Novel schemes for hearing and orientation in insects
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Severe size constraints are imposed on the hearing organs of insects, yet they perform sophisticated tasks of auditory processing. Recent research has shown how flies acoustically locate targets in space, how mosquitoes afford highly sensitive ears, and how crickets avoid deafening themselves with their songs. These findings unveil the exquisite analytical capabilities of highly specialized microscale auditory systems.

Introduction

Audition is an act of mechanoreception that demands great accuracy. Hearing relies on a cascade of biophysical events that first involve the conversion of acoustic energy into mechanical vibrations, which, in turn, are transduced and encoded by mechanosensory receptor cells. In an acoustic wave, information is essentially carried by a finite set of physical attributes and their dynamic variation: the wave's amplitude, its timing and its frequency composition. As the physical propagation of sound in the atmosphere is fast and the energies involved rather small, reliable and accurate information processing can be mechanically and computationally challenging. Audition thus relies on sensors that are mechanically well adjusted and highly sensitive [1–7]. In effect, auditory animals can detect sounds that cause mechanical disturbances of subnanometer amplitudes [8–10]; they can decipher the timing between two acoustic events separated by a few microseconds or less [11,12]; and they can discriminate between frequencies that differ by just 1% [6,13]. Much of the knowledge available on the neural and biophysical mechanisms subtending each of these feats stems from vertebrate research.

Like other auditory animals, insects are faced with challenging benchmarks to achieve adequate audition. In insects, the challenges are clearly exacerbated by the small dimensions of their ears as well as biophysical and ecological constraints. We can recommend several reviews that present the diversity of auditory anatomies and sensory mechanisms that support hearing in insects [5,14–19]. Recent research has unveiled some sophisticated auditory mechanisms in insect ears. Using behavioral, neurophysiological and biophysical methods, these investigations illustrate how specialist species can contribute novel vantage points on the mechanisms of hearing. The topics we address here pertain to the mechanisms and limits of directional hearing, to sound localization in three-dimensional space, to how sensitivity is achieved and protected, and finally, to active auditory mechanics.

Innovative mechanisms for directional hearing

The cues for directional hearing

In vertebrates and insects alike, directional sound detection relies on the positioning of the animal and its two ears in the sound field. The acoustic cues conventionally used are interaural differences in intensity (IID), in time (ITD) [20,21], and in the spectral composition of the incoming sound [22,23]. ITDs and IIDs vary with the orientation of the animal's ears (or for animals with non-moveable ears the head and body-axis) in relation to the sound source. Insect ears are usually non-moveable and are set closer together than vertebrate ears. These constraints impose short interaural distances and result, because of the physics of sound propagation, in minimal IIDs and ITDs [19]. Similarly, for small animals, interaural spectral cues tend to vanish because of reduced acoustic diffraction, especially when the wavelength of sound is large compared to body size. Some insects perform some form of frequency discrimination [24–26]. The question of whether they use binaural — and possibly monaural — spectral analysis for directional detection, as is seen in vertebrates [22], has received little consideration. Not all auditory insects need directional hearing, yet for those that do, the requirements for reliable detection and information processing can be demanding.

Small ears but large feats

Behavioral, biomechanical and neurophysiological work on a small fly has highlighted the uncommonly accurate directional sensitivity that some insect ears can achieve. Female Ormia ochracea are parasitoids that acoustically locate and attack their host, singing field crickets, [27] using miniature tympanal auditory organs [28,29]. For this fly, sound localization is no trivial feat. The largest possible acoustical IIDs and ITDs available to the auditory organs have been measured to be less than 1 decibel and 1.5 µs respectively ([30]; Figure 1a). In terms of neural coding, 1.5 µs is a short time [31,32]. By comparison, humans can resolve an acoustical ITD of 4–8 µs [21], corresponding to a midline deviation of a sound source by 1–2°. Behavioral tests using a walking treadmill have shown that Ormia orient to a 2° azimuthal deviation of a loudspeaker broadcasting a cricket song [33••]. For the fly, this 2° deviation translates into an acoustical ITD of a mere 50 ns. How does such accuracy come about? The fly seems to have dissected the problem into a series of manageable bites, each delegated to a separate step in the cascade of audition.
A mechanical process that amplifies directional auditory cues

For *Ormia*, the cascade begins with an unusual acousto-mechanical interface: two ears with mechanically coupled tympanic membranes. The tympana are connected across the midline by a flexible cuticular lever (Figure 1b). In response to the sound of a cricket, this coupled tympanal system undergoes asymmetrical mechanical oscillations. The oscillations arise from the linear combination of two resonant modes; the two tympana move out of phase and at different amplitudes, rocking like the two arms of a floppy see-saw [30,34]. As a result, a mechanical ITD of 50–60 µs and a mechanical IID of 3–12 decibels arise for sounds delivered at 90° azimuth. The mechanical ITD is thus about 40 times longer than the 1.5 µs acoustical ITD. This system thus acts as an ITD and IID amplifier, converting small acoustical cues into considerably larger mechanical cues. At present, the way these mechanical cues are conveyed from the tympanal membranes to the auditory neurons is unknown. Recent evidence reveals that these mechanical cues are sufficient for the reliable neural coding of sound direction [33••].

A neural process that amplifies auditory cues

Notwithstanding this mechanical amplification, temporal and intensity cues remain small at this stage. By extrapolation, the temporal mechanical cues available at 2° azimuth — owing to an amplification factor of 40 applied to 50 ns — would amount to 2 µs. The exact contribution of mechanical IIDs to neural coding remains uncertain for such small angles; however, the directional cues available at the stage of auditory receptor neurons have been examined in more detail [33••]. Neural ITDs (i.e. the difference in the spike latency between left and right receptor neurons) amount to 150 µs for 90° azimuth, and vary with the angle of sound incidence (Figure 1d). As the azimuthal angle decreases, neural ITD drops by 3.5 µs per degree, predicting a neural ITD of 7 µs at 2° azimuth [33••]. Somehow then, to account for the observed phonotactic behavior, the fly’s primary afferent neurons must reliably code microsecond scale events. Mason et al. [33••] provide key evidence that such a capacity is based on a remarkably fast-time spike code. First, most of the afferent neurons respond with a single, sharp spike, that is independent of stimulus duration, a form of ‘sparse coding’ [32]. Second, the spiking latency, measured as the time between stimulus onset and spiking, increases as stimulus amplitude decreases. Third, the variation in spiking time, the jitter, seems to be commensurate with microsecond resolution. Expressed as the standard deviation of absolute spiking latency, the measured jitter amounts to about 70–100 µs [33••]. The task of the population of primary afferents is thus to convey temporal events that are just 7 µs apart, a time difference that is ten times lower than the accuracy of individual afferent neurons.

In a type of sensory processing called hyperacuity, neural coding at submillisecond time scales has previously been documented theoretically [31,32], and experimentally, for
electric fish [35] and barn owls [11,36]. The underlying neural mechanisms may rely on the convergence of many sensory afferents onto an interneuron that acts as a coincidence detector. In this scheme, only the coherent firing of an ensemble of afferents within a narrow window of coincidence would elicit interneuronal spiking. In the fly, it is notable that the jitter in firing times of the afferent population increases as stimulus amplitude decreases [33••]. This is another important step in the processing cascade that may help to explain the putative hyperacute directionality of first order auditory interneurons. Critical temporal coincidence may be reached faster and more reliably for that of the two auditory channels that experiences more intense mechanical vibrations. With this respect it may be interesting to investigate the exact biophysical relationship between both ITD and IID mechanical inputs and factors including: the spiking behavior of auditory receptors, the neuroanatomy of the afferent convergence, and the integration properties of first order interneurons. Interestingly, in the locust olfactory system, time-sensitive coding and sparse information processing is carried out by Kenyon cells in the mushroom bodies [37] that are presumably acting as coincidence detectors, and that are submitted to fast and competitive excitatory and inhibitory afferent inputs [37]. The fly may offer a good opportunity to study the neural cascade of events underlying auditory hyperacuity.

**Auditory perception of space**

Whereas much is known about the detection mechanisms and the psychophysics of directional hearing in the azimuthal and elevational planes, comparatively little is known about the capacity of animals to gauge the distance of a sound source (with the notable exception of echolocation) [38]. The primary cues used to gauge the distance to a sound’s source reside in the binaural comparison of fine spectral characteristics of the incoming sound, and/or, their (also monaural) comparison over time [22]. These tasks seem challenging for insects. A series of elegant psychophysical experiments have shown that crickets can detect and discriminate between sounds delivered at different elevational angles [39]. Whether crickets or other insects can use spectral cues for the directional detection of sound in elevation remains unknown. Using a novel technique of video tracking [40], a recent study has examined the three-dimensional flight trajectories of the parasitoid fly *O. ochracea* as she homes in on the source of a cricket song placed on the ground [41•]. Flight trajectories comprise three distinct phases: a brief take-off phase, a long cruising phase in which course and altitude remain almost constant, and a landing phase that is initiated as the fly passes over the sound source. This terminal approach is characterized by a steep spiraling descent right onto the sound source. Surprisingly, the fly's phonotactic behavior is hardly affected by the interruption of the stimulus while she is in midair, cruising towards the loudspeaker. Irrespective of her position in space at the time of stimulus interruption, the fly initiates her spiraling descent at the appropriate time and place, and lands close to the silent loudspeaker. Because of the absence of visual or olfactory navigational cues, these experiments suggest that, at the time of stimulus interruption, the fly had already acquired sufficient acoustic information to navigate accurately to the sound source. This is remarkable as flies achieve successful phonotaxis without prior experience and show no experience-related improvement in their phonotactic abilities. These results seem to suggest the presence of a mechanism of acoustic detection that enables the fly to gauge both the direction and the distance of a sound source in three-dimensions. Such a capacity is strikingly reminiscent of the barn owl’s perception of acoustic targets in both azimuth and elevation [36]; yet, unlike the owl, the fly has bilaterally symmetrical ears. Discovering the nature...
acoustic sensitivity. Such damage would also prevent the detection of other, non self-generated, acoustic inputs. A remarkable recent study has revealed that neural sensitivity during song production is preserved by the action of an inhibitory corollary discharge of central origin [42••]. This discharge has at least two distinct components that act in synchrony with song production, transiently inhibiting both the primary afferent neurons presynaptically and a first-order identified auditory interneuron postsynaptically. This evidence is precious because it also provides a convenient experimental tool for the functional analysis of a neural network involved in signal recognition and selective attention.

Active auditory mechanics
Recent research on mosquitoes has documented the minuscule mechanical displacements at which insect hearing can operate [43]. Male mosquitoes hear with their antennae (Figure 2). The antenna’s distal part, the flagellum, vibrates in response to sound and constitutes the sound receiver. Flagellar vibrations, in turn, are transduced by around 15 000 mechanosensory neurons comprised by Johnston’s organ at the base of the antenna [44]. Simultaneous mechanical and electrophysiological measurements revealed that the mechanosensory neurons of Johnston’s organ electrically respond when sound displaces the flagellar tip by ±7 nanometres, deflecting the flagellum by a tenth of a millidegree [43]. According to a simple calculation taking only the antenna’s geometry into account, the ±7 nanometre displacement at the flagellar tip is reduced to a mere ±0.3 nanometres at the location of the mechanosensory neurons of Johnston’s organ. This latter figure closely corresponds to the threshold displacements of the cochlear hair cells of humans [2]. Across vertebrates, the cochlea achieves its sensitivity by means of an active process, commonly known as cochlear amplification or, more generally, as active auditory mechanics [45]. This amplification process is brought about by cochlear hair cells. These cells, in addition to transducing sound-induced vibrations, assist and amplify these vibrations through self-generated cellular motions [1–3,6–10]. Compensating for losses due to viscous damping by cochlear fluids, the motility of the hair cells sharpens mechanical resonances, thereby providing the exquisite sensitivity and frequency selectivity that characterizes the cochlea [7,45].

Active audition — diagnostic features and evidence
In mosquitoes, the detailed examination of antennal mechanics has revealed that auditory sensitivity is achieved by a process that is comparable to active amplification [46••]. The active processes in the antennal hearing organs of mosquitoes and in the vertebrate cochlea manifest themselves in surprisingly similar ways. Vertebrate ears display a combination of key mechanical characteristics that are conventionally considered to be introduced by the motility of hair cells and that together betray the presence of active auditory mechanics [7]. These characteristics are: a physiologically labile mechanical response, including intensity-dependent non-linear effects;
and, importantly, spontaneous otoacoustic emissions [45,47]. The latter sounds are particularly interesting because they originate from inside the cochlea. Most other, acoustically evoked, otoacoustic emissions are suitable probes to assess nonlinearity; but on their own they reveal little about the passivity or activity of the system, and thus the origin of its nonlinearities [45,47]. By contrast, spontaneous emissions compellingly document that something is autonomously active in the ear [1].

In both mosquitoes and vertebrates, active amplification takes place only at low stimulus intensities, and this explains why their ears behave exceedingly nonlinearly as intensity decreases. Because active auditory mechanics counteracts damping, it sharpens the resonant response to low intensity sounds, resulting in improved auditory sensitivity and frequency selectivity. This non-linear effect is only found in healthy animals; it is physiologically vulnerable and vanishes with the animal's death ([46••]; Figure 3).

In a quiet environment, excessive amplification can lead to spontaneous oscillations inside the cochlea. In mosquitoes, spontaneous oscillations of the antennal flagellum occur and can display large amplitudes (of up to 400 nm) and last for longer than one hour. The idea that these autonomous oscillations are related to an amplification process is supported by several factors including: their occurrence at the exact frequencies at which amplification takes place, their intrinsic non-linear characteristics, their susceptibility to suppression by external tones and their sensitivity to hypoxia.

Non-linear alterations of the auditory response at low stimulus intensities have been reported for several insects in addition to mosquitoes, suggesting that active auditory mechanics may be a widespread feature of insect ears. Specifically, non-linear effects have been identified in the tympanal ears of locusts [48] and moths [49•], and in the antennal hearing organs of Drosophila melanogaster [50•].

The presence of active auditory mechanics in Drosophila could be particularly enticing. First, like the antennal hearing organs of mosquitoes, the ears of Drosophila are accessible to non-invasive and detailed mechanical analyses [51]. Second, this fly is a prominent model system for the dissection of mechanisms underlying mechanosensory transduction and various hearing-impaired Drosophila mutants have already been described [3–5,51,52]. This offers the opportunity to combine mechanical investigations with genetic manipulations [4,53], providing an experimental window that may help us to understand the cascade of systemic, cellular and molecular events that bring about audition, and particularly active audition in insects.

**Conclusions**

The ears of vertebrates and insects fulfill both proximate functions of detecting acoustic waves propagating in their respective and often different sensory ecological niches, and the ultimate functions of acoustically detecting mates, predators and prey. Whereas some of the examples reviewed here constitute sharp adaptations to specific auditory tasks, subtending mechanisms may appear to be conserved across taxa. Tellingly, the ears of the fly Ormia ochracea embody such dualism: coupling tympanal membranes seems to be a unique specialisation, yet improving temporal acuity through coincidence detection seems to be a more widespread principle in sensory neuroscience. With this in view, is the apparent phenomenological convergence of active auditory mechanics between insects and vertebrates merely a result of equivalent biophysical constraints, or does it reflect fundamental properties deeply-rooted in the evolution of mechanosensory systems? Because insect ears facilitate comparative investigations at multiple levels along the cascade of hearing, the next years are likely to shed much light on the systemic, cellular and molecular mechanisms that are at work in ears.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


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