Chapter 7
Auditory Transduction

Daniel F. Eberl, Azusa Kamikouchi, and Joerg T. Albert

Abstract Auditory transduction, the process of converting acoustic energy into a nerve signal, couples the sound-evoked motion of an external receiver structure to the gate of a mechanosensitive ion channel. This chapter summarizes the physiological landscape of insect chordotonal auditory receptors, highlighting features that have informed the understanding of the central mechanisms and specializations of insect auditory transducers and their variation. Primarily based on combined genetic and functional experiments in the Johnston's organ of *Drosophila*, we present the current understanding of the molecular complexes associated with auditory transduction. The roles of the ciliary dendritic structures are integrated with those of the ion channels and associated complexes in the ciliary membrane. Finally, the chapter includes speculation on the foci of these mechanisms that may contribute to diverse physiological responses in insect auditory receptors.

Keywords Active mechanical amplification • Chordotonal organ • *Drosophila* • Johnston's organ • Katydid crista acustica • Locust Müller's organ • Mechanosensitive channel • Mechanotransduction • Moth ultrasonic hearing • NompC • Receptor lymph • Scolopale cell • Scolopidia • Stick insect • TRP channel

D.F. Eberl (✉)
Department of Biology, University of Iowa, Iowa City, IA 52246, USA
e-mail: daniel-eberl@uiowa.edu

A. Kamikouchi
Graduate School of Science, Nagoya University, Chikusa, Nagoya,
Aichi 464-8602, Japan
e-mail: kamikouchi@bio.nagoya-u.ac.jp

J.T. Albert
The Ear Institute, University College London,
332 Gray's Inn Road, London WC1X 8EE, UK
e-mail: joerg.albert@ucl.ac.uk

7.1 Introduction

For a microphone, transduction is conversion of the energy from sound waves into an electrical signal. Similarly, in sensory biology, auditory transduction is the process of converting acoustic energy into a nerve signal. The acoustic energy is captured by a physical structure that resonates with the sound. This acousto-mechanical transformation of the energy allows the subsequent transduction of the resulting mechanical signal into a change in membrane potential. Among insects, there are many kinds of receiving structures, from thinned cuticular membranes or tympana that respond to pressure oscillations or gradients to antennae that oscillate in the particle displacement of near-field sound to trichoid sensilla that respond to air currents or near-field sound (Yack 2004).

With the exception of the trichoid sensilla, each of these insect auditory receptors relies on chordotonal organs, named after their mechanistic arrangement as a string or "chord" under tension. These sensory organs are also called scolopidia, based on their structural organization with a spindle-shaped scolopale cell surrounding the neuronal dendrite to enclose it in a large extracellular cavity filled with receptor lymph (Boekhoff-Falk and Eberl 2014).

This chapter surveys major designs in insect auditory organs, with a focus on ways of activating the auditory sensory neurons. Highlighted examples illustrate salient features of auditory sensory neuron physiology. A description of the molecular features of insect auditory mechanotransducers follows, with work done primarily on *Drosophila*. The chapter ends with important outstanding questions and key molecules that have yet to be identified.

7.2 Physiology of Transduction

Rather than reviewing extensively the research on physiological responses in insect auditory neurons, which has been done elsewhere (Fullard and Yack 1993; Field and Matheson 1998; Mason and Faure 2004; Nakano et al. 2015; Pollack 2015), this section summarizes selected historical vignettes that reveal salient features of chordotonal neuron physiological properties. These examples have been extensively reviewed; here they are briefly summarized in the context of how they inform the transduction mechanism.

7.2.1 Roeder's Moth Ear Recordings

Perhaps one of the most important glimpses into the physiology of insect auditory transduction comes from the classical work of Ken Roeder on the moth auditory afferents (Roeder 1967). Many noctuid moths have a well-developed ultrasonic auditory capacity used in antipredation behavior against bats. These moths have
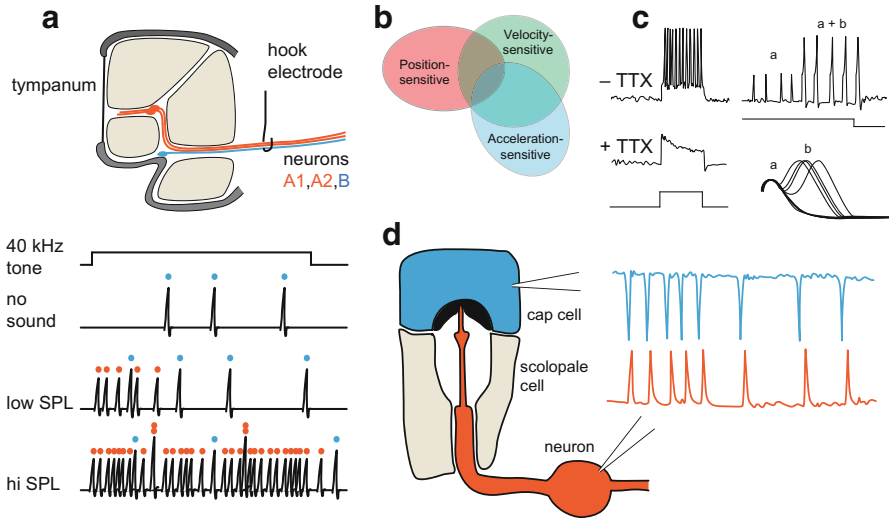
Fig. 7.1 Physiological insights into chordotonal neuron transduction. (**a**) Schematic view of Roeder's (1967) electrophysiological recordings from noctuid moth ears, which have two auditory scolopidia (A1 and A2, *orange* neurons) that innervate the ligament underlying the tympanal membrane. Using a hook electrode, he recorded activity in the auditory nerve that contains axons from these two neurons and a nonauditory neuron (B, *blue*). With no sound, only the B unit fires (*blue dots*), while a 40-kHz tone at low sound pressure level (SPL) evokes an adapting response from the A1 neuron (*orange dots*), and higher sound pressure evokes activity from both A neurons (*orange dots*), with occasional coincident firing (*double orange dots*). (**b**) Many experiments on stick insect and locust scolopidia reveal enormous variation in scolopidial response types, including position-sensitive, velocity-sensitive, and acceleration-sensitive units, as well as some combinatorial types. Not depicted are other dimensions in which these classifications can change depending on stimulus frequency or direction of movement (based on data summarized by Field and Matheson 1998). (**c**) Schematic view, based on data from Hill (1983), of intracellular recordings from locust Müller's organ auditory neuron activity in the presence of tetrodotoxin (TTX), which blocks spike formation, that reveal graded adapting receptor potentials during a 100-ms tone (*left*). Without TTX, there is no sign of adaptation. Two classes of spikes are often seen in these intracellular recordings (*right*), which Hill termed "apical" (**a**) and basal (**b**), inferred from the location of their origin in the dendrite. Apical spikes are always seen in the initial response to a tone, while basal spikes arise out of the apical spikes, evidenced by the initial shoulder on the large spikes. Superimposing a number of large and small spikes reveals a variable delay in the basal component of the large spikes. (**d**) Oldfield and Hill (1986) reported simultaneous intracellular recordings from cap cells (*blue*, also called attachment cells) and from the soma of the cognate neuron (*orange*) as schematized here from Oldfield's data. Oldfield inferred that each downward spike in the cap cell response (*blue trace*) is a negative imprint of the receptor potential, because the cap cell contacts the receptor lymph from which cations pass through mechanotransducer channels during activation. Accordingly, each cap cell negative spike is followed with a very short delay by an action potential in the neuron (*blue trace*)

metathoracic tympanal organs with two chordotonal neurons, A1 and A2 (Fig. 7.1a). Using hook electrodes to record from the tympanal nerve, Roeder took advantage of the differences in spike heights and firing patterns to identify these two units in the resulting traces, along with another nonchordotonal unit (the B cell) in the tympanic

nerve IIIN1b. The B cell extends multiple dendrites into the vicinity of the articulating cuticle under the wing and reports proprioceptively on stresses imposed on the tympanic apparatus during wing movements in flight. Whereas the B cell changes its firing rate, with little adaptation, from about 5 to 300 Hz as wing position changes, the auditory chordotonal neurons respond directly to sound. These A neurons respond to sounds in the frequency range of 3 Hz to 300 kHz (Roeder 1967; Moir et al. 2013), with highest sensitivity in the middle range of about 50–70 kHz, consistent with a bat antipredation function. The two A cells show adaptation to long or continuous acoustic stimuli but no frequency discrimination (cf. Adams 1972). The A1 cell shows a lower threshold, responding to low or moderate sound levels, with A2 beginning to fire only with sounds about 25 dB louder than the A1 threshold, reflecting the behavioral urgency of a bat at close range. Minimal adaptation of the A cells occurs under stimulation with very short tone bursts that resemble a calling bat's cries. As Roeder (1967, p. 47) summarizes, "The intensity of an ultrasonic pulse is coded in the A-axon discharge as: (i) the number of A spikes per second; (ii) the activity in one or both A cells; (iii) the duration of the after-discharge; and (iv) the response time." These coding properties hold across the 40-dB suprathreshold range, above which the A cells saturate.

What can be learned about transduction from these observations? First, the number of sensory cells in the entire tympanal organ is two, so the full auditory output of the organ is represented, while at the same time, single-unit activity is distinguishable. Second, the fact that the two A cells show a different threshold reflects physiological differentiation among the sensory neurons. This could be explained by differences in the mechanisms for action potential generation, but, alternatively, it leaves open the possibility of underlying differences in the transduction mechanism itself. Third, with such high-frequency acoustic stimulation, which transforms into tympanic membrane vibrations of the same frequency, the transduction mechanism does not result in a cycle-by-cycle pattern of action potentials in the axon. Thus, a certain amount of integration takes place in, or subsequent to, the transduction events in the sensory cell. In insects with large clusters of scolopidia, the nerve response might result in tracking a higher frequency through a population mechanism.

7.2.2 Stick Insect and Locust Femoral Chordotonal Organ Recordings

At first glance, it may seem odd to examine femoral chordotonal organs, which mediate no known hearing function in stick insects and locusts. However, these are arguably the most extensively studied chordotonal receptors at the single-unit level. Importantly for our purposes, Field and Matheson (1998) classified 22 distinct categories of physiological responses among intracellular receptor cell recordings depending on whether they respond to position, velocity, acceleration, or a combination thereof and whether they respond in the direction of flexion, extension, or

both (Fig. 7.1b). Furthermore, superimposed on these classifications is that some receptors changed categories depending on stimulation properties such as frequency (Kondoh et al. 1995). This level of receptor physiological specialization suggests that chordotonal receptors could exhibit enormous variation in transduction mechanism. It should be mentioned that there also may be mechanisms beyond the transduction apparatus that could contribute to this diversity, including mechanical and viscoelastic properties of the dendritic cap and cellular linkages to the apical and basal cuticle, subcuticular epithelium, or apodeme; the nature of intercellular adhesion junctions; the compliance of the scolopale; the ultrastructural and mechanical properties of the sensory cilium; and the composition of the receptor lymph, as well as the post-transduction physiological events in the receptor cell itself.

Because the elucidation of the molecular apparatus underlying chordotonal mechanotransduction is still in the sprouting stage, with initial insight primarily in Johnston's organ of *Drosophila* (see Sect. 7.3), it is still not clear which mechanisms could contribute to such large diversity in physiological responses. The results of single-unit analysis mentioned in this section cannot at present meaningfully enlighten the understanding of the transduction mechanisms. Importantly, this variation should strongly motivate research to discover the molecular basis of differences in transduction mechanisms.

7.2.3   Hill's Locust Müller's Organ Recordings

To approach more closely the precise events of transduction in chordotonal neurons, Hill's intracellular recordings (Hill 1983) from the locust Müller's organ are particularly revealing. On acoustic stimulation of increasing intensity, Hill observed graded potentials at the lower intensities (Fig. 7.1c). As stimulus intensity increased, evoked action potential spikes emerged, superimposed on the graded potentials, with both increasing graded potential amplitudes and increasing spike rates. This suggests a typical neuronal response from these sensory neurons. These neurons also exhibited adaptation in the spike rate during 100-ms tone stimuli. To distinguish further the receptor potentials (the transduction events) from action potentials, Hill found that tetrodotoxin (TTX) application to the preparation eliminated the action potential spikes, while the graded potentials remained (Fig. 7.1c). This allowed a more clear assessment of transduction, including verification of the graded nature of the receptor potentials and, in some of the cells, adaptation during the 100-ms tone stimuli. Although most of the recordings were from the neuron soma, Hill (1983) inferred that some electrodes penetrated the neuron in the apical dendritic regions. In these regions, the cell shows small spikes (termed apical spikes, based on inferred electrode position) that are of uniform amplitude, like an action potential, but without a baseline undershoot in the repolarizing phase, unlike a conventional action potential (Fig. 7.1c). Other records display so-called basal spikes, more like conventional action potentials in character, or a combination of apical and basal spikes. When these are combined, the basal spikes invariably arise out of the

apical spikes, suggesting that when the traveling membrane depolarization reaches a certain point along the dendrite, an enhanced action potential generation mechanism engages. It is unfortunate that the electrode locations in these experiments could not be verified by an independent method. Nevertheless, if Hill's (1983) interpretations are correct, then, taken together, the immediate transduction event results in brief spontaneous discrete depolarizations or evoked graded receptor potentials, presumably in the ciliary region of the dendrite. Given sufficient summation, these potentials become small spikes in the apical dendrite, and these small spikes may in turn become full-fledged action potentials once they pass a critical region in the basal dendrite. The action potentials will propagate along the entire cell and axon to the axon terminals.

7.2.4   Oldfield's Katydid Crista Acustica Recordings

Whereas many insects lack fine frequency discrimination, instead emphasizing recognition of temporal patterning in the acoustic stimuli, other insects exhibit specialized frequency discrimination mechanisms. One such mechanism is tonotopy, which spatially separates frequencies along an anatomical gradient. Tonotopy is exemplified by the katydid crista acustica, a specialized distal segment of the subgenual organ in the prothoracic tibia (Oldfield 1982; Oldfield and Hill 1986). Depending on species, the crista acustica contains 20–50 scolopidia arranged along the dorsal surface of a tracheal tube. In some katydids, such as *Copiphora gorgonensis*, dispersive wave propagation in the acoustic vesicle adjacent to the crista acustica is initiated through a lever mechanism from the tympanal plate, allowing both amplification and tonotopic frequency separation (Montealegre-Z et al. 2012). The crista acustica responds to frequencies in the range of 4–70 kHz, and Oldfield (1982) showed in *Caedicia simplex* that the scolopidial neurons at the proximal end of the crista acustica had their lowest thresholds at low frequencies, with a gradient of sensitivities to high frequencies at the distal end. Experiments described by Oldfield and Hill (1986) on intracellular recordings in the same species have greatly informed the understanding of the transduction process. As in Hill's (1983) work in the locust, these intracellular recordings from the receptor neuron soma generate two separable event categories, which the authors interpret as large and small spikes, where the small spike can occur in the absence of a large spike, but the large spikes always initiate with a small spike embedded as a shoulder in the rising phase. These events resemble Hill's (1983) apical and basal spikes (Fig. 7.1c). Understanding of these events is greatly enhanced by their subsequent experiments recording intracellularly from the scolopidial cap cells that form the apical attachment (Fig. 7.1d). From these cells, Oldfield and Hill (1986) recorded spikes that were antiphase to those in the receptor neurons. Importantly, simultaneous recordings from the cap cell and the receptor neurons supported a temporal coupling between the cap cell activity and the smaller of the spikes in the receptor cell, these small spikes representing the receptor potential, which may or may not elicit an action potential, the

larger spike. Because the cap cell membrane contacts the receptor lymph, Oldfield and Hill (1986) argue that the cap cell record reflects the ionic changes in the receptor lymph as ions flow through the opened mechanosensitive channels in the cilium, hence the reverse polarity in the spike from this cell. Consistent with this interpretation of the small spikes representing receptor potentials, hyperpolarization of the neuron soma by current injection blocked the large spikes but not the small ones, and negative current injection into the cap cell while the neuron soma was hyperpolarized evoked a burst of the small spikes in the neuron.

These experiments may represent, to date, the most direct electrophysiological access to the transduction event itself in chordotonal organs. The tight envelopment of the dendrite by the scolopale cell has presented a challenging obstacle to reliable insertion of an electrode in the dendrite. Perhaps the development of optical recording techniques, such as the genetic expression of Arclight, a gene-encoded fluorescent voltage sensor (Cao et al. 2013), in *Drosophila* will facilitate the spatial resolution of membrane voltage events associated with transduction.

7.3   Auditory Mechanotransducers

The molecular apparatuses of mechanotransduction, and more specifically the molecular identities of auditory mechanotransducer channels (aMETs), have been at the center of an ongoing controversy ever since a direct mechanical gating of aMETs was proposed (Corey and Hudspeth 1983). For the ears of both vertebrates (Furukawa and Ishii 1967; Corey and Hudspeth 1979) and insects (Albert et al. 2007), submillisecond response latencies have been reported, which are widely considered to be too short to result from an indirect, second messenger-mediated form of activation. Instead, a mechanical activation has been postulated in which the mechanical stimulus directly alters the free energy differences between the closed and open forms of the channels, thereby effectively coupling the channels' open probabilities to the mechanical stimulus (Corey and Hudspeth 1983). As a result, aMETs are directly gated by sound. A mechanical form of channel activation implies an elastic coupling of aMETs to external (i.e., extracellular) sound-receiving structures. The inherent reciprocity of this coupling, in turn, is bound to introduce distinct mechanical signatures of the gating, and adaptation, of aMETs into these external receiver structures: the receivers will be easier to move (i.e., more compliant) over that range of stimulus forces and displacements at which transducer gating occurs. Once the ion channels are all open or all closed, the receiver structure will be stiffer and thus more difficult to move. This phenomenon was named "gating compliance" and the serial elasticities that couple the receivers to the transducers are commonly referred to as "gating springs." Gating compliances have been reported for the auditory cells of both vertebrates (Howard and Hudspeth 1988) and insects (Albert et al. 2007). However, the molecular nature of the corresponding transducer channels, or their gating springs, has not yet been resolved.
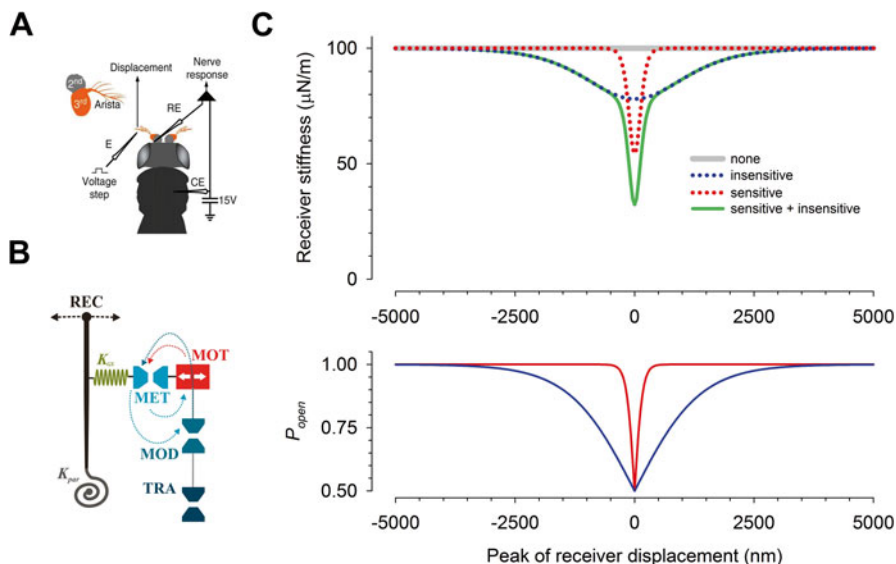
Fig. 7.2 A *Drosophila* model of auditory mechanotransduction. (**A**) Combined biomechanical and electrophysiological studies of the antennal ear of *Drosophila* have been used to dissect the mechanisms, and molecules, of auditory mechanotransduction. (**B**) The emerging view of mechanotransducer function in *Drosophila* sees external stimulus receiving structures (REC) serially coupled to mechanotransducer channels (MET) via force-transmitting elastic elements (K_{GS}). A parallel stiffness (K_{par}) summarizes all serial elasticity that does not contribute to directing forces to the MET. Motor proteins (MOT) are thought to act in series with the MET, mediating both adaptation to, and amplification of, sound-evoked signals. Downstream of transduction, further ion channels modify (MOD) the transducer signals and eventually transform them into action potentials (TRA). (**C**) Mechanotransduction in the fly's Johnston's organ (JO) has been linked to at least two independent types of MET, each of which introduces characteristic signatures into the mechanics of the antennal sound receiver. One of the two populations depends on the function of NompC and is part of a sensitive transduction pathway that contributes to sound sensation. A second population is independent of NompC and part of a less sensitive (or "insensitive") transduction pathway that contributes to the sensation of wind and gravity. The *top panel* shows the resulting dynamic receiver stiffness for four hypothetical scenarios. *Gray*: Both transducer populations are blocked, no gating whatsoever (constant stiffness); *blue*: sensitive transducers are blocked, only insensitive transducers are gated (stiffness drops over a wide range of displacements); *red*: Insensitive transducers are blocked, only sensitive transducers are gated (stiffness drops over a narrow range of displacements); *green*: both sensitive and insensitive transducers are gated (dual stiffness drop over both the narrow and the wide range). The *bottom panel* depicts the underlying open probabilities of the two transducer populations (*red*: sensitive, *blue*: insensitive)

7.3.1 Studying aMETs in **Drosophila**: Current Insights and Ongoing Controversies

The mechanosensory Johnston's organ (JO) of *Drosophila* resides in the second segment of the antenna (Fig. 7.2A). Over the past 10 years, the JO has been developed into a powerful model for the functional and molecular dissection of mechanosensation and specifically auditory mechanotransduction (Nadrowski et al. 2011;

Boekhoff-Falk and Eberl 2014; Albert and Göpfert 2015). Current models of fly auditory mechanotransduction have mainly focused on the specific roles of different transient receptor potential (TRP) channels expressed by the various cell types of JO (Fig. 7.2B). Belonging to the group of chordotonal organs (Field and Matheson 1998; Kavlie and Albert 2013), JO is formed by an array of approximately 200 scolopidia (see also Kamikouchi and Ishikawa, Chapter 10). Each scolopidium of JO typically comprises one to three neurons (JO-Ns) and three support cells (JO-SCs). At present, nine TRP channels have been linked to the function of the *Drosophila* JO (Zanini and Göpfert 2014), with expression reported for both JO-Ns and JO-SCs. Studies on auditory transduction proper have concentrated on three neuronal TRPs in particular: the TRPN1 channel No-mechanoreceptor-potential C (NompC) (Eberl et al. 2000), which localizes to the distal region of the mechano-sensory cilium of JO-Ns and the two vanilloid transient receptor potential (TRPV) channels Nanchung (Nan) (Kim et al. 2003) and inactive (Iav) (Gong et al. 2004), which localize to the proximal part of the JO-N cilium, where a Nan/Iav dimer is deemed to form, or contribute to, a heteromultimeric channel complex (Gong et al. 2004). Mechanotransducer complexes introduce multiple nonlinearities into the response behaviors of hearing organs; this is most evident perhaps in mechanical feedback amplification, where a transducer-based process adds mechanical energy to the sound-evoked motion of a stimulus-receiving structure (Göpfert et al. 2006) (see also Sect. 7.3.2 and Windmill and Jackson, Chapter 6). Loss-of-function mutations of both NompC and Nan/Iav impair mechanically evoked responses in JO: loss of Nan/Iav abolishes compound action potential (CAP) responses in the antennal nerve completely (Kim et al. 2003; Gong et al. 2004) but increases mechanical, transducer-based amplification, whereas loss of NompC leads to a strong reduction, though not a complete loss, of CAP amplitudes (Eberl et al. 2000; Effertz et al. 2011) but virtually abolishes the mechanical, transducer-based amplification (Göpfert et al. 2006; Effertz et al. 2011). Based on the near-complete loss of transducer-based feedback amplification in the ears of *nompC-null* mutant flies and the increase in auditory amplification seen in *nan/iav* mutants, it was suggested that NompC might be part of true mechanotransducer channels in auditory neurons of *Drosophila*, with Nan/Iav channels playing a downstream role in mechanical gain control and action potential generation. This suggestion is consistent with the finding that the loss of NompC leads to characteristic reductions in gating compliance, which mimic the reduction in gating compliance seen after ablation of JO auditory neurons (Effertz et al. 2012). The NompC-based model of JO auditory transduction was contested, however, by a study that reported that sound-evoked giant fiber neuron (GFN) activation persists in NompC-null mutants (Lehnert et al. 2013). As the study also found that a loss of Nan/Iav, in contrast, completely abolishes the sound-evoked GFN currents, it was suggested that Nan/Iav might form the transducer channel in JO auditory neurons. Clearly, further clarification, particularly in the form of single-cell, patch-clamp recordings of JO-Ns, is required to decide whether NompC, Nan/Iav, or a third, as yet unnamed, ion channel constitutes the JO auditory transducer channel proper. Studies on *Drosophila* touch-sensitive neurons have, however, demonstrated beyond a reasonable doubt that NompC can indeed form, or contribute to, a true mechanotransducer channel (Yan et al. 2013).

7.3.2   Active Mechanical Amplification

Although fly mechanotransducer modules are still incompletely understood on the molecular level, their contributions to sensitive hearing, which in flies just as in vertebrates relies on an active process, have been analyzed in quantitative detail. A model built on the assumption that mechanically gated ion channels act in series with adaptation motor proteins could explain the response behavior of the fly's antennal ear to small stimuli, including the characteristic intensity- and frequency-dependent nonlinearities of the active process (Nadrowski et al. 2008; Fig. 7.2B, C).

The cellular basis of the active process in the antennal ear of fruit flies has been traced to JO-Ns. In fruit flies, genes that affect the function of JO-Ns, such as *beethoven* (*btv*), *touch-insensitive-larva B* (*tilB*), and *no-mechanoreceptor-potential A* (*nompA*) are necessary for active amplification (Göpfert and Robert 2003; Göpfert et al. 2005). Mutations in *btv*, *tilB*, and *nompA* cause structural defects (Eberl et al. 2000; Chung et al. 2001; Kavlie et al. 2010), which affect the mechanical properties of, or the stimulus coupling to, the JO-N dendrites. The gene *nompA* encodes an extracellular linker protein expressed in the caps, which connect the cilia of JO-Ns to the hook (Chung et al. 2001). Mutations in *nompA* disconnect JO neurons from the antennal receiver, leading to a complete loss of active amplification and elimination of the sound-evoked nerve responses (Eberl et al. 2000; Göpfert et al. 2005). Mutations in the *btv* and *tilB* genes, in turn, cause structural defects in the axonemes, which are a characteristic structure of chordotonal cilia as well as of sperm (Eberl et al. 2000). The *btv* locus encodes the intraflagellar transport (IFT) dynein heavy chain of *Drosophila*, whereas *tilB* encodes a conserved leucine-rich repeat-containing ciliary protein (Kavlie et al. 2010). Sound-evoked electrophysiological responses of JO neurons are absent in *btv*, *tilB*, and *nompA* mutants.

In mammals, the gain of the cochlear amplifier (a summary term for the hair cell-based active process) is centrally controlled through efferent pathways (Frolenkov 2006). Axons of neurons that originate in the olivocochlear complex synapse on the outer hair cells to modulate their electrical and mechanical properties, thereby providing a mechanism for cochlear gain control. In contrast, mechanical feedback amplification in *Drosophila* is not under efferent control. Silencing transmission via chemical synapses in all neurons, which also disrupts signaling from and to JO-Ns, does not affect the amplificatory gain of the antennal ear (Kamikouchi et al. 2010). Amplification in the fly ear thus seems both generated and controlled locally within JO itself.

7.3.3   Supporting Auditory Transduction: Non-Neuronal Cell Types and Ionic Homeostasis of the Extracellular Space

Next to JO-Ns, JO-SCs have been linked to distinct mechanosensory roles in the *Drosophila* JO. One type of JO-SCs, the cap cell, has been reported to specifically express the TRP channel Pyrexia, which is thought to be required for gravity

sensation and gravity-related behaviors (such as the flies' negative geotaxis) (Sun et al. 2009). Another type of JO-SCs, the scolopale cell, has been shown to express specific α- (ATPα) and β- (Nrv2) subunits of the Na^+/K^+-ATPase. The knockdown of either subunit results in virtually complete deafness (Roy et al. 2013). The Na^+/K^+-ATPase of JO-Ns, in contrast, apparently uses a different β-subunit (Nrv3) (Roy et al. 2013). These findings not only highlight the multicellular nature of JO mechanotransduction, but they also stress the importance of ion homeostasis for auditory transducer function. The transducer sites in the JO-N cilia are tightly sealed against their environment by a cellular barrier formed of septate junction–linked epithelial and supporting cells (part of which are both cap and scolopale cells). The narrow cavity that is thus created around the transducers is thought to be filled with a receptor lymph, which differs from the common extracellular condition in that it is high in K^+ and low in Na^+. Electrogenic transport, such as through transmembrane ATPases in both neurons and associated supporting cells, appears to be a crucial requirement to keep the receptor lymph at the reported high positive potentials of +20 to +80 mV relative to the surrounding extracellular medium (Küppers and Thurm 1979; Kernan et al. 1994; Walker et al. 2000; Chung et al. 2001) and thereby providing a strong electrochemical driving force for currents through the transduction channels.

7.3.4 Mechanotransduction in JO: The Cilium and Mechanosensory Submodality

In *Drosophila*, there exist two classes of ciliated cells: spermatozoa and neurons of type I sense organs such as JO. The cilia of JO-Ns, which are located in the apical parts of their dendrites, are an essential component of the cells' mechanosensory organelles. It is the cilia that are widely deemed to host the mechanotransduction machinery proper. A key step in the differentiation of all eukaryotic cilia is the localization, and formation, of the basal body. Basal bodies, which designate the proximal end of the later cilium, serve as nucleation centers from which the microtubular axoneme can grow toward the distal end of the cilium. Ciliary development depends on the Rfx transcription factor (Durand et al. 2000; Laurençon et al. 2007). The ChO-specific transcriptional regulator Fd3F co-regulates chordotonal-specific ciliary genes in tandem with the pan-ciliary transcription factor Rfx (Newton et al. 2012). Several gene products have been localized to, and implicated in the function of, the basal bodies of JO-Ns; these include, for example, the coiled-coil domain proteins Chibby (Cby) (Enjolras et al. 2012), Uncoordinated (Unc) (Kernan et al. 1994; Baker et al. 2004), and Dilatory (Dila) (Ma and Jarman 2011). From the basal body, the sensory cilium is assembled through an intraflagellar transport (IFT) process that includes the anterograde kinesin II motor complex (Sarpal et al. 2003) and the IFT protein No mechanoreceptor potential B (NompB) (Han et al. 2003), as well as the retrograde dynein heavy chain 1b motor and the IFT protein Reduced mechanoreceptor potential A (RempA) (Lee et al. 2008). The fully differentiated

cilium of JO-Ns is a highly compartmentalized subcellular structure specialized for the transmission, and transduction, of mechanical stimuli; a vital part of JO-N ciliogenesis is therefore the generation of JO-specific ciliary compartments. RempA is crucial for this subcompartmentalization (Lee et al. 2008), and the microtubule-associated doublecortin homolog-containing DCX-EMAP is required for the differentiation (Bechstedt et al. 2010). The Fd3F-dependent transcriptional control also includes a direct regulation of Nan and Iav, the two interdependent TRVP-channel proteins required for JO auditory function (Gong et al. 2004).

As detailed in Sect. 7.3.1, multiple ion channels have been linked to the cell-type-specific properties, and overall mechanosensory function, of JO. The sequence of events that leads from a sound-induced displacement of the dendritic cap to the generation of action potentials in the JO-N axons appears to involve a signaling chain through various TRP channels located at characteristic positions within the distal, or proximal, cilium. A *Drosophila* member of the Tubby-like protein (TULP) family, dTulp, is required for the correct TRP-channel localization (Park et al. 2013).

The transmission, transduction, and amplification of sound-induced mechanical stimuli performed by JO are biophysically and molecularly complex processes, which have been the subject of intense scientific enquiry and progress during the past decade; through the identification of some of its key developmental and homeostatic requirements, its sensory complexity is becoming better understood. The different neuronal subpopulations of JO, which vary in their respective sensitivities to oscillatory (e.g., sound) or stationary (e.g., wind/gravity) stimulation have initially been defined anatomically by their distinct target zones within the fly's brain (Kamikouchi et al. 2006). The homeodomain transcription factor Engrailed (En) is a sufficient, and most likely necessary, requirement for the subpopulation-specific axonal targeting of JO auditory neurons to the GFN (Pézier and Blagburn 2013; Pézier et al. 2014). Misexpression of En in En-negative JO-Ns causes them to form ectopic chemical and electrical synapses with the GFN; RNAi-mediated knock-down of En expression in En-positive JONs, in turn, reduces the strengths of JO-N to GFN synaptic connections. This finding is of particular interest as En is also a key regulatory factor for guidance cues that pattern retinal axon terminals in the vertebrate midbrain (Fuchs et al. 2012).

7.3.5   *Beyond the Mechanosensory Canon: Unorthodox Findings from the* Drosophila *JO*

Genetic inventories of fly (Eberl et al. 1997) and nematode (Chalfie and Sulston 1981; Chalfie and Au 1989) mechanosensory organs have provided the fundament for understanding the molecular logic of mechanosensory, and also specifically auditory, transduction. The various findings have been integrated into a canonical view that rests firmly on the interaction of three key players: ion channels, motor proteins, and cytoskeletal tethers (Chalfie 2009). A microarray-based transcriptome analysis of the *Drosophila* JO (Senthilan et al. 2012) largely confirmed the

prevailing view but added a few twists to the story by reporting the expression of key components of the phototransduction cascade, including four of the fly's seven rhodopsins (Rh3, Rh4, Rh5, Rh6). Furthermore, it was shown that loss-of-function mutations of these rhodopsins greatly reduce both mechanical and electrical signatures of mechanotransducer gating in the fly's antennal ear. These findings point to specific, but as yet unknown, roles of "visual" proteins in mechanosensation and may well mark the beginning of a redefined concept of sensory specificity and its molecular requirements. The same screen, most notably, also found the pheromone-binding protein Os-C, and several ionotropic receptors, currently associated with chemosensory transduction, expressed in the *Drosophila* ear; their roles, however, have not yet been tested.

7.4 Summary

The hearing organs of insects, and the acoustic communication systems they are part of, have been the subject of intense scientific enquiry for many decades. But it was only during the last 15 years or so that we have seen substantial, and rapidly growing, progress in our understanding of the very process that brings about hearing, namely, the process of auditory transduction. Fueled by the genomic revolution and pioneered by an insect, *Drosophila melanogaster*, new ion channel families (such as TRP channels) have been identified and linked to specific roles within the auditory transduction chain. But in research as in life, the devil is in the details and the proof is in the pudding and so we are still lacking unequivocal evidence demonstrating which proteins actually form the auditory transducer channels proper. The current controversy has it that this is either NompC or a heteromeric complex built of Nan and Iav. For both possibilities there are supporting as well as contradicting data. Recent research has zoomed in on auditory transducer complexes in unprecedented molecular detail but, it seems, still not close enough to clearly resolve the component identities and their specific mechanisms. On a much more general level, and light years away from the missing final details, the molecular inventory of the *Drosophila* ear has brought to light substantial, but mechanistically as yet elusive, contributions of visual proteins, such as rhodopsins, to the process of auditory mechanotransduction. These findings shake up our concepts of sensory specificity and will clearly be hot spots of future auditory research.

Various insect ears of both the tympanal and flagellar type have been shown to display the hallmarks of active amplification (as first proposed for the human cochlea). It is currently not clear if a transducer-based feedback mechanism, such as the one reported for *Drosophila*, is the common molecular source of the observed amplification; if this turns out to be the case, it will be very interesting to see if transducer-based feedback amplifiers have evolved independently in different insect groups or rather share a common origin. Furthermore, among the JO-Ns in *Drosophila*, only two major classes have been identified, those that underlie hearing and are likely to be phasic (very fast adapting) and those that subserve wind and

gravity and are likely to be more tonic (slowly adapting). This relative simplicity is an advantage for fundamental investigations of transduction mechanisms and their genetic basis, but in time one hopes to understand the complexities that contribute to the broader scolopidial physiological variation. All taken together, research on insect auditory transduction is thus a prime example to show that basic research, more than anything else, deals with the unknown unknowns. If conducted properly, it will often discover more new questions than the many answers to old ones.

References

Adams, W. B. (1972). Mechanical tuning of the acoustic receptor of *Prodenia eridania* (Cramer) (Noctuidae). *Journal of Experimental Biology, 57*, 297–304.

Albert, J. T., & Göpfert, M. C. (2015). Hearing in *Drosophila. Current Opinion in Neurobiology, 34*, 79–85.

Albert, J. T., Nadrowski, B., & Göpfert, M. C. (2007). Mechanical signatures of transducer gating in the *Drosophila* ear. *Current Biology, 17*, 1000–1006.

Baker, J. D., Adhikarakunnathu, S., & Kernan, M. J. (2004). Mechanosensory-defective, male-sterile *unc* mutants identify a novel basal body protein required for ciliogenesis in *Drosophila. Development, 131*, 3411–3422.

Bechstedt, S., Albert, J. T., Kreil, D. P., Müller-Reichert, T., Göpfert, M. C., & Howard, J. (2010). A doublecortin containing microtubule-associated protein is implicated in mechanotransduction in *Drosophila* sensory cilia. *Nature Communications, 1*, 11.

Boekhoff-Falk, G., & Eberl, D. F. (2014). The *Drosophila* auditory system. *WIREs Developmental Biology, 3*(2), 179–191.

Cao, G., Platisa, J., Pieribone, V. A., Raccuglia, D., Kunst, M., & Nitabach, M. N. (2013). Genetically targeted optical electrophysiology in intact neural circuits. *Cell, 154*(4), 904–913.

Chalfie, M. (2009). Neurosensory mechanotransduction. *Nature Reviews Molecular Cell Biology, 10*(1), 44–52.

Chalfie, M., & Sulston, J. (1981). Developmental genetics of mechanosensory neurons of *Caenorhabditis elegans. Developmental Biology, 82*, 358–370.

Chalfie, M., & Au, M. (1989). Genetic control of differentation of the *Caenorhabditis elegans* touch receptor neurons. *Science, 243*, 1027–1033.

Chung, Y. D., Zhu, J., Han, Y.-G., & Kernan, M. J. (2001). *nompA* encodes a PNS-specific, ZP domain protein required to connect mechanosensory dendrites to sensory structures. *Neuron, 29*, 415–428.

Corey, D. P., & Hudspeth, A. J. (1979). Response latency of vertebrate hair cells. *Biophysical Journal, 26*(3), 499–506.

Corey, D. P., & Hudspeth, A. J. (1983). Kinetics of the receptor current in bullfrog saccular hair cells. *Journal of Neuroscience, 3*(5), 962–976.

Durand, B., Vandaele, C., Spencer, D., Pantalacci, S., & Couble, P. (2000). Cloning and characterization of *dRFX*, the *Drosophila* member of the RFX family of transcription factors. *Gene, 246*, 285–293.

Eberl, D. F., Duyk, G. M., & Perrimon, N. (1997). A genetic screen for mutations that disrupt an auditory response in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA, 94*, 14837–14842.

Eberl, D. F., Hardy, R. W., & Kernan, M. (2000). Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. *Journal of Neuroscience, 20*, 5981–5988.

Effertz, T., Wiek, R., & Göpfert, M. C. (2011). NompC TRP channel is essential for *Drosophila* sound receptor function. *Current Biology, 21*, 592–597.

Effertz, T., Nadrowski, B., Piepenbrock, D., Albert, J. T., & Göpfert, M. C. (2012). Direct gating and mechanical integrity of *Drosophila* auditory transducers require TRPN1. *Nature Neuroscience, 15*(9), 1198–1200.

Enjolras, C., Thomas, J., Chhin, B., Cortier, E., Duteyrat, J. L., Soulavie, F., et al. (2012). *Drosophila chibby* is required for basal body formation and ciliogenesis but not for Wg signaling. *Journal of Cell Biology, 197*(2), 313–325.

Field, L. H., & Matheson, T. (1998). Chordotonal organs of insects. In P. D. Evans (Ed.), *Advances in insect physiology* (Vol. 27, pp. 1–228). San Diego: Academic Press.

Frolenkov, G. I. (2006). Regulation of electromotility in the cochlear outer hair cell. *Journal of Physiology, 576*, 43–48.

Fuchs, J., Stettler, O., Alvarez-Fischer, D., Prochiantz, A., Moya, K. L., & Joshi, R. L. (2012). Engrailed signaling in axon guidance and neuron survival. *European Journal of Neuroscience, 35*(12), 1837–1845.

Fullard, J. H., & Yack, J. E. (1993). The evolutionary biology of insect hearing. *Trends in Ecology & Evolution, 8*, 248–252.

Furukawa, T., & Ishii, Y. (1967). Neurophysiological studies of hearing in goldfish. *Journal of Neurophysiology, 30*(6), 1377–1403.

Gong, Z., Son, W., Chung, Y. D., Kim, J., Shin, D. W., McClung, C. A., et al. (2004). Two interdependent TRPV channel subunits, Inactive and Nanchung, mediate hearing in *Drosophila*. *Journal of Neuroscience, 24*, 9059–9066.

Göpfert, M. C., & Robert, D. (2003). Motion generation by *Drosophila* mechanosensory neurons. *Proceedings of the National Academy of Sciences of the USA, 100*, 5514–5519.

Göpfert, M. C., Humphris, A. D. L., Albert, J. T., Robert, D., & Hendrich, O. (2005). Power gain exhibited by motile mechanosensory neurons in *Drosophila* ears. *Proceedings of the National Academy of Sciences of the USA, 102*, 325–330.

Göpfert, M. C., Albert, J. T., Nadrowski, A., & Kamikouchi, A. (2006). Specification of auditory sensitivity by *Drosophila* TRP channels. *Nature Neuroscience, 9*, 999–1000.

Han, Y.-G., Kwok, B. H., & Kernan, M. J. (2003). Intraflagellar transport is required in *Drosophila* to differentiate sensory cilia but not sperm. *Current Biology, 13*, 1679–1686.

Hill, K. G. (1983). The physiology of locust auditory receptors. II. Membrane potentials associated with the response of the receptor cell. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 152*, 483–493.

Howard, J., & Hudspeth, A. J. (1988). Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog saccular hair cell. *Neuron, 1*(3), 189–199.

Kamikouchi, A., Shimada, T., & Ito, K. (2006). Comprehensive classification of auditory sensory projections in the brain of the fruit fly *Drosophila melanogaster*. *Journal of Comparative Neurology, 499*, 317–356.

Kamikouchi, A., Albert, J. T., & Göpfert, M. C. (2010). Mechanical feedback amplification in *Drosophila* hearing is independent of synaptic transmission. *European Journal of Neuroscience, 31*(4), 697–703.

Kavlie, R. G., & Albert, J. T. (2013). Chordotonal organs. *Current Biology, 23*(9), R334–R335.

Kavlie, R. G., Kernan, M. J., & Eberl, D. F. (2010). Hearing in *Drosophila* requires TilB, a conserved protein associated with ciliary motility. *Genetics, 185*, 177–188.

Kernan, M., Cowan, D., & Zuker, C. (1994). Genetic dissection of mechanosensory transduction: Mechanoreception-defective mutations of *Drosophila*. *Neuron, 12*, 1195–1206.

Kim, J., Chung, Y. D., Park, D.-Y., Choi, S., Shin, D. W., Soh, H., et al. (2003). A TRPV family ion channel required for hearing in *Drosophila*. *Nature, 424*, 81–84.

Kondoh, Y., Okuma, J., & Newland, P. L. (1995). Dynamics of neurons controlling movements of a locust hind leg: Wiener kernel analysis of the responses of proprioceptive afferents. *Journal of Neurophysiology, 73*(5), 1829–1842.

Küppers, J., & Thurm, U. (1979). Active ion transport by a sensory epithelium. I. Transepithelial short circuit current, potential difference, and their dependence on metabolism. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 134*, 131–136.

Laurençon, A., Dubruille, R., Efimenko, E., Grenier, G., Bissett, R., Cortier, E., et al. (2007). Identification of novel regulatory factor X (RFX) target genes by comparative genomics in *Drosophila* species. *Genome Biology, 8*, R195.

Lee, E., Sivan-Loukianova, E., Eberl, D. F., & Kernan, M. J. (2008). An IFT-A protein is required to delimit functionally distinct zones in mechanosensory cilia. *Current Biology, 18*, 1899–1906.

Lehnert, B. P., Baker, A. E., Gaudry, Q., Chiang, A.-S., & Wilson, R. I. (2013). Distinct roles of TRP channels in auditory transduction and amplification in *Drosophila*. *Neuron, 77*(1), 115–128.

Ma, L., & Jarman, A. P. (2011). Dilatory is a *Drosophila* protein related to AZI1 (CEP131) that is located at the ciliary base and required for cilium formation. *Journal of Cell Science, 124*(Pt 15), 2622–2630.

Mason, A. C., & Faure, P. A. (2004). The physiology of insect auditory afferents. *Microscopy Research & Technique, 63*, 338–350.

Moir, H. M., Jackson, J. C., & Windmill, J. F. C. (2013). Extremely high frequency sensitivity in a 'simple' ear. *Biology Letters, 9*(4), 20130241.

Montealegre-Z, F., Jonsson, T., Robson-Brown, K. A., Postles, M., & Robert, D. (2012). Convergent evolution between insect and mammalian audition. *Science, 338*, 968–971.

Nadrowski, B., Albert, J. T., & Göpfert, M. C. (2008). Tranducer-based force generation explains active process in *Drosophila* hearing. *Current Biology, 18*, 1365–1372.

Nadrowski, B., Effertz, T., Senthilan, P. R., & Göpfert, M. C. (2011). Antennal hearing in insects: New findings, new questions. *Hearing Research, 273*, 7–13.

Nakano, R., Takanashi, T., & Surlykke, A. (2015). Moth hearing and sound communication. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 201*(1), 111–121.

Newton, F. G., zur Lage, P. I., Karak, S., Moore, D. J., Göpfert, M. C., & Jarman, A. P. (2012). Forkhead transcription factor Fd3F cooperates with Rfx to regulate a gene expression program for mechanosensory cilia specialization. *Developmental Cell, 22*(6), 1221–1233.

Oldfield, B. P. (1982). Tonotopic organisation of auditory receptors in Tettigoniidae (Orthoptera: Ensifera). *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 147*, 461–469.

Oldfield, B. P., & Hill, K. G. (1986). Functional organization of insect auditory sensilla. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 158*, 27–34.

Park, J., Lee, J., Shim, J., Han, W., Lee, J., Bae, Y. C., et al. (2013). dTULP, the *Drosophila melanogaster* homolog of Tubby, regulates transient receptor potential channel localization in cilia. *Public Library of Science Genetics, 9*(9), e1003814.

Pézier, A., & Blagburn, J. M. (2013). Auditory responses of engrailed and invected-expressing Johnston's organ neurons in *Drosophila melanogaster. PLoS ONE, 8*(8), e71419.

Pézier, A., Jezzini, S. H., Marie, B., & Blagburn, J. M. (2014). Engrailed alters the specificity of synaptic connections of *Drosophila* auditory neurons with the giant fiber. *Journal of Neuroscience, 34*(35), 11691–11704.

Pollack, G. S. (2015). Neurobiology of acoustically mediated predator detection. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 201*(1), 99–109.

Roeder, K. D. (1967). *Nerve cells and insect behavior* (rev. ed.). Cambridge, MA: Harvard University Press.

Roy, M., Sivan-Loukianova, E., & Eberl, D. F. (2013). Cell-type–specific roles of Na^+/K^+ ATPase subunits in *Drosophila* auditory mechanosensation. *Proceedings of the National Academy of Sciences of the USA, 110*(1), 181–186.

Sarpal, R., Todi, S. V., Sivan-Loukianova, E., Shirolikar, S., Subramanian, N., Raff, E. C., et al. (2003). *Drosophila* KAP interacts with the kinesin II motor subunit KLP64D to assemble chordotonal sensory cilia, but not sperm tails. *Current Biology, 13*(19), 1687–1696.

Senthilan, P. R., Piepenbrock, D., Ovezmyradov, G., Nadrowski, B., Bechstedt, S., Pauls, S., et al. (2012). *Drosophila* auditory organ genes and genetic hearing defects. *Cell, 150*(5), 1042–1054.

Sun, Y., Liu, L., Ben-Shahar, Y., Jacobs, J. S., Eberl, D. F., & Welsh, M. J. (2009). TRPA channels distinguish gravity sensing from hearing in Johnston's organ. *Proceedings of the National Academy of Sciences of the USA, 106*, 13606–13611.

Walker, R. G., Willingham, A. T., & Zuker, C. S. (2000). A *Drosophila* mechanosensory transduction channel. *Science, 287*, 2229–2234.

Yack, J. E. (2004). The structure and function of auditory chordotonal organs in insects. *Microscopy Research & Technique, 63*, 315–337.

Yan, Z., Zhang, W., He, Y., Gorczyca, D., Xiang, Y., Cheng, L. E., et al. (2013). *Drosophila* NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature, 493*(7431), 221–225.

Zanini, D., & Göpfert, M. C. (2014). TRPs in hearing. *Handbook of Experimental Pharmacology, 223*, 899–916.