechanisms in mechanoreceptive structures [43-45], and most recently, responses to noise trauma [46].

Superficially, the auditory organs of flies and mammals appear morphologically unrelated (Fig. 2a, b). Nevertheless, the developmental genetic association between these organs is indisputable based on the shared developmental cascades of genetic regulation required for their development and specification [39, 47]. In each antenna, the *Drosophila* Johnston's organ (JO) is a cluster of about 225 chordotonal sensory organs, called scolopidia (Fig. 2a). Most JO scolopidia contain two sensory neurons, though a small fraction have three sensory neurons, for a total of close to 500 sensory neurons in each JO. Support cells in these organs include the scolopale cell (Fig. 2a), surrounding the sensory neuron dendrites with an enclosed extracellular cavity of receptor lymph [48], as well as cap cells and ligament cells that facilitate apical and basal attachments respectively. Mechanical activation of the sensory dendrites occurs through transfer of vibrations from the antennal joint (or the

tympanum in insects with tympanal organs) through the dendritic cap, an extracellular matrix structure associated with the dendritic tips and extending to the relevant cuticle. Thus, chordotonal activation is along the longitudinal axis, in contrast to hair cells, which are activated by transverse force (Fig. 2a,b).

Using scolopidia, insects can detect a broad range of frequencies that rivals vertebrates, from less than 1 kHz in *Drosophila* [40] up into ultrasonic frequencies of 80-100 kHz in moths and other insects with effective bat anti-predation [49]. In *Drosophila* species, auditory receptors are tuned by active physiological mechanisms for robust sender/receiver coupling [50]. These active mechanisms appear to be in the ciliated sensory dendrites of the JO neurons [45], and like the mammalian cochlear amplifier, include mechanical feedback from transduction channel gating [51] as well as cellular motility, in this case active movement of the sensory cilium in contrast to mammalian outer hair cell electromotility. The *Drosophila* mechanotransduction channels include NompC (a TRPN channel with homologs in frogs and fish but not birds and mammals), and the TRPV channel subunits encoded by *iav* and *nan* genes [52]. For more extensive review of *Drosophila* auditory mechanisms, please see reviews by [39, 47, 53-55] and others.

Noise-Induced Hearing Loss in *Drosophila*

To understand how research in *Drosophila* hearing mechanisms could illuminate the molecular pathways underlying noise-induced hearing loss, we began to study the responses of flies exposed to loud noise [46]. To achieve the goal of maximal duty cycle stimulation in the antenna, which is not tonotopically organized, we elected to use a pure tone trauma stimulus of 250 Hz which approximates the antenna's best frequency [46]. Flies subjected to this tone at 120 dB for 24 hr showed significantly reduced hearing immediately after the trauma, as measured by extracellular potentials in the antennal nerve, which carries the axons of the sensory neurons. These sound-evoked potentials (SEPs) are roughly equivalent to compound action potentials recorded from the mammalian auditory nerve. The SEP amplitudes were reduced up to 50% immediately after trauma, relative to untraumatized controls, with partial recovery after 1 day and full recovery by 7 days post-trauma [46]. Remarkably, the sensory neurons did not die, as would mammalian hair cells subjected to similar levels of noise trauma. Nevertheless, in addition to effects on SEP amplitude, noise trauma also resulted in significant SEP latency increases on the order of 50 μ s, similar to mammalian results [46].

Effects of Noise Trauma on *Drosophila* Auditory Cells

To understand the effects of noise on the biology of the sensory neurons, we compared fixed antennae from traumatized and control animals prepared for TEM [46]. While no gross morphological abnormalities were found, noise trauma significantly reduced mitochondrial size in auditory neurons, suggesting mitochondrial fission, a common outcome of cell stress including in noise-exposed mammalian auditory hair cells. Interestingly, mitochondrial size reduction appeared to be a delayed effect, only seen 7 days post-trauma but not in the 0 day or 1 day post-trauma animals. Hair cell mitochondrial dysfunction has been described in mammalian noise-induced hearing loss, suggesting parallel pathways in noise damage in flies and mammals [56].

To begin evaluating genetic susceptibilities to noise-induced hearing loss in *Drosophila*, we performed experiments on flies with a reduced gene dosage of a Na^+/K^+ -ATPase β subunit that is specifically expressed in JO neurons, compared to control flies with both copies intact [46]. While these heterozygous flies had normal SEP amplitudes without noise trauma, noise-exposed flies exhibited exacerbated SEP amplitude reductions that recovered at a slower rate. Furthermore, they had smaller JO neuron mitochondria even in untraumatized flies, with exaggerated mitochondrial reduction at all time points after trauma [46]. These findings indicate that in this model system it is feasible to identify subtle genetic factors with no discernible effects under control conditions, but whose effects are revealed only under conditions of noise trauma. Such factors represent genetic pre-dispositions to noise-induced hearing loss, which have been very difficult to identify in human genetics [57].

Because this was the first study of noise-induced hearing loss in an insect system, much work still needs to be done to understand the mechanisms through which the JO neurons respond to noise damage, why they recover within a few days, and how disruption of molecular pathways can reveal the biology of overstimulation in this system. Then it will be possible to take advantage of the strongest feature of the *Drosophila* model system, namely the ability to apply sophisticated genetic manipulations to dissecting the pathways. Furthermore, the high throughput possible in this system may lend itself to small molecule screens and drug screens that could interfere with the damage or progression of noise-induced hearing loss.

Conclusion

Despite their divergent anatomy, physiology, and evolutionary history, anemone hair bundles and *Drosophila* Johnston's organ share many fundamental traits with vertebrate hair cells. They perform similar functions with mechanotransduction machinery having a significant degree of homology. Yet the anemone and fly models each possess unique strengths that make them useful for studies of noise damage and repair.

Sea anemone hair bundles and vertebrate hair cells are vulnerable to similar chemicals known to mimic various forms of trauma, and perhaps surprisingly may share similar mechanisms of repair. Our knowledge of the repair process in anemones still remains rudimentary, with many questions unanswered. We still do not know the exact biochemical mechanism of recovery from Ca^{2+} -free exposure mediated by fractions β and γ . The identity of the myriad other factors in RP is unknown. The temporal discrepancy between behavioral recovery of hair bundle function (~8 min) and morphological/electrophysiological recovery (42-6 min) remains a mystery. Finally, the exact nature of anemone RP-mediated repair of lateral line neuromast function in teleosts must be elucidated and expanded upon. For example, do anemone RP aid in the repair of other vertebrates – avians, and especially mammals?

Similarly, the *Drosophila* model has now been shown to display physiological and anatomical sequelae upon noise trauma, including reduced auditory response amplitudes with increased latencies, and mitochondrial changes consistent with metabolic and oxidative stress, which we know occurs in noise-exposed vertebrate hair cells. The analysis of noise-

induced hearing loss in *Drosophila* is very recent, so there are still many urgent questions. What gene regulatory changes in JO neurons result from noise damage, and what molecular pathways contribute to the recovery? What is the significance of mitochondrial changes to the response and the recovery? The myriad *Drosophila* genetic tools to express markers, RNAi constructs, and study mutations open the door to unlimited possibilities for exploration of the fundamental mechanisms of noise response and recovery.

It would appear that mechanosensory cell trauma and repair share an ancient lineage, which gives the anemone and fly model systems a unique advantage – the ability to comparatively study a genetic/physiological process in taxa far removed from most models of hair cell trauma. This enables the analysis of highly fundamental and conserved processes of hair cell trauma and repair, which in turn sheds light on current work in studying both the molecular and cellular mechanisms of noise trauma and potential therapeutic interventions.

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Key Point Summary

- Sea anemone hair bundles and *Drosophila* chordotonal organs are ancestrally related to mammalian auditory hair cells and fish lateral line neuromasts.
- Noise-induced mammalian auditory hair cell damage resembles noise damage in fly chordotonal organs and sea anemone hair bundle damage induced by low Ca^{2+} .
- Sea anemone hair bundles exhibit remarkably rapid repair with secreted proteins, which, when applied to damaged fish lateral line neuromasts, can accelerate their repair and recovery, despite the enormous phylogenetic distance between these organisms.
- Discovery of noise-induced hearing loss in *Drosophila* promises inexpensive and high throughput approaches for systematic genetic dissection of molecular and cellular mechanisms underlying noise-induced damage and recovery.
- These models reveal ancient homologies in response and recovery pathways that can be exploited for therapeutic design to mitigate human noise-induced hearing loss.

Figure 1.

Anemone hair bundle anatomy and mechanotransduction. (a) The hair bundle is a multicellular complex consisting of a single sensory neuron (sn), from which projects a central kinocilium (k) and several large-diameter stereocilia (ls). Surrounding the neuron are multiple hair cells (hc) which project many small-diameter stereocilia (ss) from their apical surfaces, which connect to the large-diameter stereocilia by inter-stereociliary linkages (not shown). Modified from [6]. (b) Vibration arising from prey/probe movement (indicated by left arrow) cause deflection of linked stereocilia. This movement causes hydrodynamic shearing forces creating strain on tip-links (tl) connecting stereocilia on hair cells on the side closest to the 'positive' (left) direction, opening force-gated mechanotransduction channels, allowing increased cation influx (represented by open circles with '+' symbols) and depolarization of the hair cell. Simultaneously, stereociliary deflection of hair cells lying on the opposite side (right) of the neuron experience a 'negative' deflection, slackening tip-link tension, closing mechanotransduction channels and decreasing cation influx, and hyperpolarizing the hair cell. ax – axon of sensory neuron; n – nucleus. Other abbreviations as in (a). Modified from [3].

Figure 2.

Schematic overview of *Drosophila* scolopidium (a) and vertebrate hair cell (b) anatomy. Scolopidia consist of 2-3 sensory neurons each projecting a single sensory ciliated dendrite with biochemically and functionally distinct proximal and distal regions. The dendrites are encapsulated by scolopale and cap cells. Acoustic stimulation of the fly's antenna results in oscillatory extension/compression of scolopidia along the longitudinal axis (indicated by diagonal arrow in a), engaging the mechanotransduction apparatus to depolarize the neurons. This is functionally analogous to vertebrate hair cell depolarization by acoustically-generated forces (indicated by horizontal arrow in b) acting on stereocilia along the auditory sensory epithelium. Modified from [47].