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Comparative Aspects of Hearing in Vertebrates and Insects with Antennal Ears

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The evolution of hearing in terrestrial animals has resulted in remarkable adaptations enabling exquisitely sensitive sound detection by the ear and sophisticated sound analysis by the brain. In this review, we examine several such characteristics, using examples from insects and vertebrates. We focus on two strong and interdependent forces that have been shaping the auditory systems across taxa: the physical environment of auditory transducers on the small, subcellular scale, and the sensory-ecological environment within which hearing happens, on a larger, evolutionary scale. We briefly discuss acoustical feature selectivity and invariance in the central auditory system, highlighting a major difference between insects and vertebrates as well as a major similarity. Through such comparisons within a sensory ecological framework, we aim to emphasize general principles underlying acute sensitivity to airborne sounds.

Introduction

Auditory physiology offers a distinctive perspective on the interaction between a sensory system and its environment. On the one hand, auditory systems in vertebrates and insects with sensitive hearing are capable of remarkable performances on multiple levels, both within the sensory periphery where the minute energies associated with sound are converted into electrical signals, as well as within higher-order brain areas where complex natural stimuli such as human speech are processed. For example, displacements caused by acoustical stimuli in the inner ear at the threshold of hearing are sub-nanometer and comparable to the distance between atoms in molecules [1]. Furthermore, thermal fluctuations in the ear's mechanotransduction apparatus not only are significant, but also can be larger than the faintest audible signals, making signal detection a challenging task [2]. Ascending the sensory hierarchy, one encounters other marvels of evolution, such as the ability of individual neurons to encode — using millisecond-long action potentials — inter-aural time differences of only about ten microseconds, and to use this information to localize the source of the sound [3,4]. No engineered system has yet been designed that could understand distorted speech in a noisy and reverberating environment with multiple speakers. That our auditory system achieves this feat is testament to its remarkable performance.

On the other hand, an engineer could argue that the auditory system's performance is objectively poor, even in animals with sensitive hearing: at the very first step, during the mechanoelectrical transduction in the inner ear, external sounds are distorted, or even completely suppressed, while new tones are generated by the ear itself [5]. Forward and backward masking, illusory percepts of nonexistent tones (such as the Zwicker illusion [6]), perceptual merging of separate auditory streams, the precedence effect suppressing the perception of echoes that has been demonstrated in insects and vertebrates [7,8] (and which some blind people can unsuppress), auditory hallucinations, and a frustrating inability to distinguish between distinct phonemes of a foreign language — all of these phenomena indicate that evolution has not shaped the auditory system as an objective detector of acoustical reality. Instead, the auditory system, like any biological system, has evolved to help animals to find food, escape predators, and mate. The sensory ecology of each species, together with the laws of physics, are therefore the major factors controlling animal hearing.

Because sensitive hearing evolved independently multiple times in different animals, a comparison of hearing in these animals is useful for understanding the fundamental principles that govern the structure and function of the auditory system [9,10]. In this review, we use this comparative approach to highlight several fundamental mechanisms of hearing in the peripheral and central auditory systems of insects and vertebrates, discussing similarities as well as differences in the context of the animals' sensory ecology.

Peripheral Auditory System

Insects and vertebrates both transduce acoustic energy into electrical signals using highly specialized structures that, although implemented differently, follow the same biophysical principles. In both groups of animals, the need for a fast transduction of sound-evoked vibrations must be achieved through a direct coupling of the mechanical stimulus to the mechanosensitive ion channels [11]. The direct coupling assures speed but leads to nonlinearity — and therefore sound distortions — because thermodynamics imposes a nonlinear dependence of the channels' open probability on the stimulus intensity. Yet the advantage of speed is apparently greater than the disadvantage of sounds being distorted by the ear (in some cases, as we will discuss below, these distortions may even be exploited as signals in their own right). Likewise, the functional advantage offered by frequency tuning, the amplification of weak sounds, and (at least in vertebrates) the ear's ability to convert a million-fold range of sound intensities into a hundredfold range of mechanical or neural responses outweighs the disadvantages of the inner ear's instability and metabolic vulnerability (i.e., the requirement for a self-regulatory energy-expending mechanism

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to maintain the system's control parameters in the desired range in order to benefit functionally from the oscillatory instability) [12]. Thus, natural selection has produced ears that operate close to a bifurcation [12], which produces amplification, frequency selectivity, and a gain control — characteristics that underlie sensitive hearing in vertebrates as well as in some insects.

Insects, Sound and the Auditory Periphery

The Encyclopedia Britannica defines sound as "*a mechanical disturbance from a state of equilibrium that propagates through an elastic material medium*". The term 'sound' thus simply describes mechanical forces travelling through a (gaseous, liquid or solid) substrate, whereby the transmission of these forces occurs through series of elastic collisions between the particles within that substrate.

In animal hearing one major task of the auditory system is to secure the audibility of a distinct spectrum of such sound-associated forces, namely those of biological relevance. Sorting biologically relevant signals from biologically irrelevant noise involves the active filtering and partial amplification — or suppression — of distinct spectrotemporal features of sound. The auditory systems of different species thus display distinct degrees of auditory specificity, i.e., species-specific adaptations of their hearing ranges. Corresponding functional properties have been studied and reported for a wide range of auditory systems, including the mammalian cochlea [13] as well as insect ears [14].

In insects, where evolutionary pressures for miniaturization and 'concurrent engineering' are particularly high as a result of their small size, much of the required filtering and pre-processing already takes place in the auditory periphery, namely at the level of the auditory transducer modules. By auditory transducer modules, we mean the auditory mechanotransducer channels proper, together with elastic components (collectively referred to as gating springs), which funnel forces to the mechanotransducer channels thus controlling their gating, as well as adaptation motors, acting in series with the mechanotransducer channels and providing the forces mediating adaptation and amplification.

Insect ears can be grossly divided into two major types: tympanal ears, in which sound-associated forces are detected by, and exert pressure on, a thin membrane area (analogous to the eardrum of the mammalian ear); and antennal ears, which act as pendulums driven by sound-induced particle motion of the surrounding air. Whereas the pressure-sensitive tympanal ears act as far-field sensors that can operate at very high frequencies (up to 100 kHz or even higher) over long distances (tens of meters), the particle-velocity-sensitive antennal ears act as near-field sensors operating at lower frequencies (typically <1 kHz) over shorter distances that are not expected to exceed a few centimeters. We will focus our discussion in this review almost exclusively on antennal ears, which in many ways are intriguingly similar to the cellular substrates of vertebrate hearing, i.e., the mechanosensory hair cells. Considering the evolutionary distance between insects and vertebrates, it is remarkable how well hair-cell-based models of mechanotransduction [15] can capture mechanotransduction in the antennal ears of *Drosophila* [16]. We will here use the antennal ears of two dipteran insects ('true flies'), namely drosophilids and mosquitoes, to illustrate how the auditory periphery can play a

leading role within the sensory ecology of an entire acoustic communication system (Figure 1). The auditory transducer modules, as we will show, confer substantial amounts of auditory specificity to the antennal ears of both mosquitoes and drosophilid flies. Most interestingly, a recent study has directly implicated the auditory transducer channels of a bush cricket species (*Mecopoda elongata* L.) in frequency discrimination [17], suggesting that mechanotransducer channels also make substantial contributions to the filtering and pre-processing of sound in tympanal ears.

The Sensory Periphery of *Diptera*

Auditory Anatomy and General Principles of Function

The antennal ears of *Drosophila melanogaster* are formed by two functionally distinct anatomical compartments: the second antennal segment (A2 or pedicellus) houses the partially sound-sensitive neurons of the Johnston's organ (JO); and the third antennal segment (A3 or funiculus), together with a unilateral appendage named the arista, acts as the sound receiver (Figure 1B). Upon sound stimulation, A3 starts to rotate about its longitudinal axis [18], leading to the alternate stretching and compressing of two populations of JO neurons with opposite response polarity [19]. JO neurons are components of multicellular organules called scolopidia. The scolopidia of antennal ears consist of typically two (occasionally three) neurons plus three to four support cells [20,21]. The *Drosophila* JO houses ~200 scolopidia (corresponding to ~500 JO neurons), whereas the JOs of male mosquitoes can host up to ~7,500 scolopidia (corresponding to ~ 15,000 JO neurons as ~97% of mosquito scolopidia possess two neurons) [22]. As well as containing a much larger number of neurons, the mosquito antenna differs in another important aspect from the ear of the fruit fly. The *Drosophila* antenna, as described above, forms a rotating pendulum (with only one mechanical degree of freedom), whereas the mosquito antenna conforms to the ancestral 'flagellar' condition, where the antenna's flagellum acts as an inverted pendulum that can swing in all directions within its plane of suspension, thus having two mechanical degrees of freedom (Figure 1E). The functional anatomy of the mosquito ear is likely to reflect the major role that is played by the sense of hearing in mosquito mate localization [23]. No phonotaxis, or other directional auditory behavior, has yet been reported for *Drosophila* (despite the distinct directionality of its antennal sound receiver; see [24] and below).

Sensory Ecology of Hearing I — Drosophila *Antennae as Active, Mechanical Pulse Extractors*

With regard to the passive vibrational properties of its cuticular joint (as manifest in freshly dead or CO_2-sedated animals), the *Drosophila* antennal sound receiver can be approximated as a moderately damped, simple harmonic oscillator with a linear response behavior [25]. Mechanically coupled to the receiver, however, are directly gated ion channels situated in the membranes of JO neurons. Essential nonlinearities, associated with the gating of mechanotransducer channels, in tandem with energy input from adaptation motors, render the antennal ear as a whole both active and nonlinear [25]. Even more than that, with regard to key mechanical properties, the antennal ears are in effect dominated by the properties of their auditory transducer modules [26]. In the following discussion we will briefly illustrate
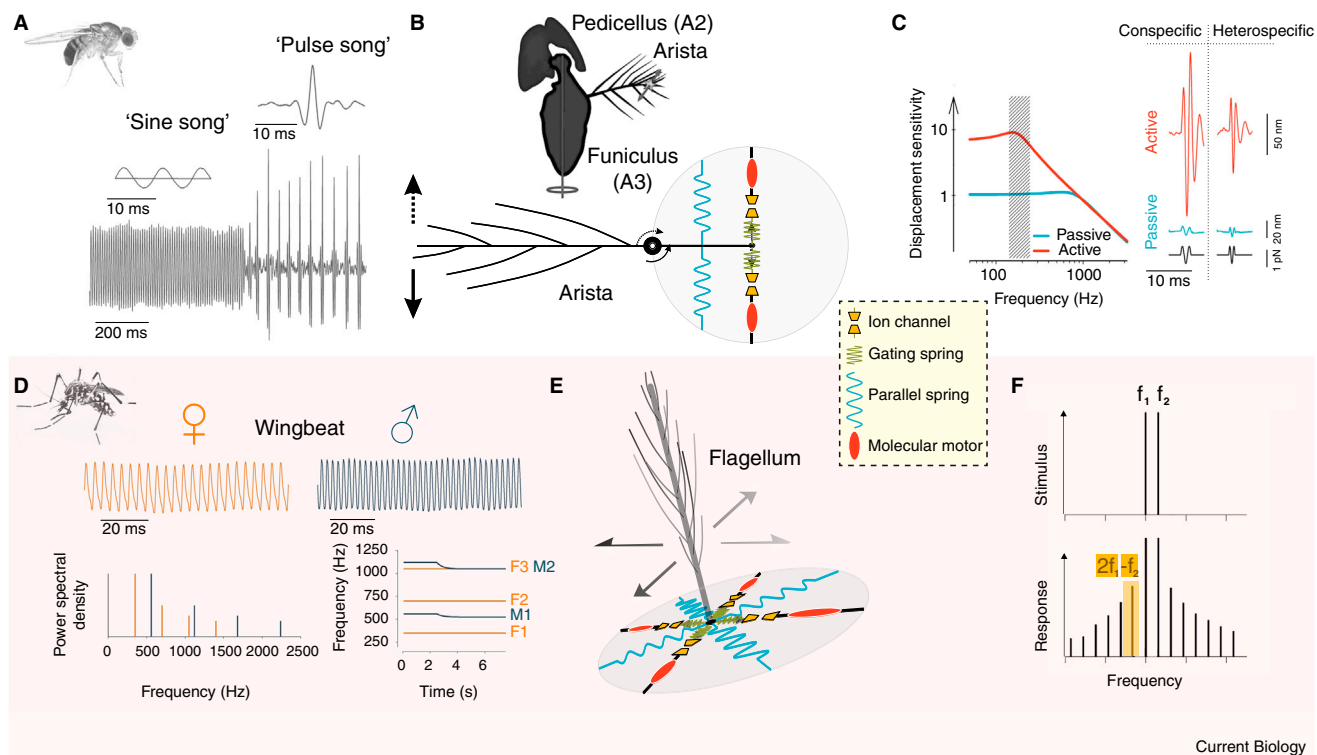
Figure 1. The sensory ecology of auditory transduction: antennal ears of Diptera.
(A–C) *Drosophila*; (D–F) mosquitoes. The landscape of signals used in the acoustic communication systems of fruit flies and mosquitoes is spectrally simple. It involves either pure tones (left panel in (A), *Drosophila* sine songs; upper panels in (D), mosquito wingbeat) or minor modifications of sinusoidal oscillations (right panel in (A), *Drosophila* pulse song). In some cases the emitted signal contains prominent higher-order harmonics next to its fundamental frequency (lower panels in (D), mosquito wingbeat). In both males (M) and females (F) the emitted signal (lower panels in (D), mosquito wingbeat) contains prominent higher-order harmonics (shown: M2, F2, F3) next to its fundamental frequency (M1, F1). (B,E) Sound signals in both fruit flies and mosquitoes are transduced by the mechanosensory neurons of the Johnston's organ in the second antennal segments (A2) of their antennae, which are coupled to specialized sound receiver structures formed by more distal antennal parts. Within the membranes of these neurons, directly gated mechanotransducer channels are thought to associate with molecular motors to provide stimulus adaptation and amplification. In *Drosophila*, the third antennal segment (A3 or funiculus), together with a unilateral appendage named the arista, acts as the sound receiver, whereas in mosquitoes, the sound-receiving role is taken over by the flagellum. (C) Left: In *Drosophila*, transducer-based mechanical feedback amplification increases the antennal displacement sensitivity within the spectral range of courtship song components (hatched area) by a factor of ~10 (red) compared with the passive system (blue). Right: The example shows the antennal deflection in response to a song pulse for an entirely passive antenna of *D. teissieri* (e.g., dead or CO_2-sedated, without contributions from active transducer modules; blue traces) and the active system (with contributions from active mechanotransducers; red traces). Note the marked increase of antennal deflection for the active state and the relative increase seen for a conspecific courtship pulse compared with a (higher frequency) pulse of a different species (*D. yakuba*). Corresponding stimuli are shown in the bottom traces (black). (F) In the ears of mosquitoes, a nonlinear process (most likely linked to transduction) generates distortion products. Even when stimulated with only two pure tones, f_1 and f_2 (e.g., male and female wingbeat), the antennal response will display additional tones. As these additional tones can be particularly prominent in the lower frequency range (highlighted is the cubic distortion product $2f_1–f_2$), this behavior has been suggested as a mechanism to communicate beyond the actual auditory range (data recalculated from [30,130]).

how these molecular properties are matched to and enable the specific auditory tasks of the fly ear.

Behaviorally, hearing in flies is most closely linked to the acoustic communications that take place during the mating ritual [27]. Male flies vibrate their wings to 'sing' songs to the females. In *D. melanogaster*, these songs are spectrally very simple, containing longer (typically 0.2–1 second), sinusoidal, pure-tone-like components ('sine songs') alongside shorter (~5–10 millisecond) pseudo-sinusoidal waveforms ('pulses'). Pulses are repeated at species-specific inter-pulse intervals (IPIs) to form trains. The IPIs are thought to represent the major signal that mediates species recognition during courtship [28]. For the fly's auditory brain to be able to analyse the IPIs of the pulse trains, however, the ear must have detected these pulses in the first place. Individual pulses represent waveforms of carrier frequencies of between approximately 150 and 250 Hz [29,30]. In its linear regime, i.e.,

without any contributions from mechanotransducer gating, the fly's sound receiver is tuned to best frequencies of around 800 to 1,000 Hz. In its active state, the receiver's best frequency is shifted into the range of pulse carrier frequencies. But far more than simply spectrally matching a receiver to its biologically most relevant signals, the auditory transducer modules provide active amplification, increasing the antennal displacement response to individual conspecific pulses by around 10-fold (Figure 1C). By virtue of the underlying transducer-based process, the antennae are thus able to extract pulses out of a noisy environment in an efficient and frequency-dependent way.

One key feature of the essential nonlinearities that support hearing in both insects and vertebrates is that they become relatively more prominent the smaller the stimulus (and thus the fainter the sound) [31]. The dynamic range of system responses (both mechanical and electrical) is therefore heavily

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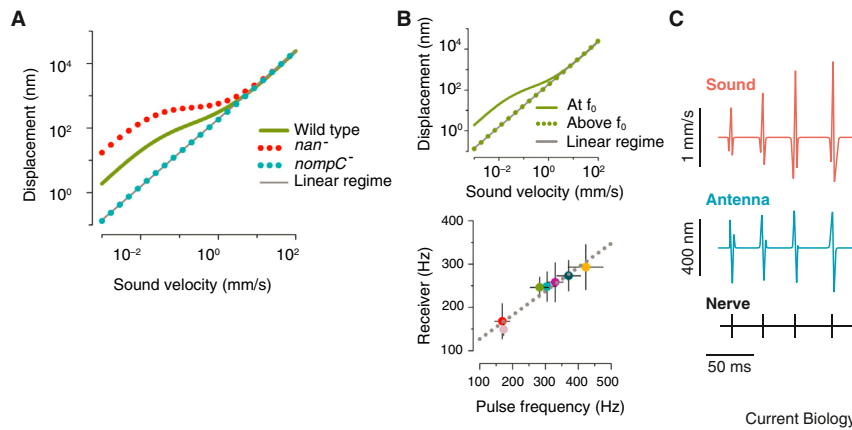
Figure 2. Transduction-dependent compressive nonlinearities reflect sensory ecological trade-offs in *Drosophila* acoustic communication.
(A) When stimulated by pure tones at its best frequency, the wild-type *Drosophila* antennal receiver displays a compressive nonlinearity, which produces relatively larger displacements for smaller stimuli (particle velocities), depicted by the green curve. This nonlinear compression originates from underlying saturating nonlinearities of mechano-transducer gating. Removing distinct functional components of the fly's auditory transduction chain, such as the key mechanotransduction channels NompC and Nanchung, can abolish (e.g., through loss of *nompC* function; *nompC*⁻, blue) or enhance (e.g., through loss of *nanchung* function; *nan*⁻, red) the extent of nonlinearity. The grey line depicts the linear regime of the passive antenna for comparison. (B) Top: Transduction-related nonlinear amplification is frequency-dependent; a loss of nonlinearity for stimulation ∼ 2.5-times above the receiver's best frequency (f_0) is shown. Bottom: The receivers of different *Drosophila* species (shown by the different coloured circles) have been shown to display differential best frequencies, which correlate almost linearly (grey line) with the spectral content of pulses produced by the corresponding conspecific males. (C) One direct result of nonlinear compression is the normalizing effect it has on response amplitude. Within the working range of the corresponding transducers, antennal deflections (middle trace, blue) will be larger for small sound intensities (upper trace, red). This effect is intensity dependent and becomes negligible for greater sound intensities, thereby reducing the effects of potential sound-amplitude differences. As stimulus amplitudes beyond the levels that saturate the transducers will not contribute to the nerve responses, this 'normalization' will be even greater at the level of the auditory nerve (bottom, black). As a result, a pulse train with natural amplitude variations will be converted into a train of nearly 'unitary' pulses, facilitating the robust encoding of inter-pulse intervals. Data recalculated from [30,130].

compressed and the ability to resolve amplitude differences is sacrificed in favour of enhanced absolute sensitivity (Figure 2). In the context of a real-world pulse train this means that, by amplifying pulse-evoked receiver displacements in an intensity-dependent way, the transducer-based process levels out amplitude differences between individual pulses, effectively 'normalizing' pulse amplitudes (although it should be noted that, within certain ranges, amplitude differences can still be resolved and that this process might be further facilitated by a range fractionation between different JO neurons).

In a communication system that operates with a rate code of relatively sparse events (trains typically consist of only 5–10 individual pulses) as well as in mechanically noisy conditions, the above system properties are likely to be adaptive. Most notably, the female ear must function during the actual mating ritual, i.e., when being chased by a singing male. Fly courtship has proven to be an interactive and dynamic behavior involving a continuous adjustment of signals [32,33], in some species even male–female duetting [34], as well as fast movements and turns, which will by themselves impose a considerable load of mechanical background noise on the ear. The job of the female's ear is made even more challenging by the fact that it is, by construction, acutely sensitive to variations in the angle of incidence of the sound stimulus [24]. Under these circumstances, a built-in, frequency-specific hearing aid, such as the one provided by the transducer-based process, will be of considerable value to boost the audibility of the biologically most relevant signals, i.e., individual song pulses. The widely reported intensity dependence of both frequency tuning and amplification in the *Drosophila* ear [30,35,36], which maximises the sensitivity and frequency match specifically for faint song components, increases the female's chance to catch every single pulse, however small. For larger pulse amplitudes, in turn, neither active amplification nor frequency optimization are required; the mechanical properties of the (higher-frequency) passive antenna will then dominate the antennal response and
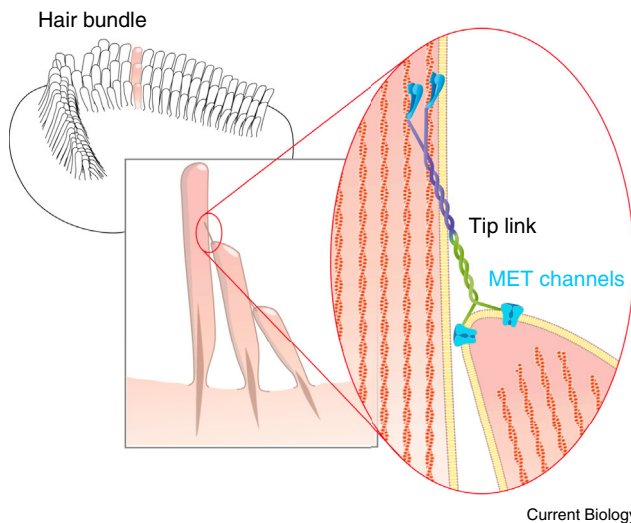
the (lower-frequency) song pulses will simply fall into the linear part of the passive antenna's displacement response. The fly's antenna was aptly named a love song receptor almost 40 years ago [37]. Today we can add that it does this job by acting as an active and efficient pulse extractor. Most remarkably, the receiver's fight for audibility seems to be fought on the sender side as well, as it has been reported that *Drosophila* males continuously and rapidly adjust courtship song intensity to the perceived distance from the females [32].

Beyond a pioneering study [38], which suggested that differential low-pass filtering properties of second order neurons might contribute to the decoding of species-specific IPIs, still very little is known about how pulse songs (or courtship songs more generally) are represented, and decoded, within the fly's auditory brain. Much progress in this regard, however, has been made over the last decades by seminal studies conducted in insects with tympanal ears, such as crickets. It would exceed the scope of this review to describe these advances here in detail but the interested reader is referred to a recent conceptual review of the topic by Hedwig [39].

Sensory Ecology of Hearing II — Mosquito Antennae Detect, and Transpose, Pure Tones

Above we have discussed how the antennal ears of *Drosophila* have been shaped by, and thus betray, the specific sensory-ecological needs of the flies' courtship behavior. In this regard, the flagellar ears of mosquitoes truly sing a song of their own.

Hearing in mosquitoes is mainly about hearing another mosquito's wingbeat. Males detect, locate and chase females by detecting, locating and chasing a female flight tone [40]. Both sexes, in turn, have been reported to respond to each other's wingbeat frequencies by modulating their own [41–43], an acoustic behavior thought to mediate male–female interactions within larger mating swarms. Just as in *Drosophila*, mosquito antennae have been reported to be active, nonlinear oscillators of exquisite nanometer-range sensitivity [23,44–46], with the source of the observed activity and nonlinearity likely

Figure 3. A schematic of a hair bundle, stereocilia, and a tip link connected to MET channels.
Left: A hair bundle of an outer hair cell (the cell body is not shown) with three rows of stereocilia. Middle: A column of three stereocilia of progressively varying height. Right: A magnification of the tip-link region, showing a pair of stereocilia filled with actin filaments (red), and a tip link composed of a cadherin-23 dimer (blue) and a protocadherin-15 dimer (green) attached to two MET channels (light blue).

being the same as in the fly, i.e., the auditory neurons proper and their presumably active auditory transducer modules. Rather than giving a comprehensive view on acoustic communication in mosquitoes, and on the role of the transducer machinery therein, we would like to concentrate on one particular example that showcases how the very periphery of hearing may pre-process and analyse sound. For three species it has been reported that the wingbeat-matching behavior results in a convergence of higher harmonics rather than of the fundamental frequencies [41,43,47]. In all three cases a match appears to be achieved around the second harmonic of the male wingbeat (M2) and the third harmonic of the female wingbeat (F3; Figure 1D). For a 'prototypical' mosquito species with a male wingbeat of ~600 Hz and a female wingbeat of ~400 Hz, this would result in a convergence at a frequency of ~1,200 Hz, which is commonly assumed to be considerably above the range of antennal mechanical sensitivity and thus above the mosquito hearing range.

Intriguingly, the solution to this sensitivity dilemma that the sensory periphery faces has been suggested to come from the sensory periphery itself [43,48]. It is a key feature of active hearing organs that the nonlinearities of the system together with an inherent reciprocity of force transmission must lead to the generation of distortion products [5]. In the case of the mosquito ear this means that, even when stimulated with only two tones f_1 and f_2 (representing, for example, male and female flight tones), the antennal displacement response will display peaks at a set of mathematically predicted additional frequencies, i.e., distortion products. One of them, the cubic distortion product $(2f_1-f_2)$, has been found to be particularly prominent in many auditory systems, including the mosquito ear [48]. As the frequency of this cubic distortion product is always lower than that of the primary tones (f_1 and f_2), it may act to transpose an otherwise 'inaudible' pair of tones into an audible tone. The hypothesized mechanism has particular traction in *Culex* mosquitoes, as the antennae in female *Culex* are tuned to frequencies well below the male and female wingbeats but close to the predicted cubic distortion product. Alternatively, as has been reported for *Aedes* mosquitoes [41], the antennal nerve may respond to higher frequencies of stimulation with a sustained DC-like response component, thus transposing the higher stimulus frequency even further into the lower frequency range. Future research is needed to resolve these questions, but in either case, it seems, the sensory periphery will take a centre-stage role. It will be particularly interesting to explore how the diverging sensory-ecological contexts of pure-tone-dominated hearing in mosquitoes and pulse-dominated hearing in fruit flies have shaped the molecular mechanisms behind these auditory functions.

Mechano-electrical Transduction in Vertebrates

Vertebrates convert sound energy into electrical signals using a single, evolutionarily conserved type of cellular transducer. Mechano-electrical transduction (MET) occurs in hair cells, the receptor cells of the inner ear. Each hair cell has a bundle of clavate microvilli, called stereocilia, densely filled with cross-linked actin filaments and surrounded by the plasma membrane (Figure 3). Their height increases along the axis of symmetry of the hair bundle. A molecular 'string' called a tip link connects two adjacent stereocilia of different heights and is composed of specialized cadherin molecules [49]. Its lower end (bearing protocadherin-15) is anchored at the tip of the shorter stereocilium where the MET channels are located [50] and its upper end (containing cadherin-23) is attached to the side of the taller stereocilium where myosin motors are found. The movement of the hair bundle towards the taller stereocilia stretches tip links and opens MET ion channels.

The gating-spring model describes the operation of this system quantitatively [15,51]. Tension in a gating spring (tip link) opens a MET channel. The open probability (P_0) as a function of displacement (X) follows the Boltzmann relation. Channel opening produces a movement, termed the gating swing, which relaxes the gating spring. A product of the gating-spring stiffness and the gating swing defines the single-channel gating force, determines the slope of the P_0–X relation, and sets the ear's sensitivity to sound. Sensitive hearing requires a steep P_0–X relation and therefore a large gating swing [52], which is supposed to originate somehow from the conformational rearrangement of the MET channel.

The gating spring is a key element of the gating-spring model and represents an elastic component, or a series of elastic components, whose tension determines the open probability of the MET channel. Other elastic elements, which lie in parallel with the gating springs (such as stereociliary pivots), are called parallel springs; their tension does not determine the open probability of the channel directly. What is a biological correlate of this model concept? Can the tip link be the gating spring? By comparing a hair bundle's stiffness before and after disrupting tip links with a calcium chelator, the gating-spring stiffness was estimated experimentally to be between 0.4 and 4 mN·m^{-1} [15,53]. Although tip links are conspicuous morphological candidates for gating springs, a crystal structure of

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Box 1. Can a tip-link polymer be a hair cell's gating spring?

All polymers can be divided into three classes depending on the ratio between their total length (contour length), L, and the persistence length, Lp, that determines the distance over which the thermal forces decorrelate the tangent along the contour, i.e., oppose the tendency of the polymer to point in the same direction.

- If $L \gg Lp$ then a polymer is flexible: it can form loops, knots, and hairpins. Because there is only a single straight conformation of a molecule, pulling on it and straightening it decreases the entropy: the stiffness of a flexible polymer has therefore a strong entropic component.
- If $Lp \gg L$, a polymer is stiff. Because $Lp \gg L$, the molecule is already straight, and therefore its stiffness is enthalpic and determined by the longitudinal compliance characterized by the elastic modulus.
- If L and Lp are approximately equal, however, a polymer is neither too flexible (does not form knots, for example) nor too stiff (is not a straight rod); it is semi-flexible. Most biological polymers, such as actin, tubulin, tropocollagen, and DNA are semi-flexible. For such polymers, both the entropic and the enthalpic components will contribute to the total stiffness.

To determine an order of magnitude for the stiffness of a tip link, its L, Lp, and the Young's modulus (E) of cadherins need to be known. The molecular dynamics force-extension curves (Figure S13 in Sotomayor and Schulten [54]) give a stiffness κ of C-cadherin in the presence of Ca^{2+} that is equal to 50 mN·m^{-1} (400 pN force for 8 nm extension). From which, using $E = \frac{\kappa L}{\pi r^2}$, the Young's modulus of $\sim$ 0.37 GPa follows (Sotomayor and Schulten [54] used L = 23 nm, and r = 1 nm). Thermal forces from the fluid will produce some bending and randomize the orientation of the polymer over the characteristic distance Lp. Unless the polymer is a homogeneous isotropic rod of a constant cross-section, the relationship between E and Lp is unknown. Assuming a Lp in the range of 20 to 100 nm from the EM micrographs in [49] — based on the observation that reconstituted tip links were not straight but had several bends — one can estimate the combined stiffness of a tip link under tension using an equation originally derived by Odijk [131] in the form given in Table 1 in [132]: $x = L\left[1 - \frac{1}{2}\left(\frac{k_B T}{F L_p}\right)^{1/2} + \frac{F}{K}\right]$ where x is the end-to-end distance, L is the contour length, F is force, and $K = \frac{\pi}{2}a^2 E$, where a is the radius of the tip link (4.5 nm). Adaptation motors provide several pN of tension on the tip link. Taking the tension of 10 pN from [133] and assuming Lp = 50 nm, one obtains the end-to-end distance of 162 nm (contour length 170 nm). The difference between the two lengths is stored in the transverse fluctuations. Then, a 1 pN increment of force elongates the polymer to 163 nm, corresponding to the stiffness of 2.7 mN·m^{-1}. With the tension of 5 pN and Lp = 50 nm, the end-to-end distance equals 159 nm (compared to 162 nm with 10 pN of tension). As expected, less tension corresponds to more transversal fluctuations. An additional 1 pN of force (making the total force 6 pN) gives the end-to-end distance of 160 nm; and the stiffness is 964 μN·m^{-1}. This value accords well with the experimentally estimated 0.4–4 mN·m^{-1} stiffness of the gating spring. Therefore, a tip link can be a hair cell's gating spring [134].

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cadherin-23 repeats indicates a value for a tip-link stiffness of 50 mN·m^{-1}, significantly greater than the experimentally estimated values [54]. Although it was concluded based on these data that the tip link could not be the gating spring, a polymer of the size of the tip link with the Young's modulus of cadherins and with a physiological level of tension has a stiffness that matches the experimentally estimated values (Box 1), suggesting that tip links could be the gating springs of hair cells. In order to resolve this question, it will be important to perform single-molecule force-extension experiments using isolated or reconstituted tip links to test their stiffness directly as a function of applied force [55].

The gating-spring model describes mechanotransduction both in vertebrate and in insect ears [51,56,57]. Since insects do not have tip links, other elements of the mechanotransduction complex, for example, the lipid bilayer and intracellular proteins in series with the transduction channels, such as the ankyrin repeat domains of the NompC mechanotransduction channel in insects, can perform the gating-spring role as well [58–62].

Although it is possible to evoke a physiological response during signal transduction by changing the activity of only a single protein — a notable example being the detection of individual photons by opsins in the retina — the collective activity of an ensemble usually provides a better signal-to-noise ratio. One hair cell may use over a hundred MET channels, located on a hair bundle's many stereocilia. Doing so, however, poses a formidable physical challenge because, unlike the insect antennae, hair cells are immersed in water, a viscous fluid. Viscous friction dissipates energy and dampens oscillations. Sharp frequency selectivity — a fundamental property of the vertebrate auditory system — is impossible due to these fluid–structure interactions unless stereocilia are grouped in a hair bundle [63]: we discuss the importance of these interactions in Box 2 and Figure 4.

Some hair bundles are directly stimulated by viscous forces, and their shape reflects this fact. The alligator lizard, for example, has free-standing hair bundles with extraordinarily long and thick stereocilia [64]. In mammals, inner hair cells are also stimulated hydrodynamically, and their stereocilia are twice as thick as those in outer hair cells, having a diameter of 500 nm compared with 250 nm. An outer hair cell would benefit from having as many stereocilia as possible in order to maximize the force produced by the hair bundle and to have as many MET channels per cell as possible to drive the electromotility. This probably accounts for why the outer hair cell stereocilia are arranged not in a line or semicircle as they are in inner hair cells but in a V- or

Box 2. How liquid in the inner ear has shaped the hair bundle.

A hair bundle operates at small Reynolds numbers on the order of 10^{-4}. The Reynolds number (Re) is defined by: Re = $uL\rho/\mu$, where u is the velocity, L is a linear dimension (e.g., a hair bundle's size), ρ is the density and μ is the dynamic viscosity of the fluid. The Reynolds number indicates the relative importance of inertia over viscous forces for a particular type of flow. For the hair bundle, a Reynolds number of much lower than 1 indicates the relative importance of viscous forces.

When a solid object moves through a viscous fluid, the velocity of the fluid directly in contact with the object's surface is zero: the fluid sticks to the wettable surface (the 'no-slip' condition). This condition usually applies to hydrophilic surfaces [135]. Therefore, a gradient of fluid velocity develops in the moving object's neighbourhood. A layer of fluid is dragged along with the moving object. How far this influence spreads into the fluid, i.e., how thick the 'boundary layer' is, depends on the velocity difference between the object and the fluid, on the size of the object, and on the fluid's density and viscosity (the same factors that determine the Reynolds number). For example, a cylinder of the size of a single stereocilium oscillating at 30 Hz in water will create a boundary layer that is about 73 μm thick. Oscillating, it drags a thick layer of fluid with it and creates a gradient of fluid velocity spreading to distances much larger than its own diameter — in fact, even larger than the size of the whole hair bundle.

A hair bundle is a micrometer-sized array of cylinders that are very closely spaced: at the top of the hair bundle, the ratio of the gap between two adjacent stereocilia to the diameter of a stereocilium is less than 0.1. Stereocilia are therefore completely immersed in each other's boundary layers. If fluid were forced to flow through a narrow gap between two adjacent stereocilia, a steep velocity gradient would have to develop in the fluid because of the 'no-slip' condition, and there would be a strong resistance, due to viscosity, to the flow through the gap.

The problem of flow through an infinite array of parallel cylinders at small Reynolds number was first solved in 1957 by Tamada and Fujikawa [136], who found that, because of the steep velocity gradients, the drag on each cylinder in the array strongly increases compared with the drag on the same cylinder in isolation. In contrast, as they note, if an array of cylinders is finite, the fluid has an alternative to flow around the array rather than through it. In this case, very little fluid may pass between the cylinders and the drag on each of them can be much smaller than the drag on an identical isolated cylinder.

The same situation applies to a hair cell (Figure 4): by grouping stereocilia in a tightly packed hair bundle, evolution reduced the drag per stereocilium dramatically [63], making the sensitive hearing in vertebrates possible.
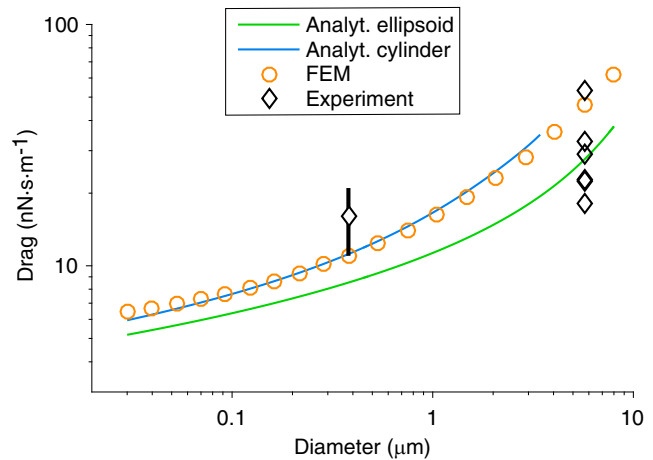
Figure 4. Grouping of stereocilia in a hair bundle dramatically reduces the drag per stereocilium.
Drag coefficient is a function of radius r of a cylinder displaced by a linear profile over the height on a fixed plate. The height of a cylinder corresponding to a stereocilium is $h = 8$ μm. The drag coefficient is given for an equivalent force at the cylinder's tip. For the finite-element result (FEM) the liquid domain has an outer diameter of 62 μm and a height of 37 μm. For comparison, the analytical solutions for a rotating semi-ellipsoid (Analyt. ellipsoid) and for a cylinder (Analyt. cylinder) are also shown. The latter is provided within the valid range of r < 0.22 h. Experimental values of the drag coefficient for an isolated stereocilium and for an entire hair bundle without tip links are shown as black diamonds (n = 6 in each case). Note that the drag on N stereocilia in a hair bundle is only about three times greater than the drag on a single isolated stereocilium. This means that, when stereocilia are grouped in a bundle, N–3 stereocilia move as if they did not experience any viscous drag at all. With a typical N of 50–100 stereocilia, this effect decreases viscous dissipation dramatically. Adapted with permission from Macmillan Publishers Ltd: Nature [63], © 2011.

which would experience a smaller hydrodynamic force for the same flow, whereas a greater number of thinner stereocilia would increase the fluid coupling but also would make the hair bundle stiffer, diminishing its deflection and thus reducing the magnitude of the tip-link extension in response to the hydrodynamic force. Wider hair bundles can therefore be assumed to be more sensitive to hydrodynamic forces than narrower hair bundles of identical stiffness.

Crucially, water filling the gaps between stereocilia will allow their relative shear. Indeed, water is known to be a very good lubricant [65]. At the same time, its viscosity will suppress internally generated 'squeezing' modes of motion and will therefore diminish sound distortions generated by the ear [66], making it a better sound detector, at least from an engineer's perspective.

Central Auditory System

How sensory neurons represent or encode natural signals is one of the classical problems in neuroscience. Using stimuli that are relevant to the animal has produced the clearest descriptions of how sensory systems work in 'specialized animals', such as bats [67], electric fish [68], or barn owls [69,70]. In each of these examples, the stimuli were both natural and simple, which was key to understanding their representations. Most of the sensory cortex in humans and other animals, however, deals with natural stimuli that are statistically complex. This complexity has slowed progress in understanding central sensory representations. That said,

W-shape, and why they are smaller, because otherwise they would not all fit in three or four rows atop a hair cell. In contrast, it would make sense for an inner hair cell to have thick stereocilia to maximize coupling to the moving fluid. Indeed, the same number of thinner stereocilia would result in a smaller hair bundle,

recent advances in computational methods have enabled the characterization of auditory receptive fields using behaviorally relevant stimuli, both in insects and in vertebrates, revealing an interesting similarity between them. Before we address this point, however, one key difference between insect and vertebrate central auditory systems is worth noting.

In many insect species, the number of auditory neurons decreases from the periphery to the centre. For example, the *Drosophila* JO contains about 500 mechanosensory cells, ~75% of which (subgroups A, B and D) have been linked to auditory function [71], but the ascending mechanosensory pathways for conspecific song detection are likely to have substantially fewer neurons [71–73]. In insect auditory systems, individual — i.e., unique — neurons can be identified, and in some species a single neuron alone can drive important behaviors, such as the bat-evasion reflex in noctuid moths [74]. Computational network models based on data from individual auditory neurons in genetically engineered *Drosophila* have been built and have faithfully predicted key aspects of the fly's auditory behavior [75].

In vertebrates, in contrast, the number of auditory neurons always increases from the periphery (cochlea) to the centre (auditory cortex), by as much as four orders of magnitude [76]. These neuroanatomical differences suggest associated functional differences: insect auditory systems are listening for sets of well-defined and species-specific sounds, whereas vertebrates — for example, humans — use their auditory systems to extract broader, and often less predictable, information about the environment. Indeed, we can hear anything between 20 Hz and 20 kHz, the typical frequency range of our (healthy) inner ears. The auditory system of insects might thus be thought of as a special-purpose, rigid system, adapted to detect only a handful of signals, mostly mate and predator calls. This view accords with the finding that information within the insect brain is quickly distributed into multiple parallel (and decorrelated) streams for the separate extraction of individual stimulus features [77]. The vertebrate auditory system, in contrast, is a general-purpose and more flexible system that is shaped by learning [78–81], affected by mood [82], focused by attention [83–85], and one that influences and is influenced by other parts of the brain, both sensory [86] and motor [87] areas, to detect and interpret any sound that may be subjectively important at any particular moment. Our auditory system reconfigures its functional connectivity 'on the fly' [88,89].

This property is important because, according to the principle of efficient coding, if an animal is to extract the maximal amount of information about a stimulus whose statistics vary in time, as they usually do in natural settings, then neuronal ensembles must adjust their responses to match the varying stimulus statistics [90]. On the other hand, from the point of view of a decoder, an important property of the neuronal response is its invariance, two good examples being contrast-invariant responses to gratings in the visual cortical area V1 [91] and a time-warp-invariant processing of auditory cues [92–94]. What this means is that cortical circuits must efficiently and flexibly combine selectivity with invariance in pattern recognition.

Natural sounds, including speech, are produced by vibrating bodies and are characterized by a rapid onset and a slow decay of the envelope. The basilar membrane impulse response [95], the mechanical and electrical responses of a single hair cell [96], and kernel functions representing natural sounds in efficient coding algorithms [97] all share the same asymmetrical Gabor-like shape, i.e., a sine wave multiplied by a Gaussian function. Gabor filters occur at various stages of visual and auditory processing in insects and vertebrates [98,99]. Why are they so widespread?

One reason for a Gaussian envelope may be that a (normalized) dot product of a stimulus vector and a synaptic weight vector combined with a sigmoidal nonlinearity produces an approximately Gaussian tuning [100]. The dot product (the inner product between two vectors) would measure the similarity between a stimulus and a neuron's receptive field, and the nonlinearity comes from the action-potential generation mechanism. This is a biologically plausible way to obtain Gaussian tuning. In vision, it is well known that the Laplacian of the two-dimensional Gaussian distribution, which can be approximated by the difference of two Gaussians, e.g., with inhibition tuned more broadly than excitation, is an optimal operator to detect intensity changes in an image [101]. As a second-order differential operator, it produces filters that resemble Gabors. Crucially, the same neuronal circuit that can implement Gaussian tuning for selectivity can also implement, by using different parameter values, a MAX-like operation (output = maximum(input)), which is important for invariance and has been observed experimentally in the mammalian visual cortex [102,103] and in the central auditory system of a songbird [104]. The predicted computational flexibility was experimentally verified in the European starling, a species of songbird, where individual central auditory neurons could switch between the MAX-like operation for invariance and a tuning operation for selectivity, depending on the stimulus and network state [104], displaying sparse responses and multidimensional selectivity [105].

A Gaussian tuning function is also useful for generalization after training on a limited data set. Specifically, radial Gaussian basis functions are smooth and interpolate well from training data to new data [106]. This property is useful for learning. Mathematically, Gabor filters have an optimal concentration both in space and time (frequency), thus introducing minimal distortions. Furthermore, Poggio *et al.* [107] have argued that learning invariances in the visual cortex to the group $SO_2 \times R^2$, a type of symmetry dealing with rigid rotation and translation, produces Gabor-like tuning. In other words, this particular selectivity property may be a consequence of having to deal with (and discard) such affine image transformations, which is an invariance property. If this argument proves correct, then it would indicate that selectivity and invariance, two pillars of natural object recognition, are not independent but are intimately connected as two parts of a whole. The similarity in central representations — across sensory modalities and animal classes — could then be linked to the conservation laws of physics, for any symmetry is associated, as Noether's theorem proves, with a conservation law.

Conclusions and Future Directions

Reflecting both evolutionary and functional kinship, the chordotonal-organ-based ears of insects share multiple similarities with the hair-cell-based ears of vertebrates. Starting with the fact that their cellular substrates, namely chordotonal neurons and hair cells, arise from their respective precursor cells through a series

of asymmetric mitoses dependent on Notch and basic helix-loop-helix transcription factors [20,108], the similarities extend further to the elementary process of auditory mechanotransduction, which in both hair cells [109] and chordotonal neurons [110] is mediated by mechanically gated, mechanically adapting ion channels. Many features that are considered hallmarks of mammalian, or more generally vertebrate, auditory systems have been found to be phenocopied in the auditory systems of insects. Receptors in the auditory periphery of bush crickets [111,112], for example, are tonotopically arranged. Tonotopy is one of the fundamental organizational principles of the vertebrate inner ear, including the mammalian cochlea. As in vertebrates, the projection patterns of first-order sensory neurons in insect auditory systems can retain this tonotopic organization [113], but, unlike the situation in vertebrates, the tonotopy does not appear to extend to downstream interneurons [77]. Perhaps most strikingly of all, even the spectral decomposition of sound by way of dispersive wave propagation, which enables frequency analysis in the cochlea, has been found in an insect (*Copiphora gorgonensis*) [114]. Insect ears, like their vertebrate counterparts, can produce 'phantom tones' when presented with two stimulus tones [115,116], a phenomenon well known from the mammalian cochlea [117] and also demonstrated at the single hair-cell level [63,118]. Even higher-order, cognitive performances, such as the categorical perception of sound frequency, have been reported in insects (*Teleogryllus oceanicus*) [119].

One of the reasons for the multiple similarities simply lies in the stunning diversity of insect hearing organs and auditory systems [120]. Based only on species with tympanal ears, it has been suggested that hearing has evolved independently at least twenty times across the various insect orders [121]. The rich diversity of insect hearing organs, in turn, largely reflects the richness and diversity of insects themselves. Only a fleetingly small number of insect species is expected to possess a sense of hearing [122], but, accounting for an expected 5.5 million species alone [123], insects have served and will continue to serve as a near inexhaustible treasure trove for research into hearing and acoustic communication.

A recent study may exemplify these relationships and also indicate some directions that future research could take. Hearing in *Drosophila* was found to be independent of efferent control [36], and no efferent innervation had previously been reported for any other insect; however, it has now been detected in the antennal ears of mosquitoes, where it modulates both frequency tuning and amplificatory gain [124]. Efferent gain control is one of the key features of vertebrate, and specifically mammalian, hearing [125] and is thought to be key to the ear's exquisite auditory performance, especially in noisy environments (possibly contributing to the 'cocktail party effect'). Acoustic interactions between mosquitoes have proven more varied than previously anticipated (compare [126] and [127]), and most of these interactions are likely to take place in larger swarms as part of the animals' mating behavior [128]. From an auditory perspective, such a mosquito mating swarm may well be described as the mother of all cocktail parties. It will be fascinating to see if — and if so how — efferent modulation can help to prime the mosquitoes' antennal ears for their challenging tasks. Some of the major challenges for hearing research, in turn, will be of a translational nature — to translate insect studies into vertebrate research, translate findings from mosquitoes into experiments in *Drosophila*, translate studies on tympanal ears into studies on antennal ears, and translate from molecular to mechanistic (and always *vice versa*).

To understand how hearing works at the most basic level — a spring pulling on an ion channel — it will be important to identify the molecular composition of both the gating spring and the MET channel. (For a discussion of potential MET channel candidates in vertebrates, see [129].) Identifying and characterizing molecular components of the mechanotransduction complex and their interaction with each other will aid our understanding of how sounds perform work on the MET channels, i.e., the nature of the movement that transforms a sound's mechanical energy into the channel opening. This knowledge will also help us to figure out how channel gating contributes to sound amplification.

To understand how hearing works at the central level, i.e., at the level of algorithms and their implementation in the central auditory system, it will be important to establish more connections not only between insect and vertebrate hearing but also between insect and vertebrate vision. Indeed, selectivity and invariance are generic and sensory-modality-independent principles underlying natural pattern (object) recognition, but they have been mostly explored in natural and computer vision. Bringing that knowledge to bear on problems in hearing research will be a major task of future studies. Finally, comparing biological representations of natural sounds with representations learned by artificial neural networks (i.e., adaptive computer programs) will help to reveal general principles of hearing in insects, vertebrates … and machines.

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