



Auditory tuning in the bushcricket miniature hearing organ

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When the air of warm summer nights is buzzing and whirring with the songs of male bushcrickets (also known as katydids), our ears perform a series of tasks enabling us to perceive these acoustic communication signals. Airborne sound waves travel through the ear canal of the outer ear and impinge on the tympanic membrane, causing mechanical vibrations. These are transformed via the three ossicles in the middle ear into fluid vibrations of the inner ear cochlea, which in turn, activate sensory receptor cells, thereby transforming the mechanical energy into electrical signals (1). In our ears, this transduction process involves an ~25-mm-long ear canal, a tympanic membrane 8 to 10 mm in diameter, and ~3,500 inner hair cells distributed along the 35-mm-long basilar membrane (2). Female bushcrickets, the males' intended audience, have to perform essentially the same feat but with miniature hearing organs located not on the head but on the upper tibia of the forelegs. These ears are typically only around 1 to 2 mm in size and contain rarely more than 100 neurons (3). Nevertheless, these tiny insect ears share a surprising amount of structural and functional similarities with our ears, some of which Vavakou et al. (4) uncover.

Each bushcricket ear consists of two ear drums—the anterior tympanic membrane (ATym) and the posterior tympanic membrane (PTym)—located on opposite sides of the leg. Both tympana are backed internally by two branches of an air-filled tube connecting the ear to a large spiracular opening at the thorax. This acoustic trachea fulfills the role of an (outer ear) ear canal, with sound traveling from the acoustic spiracle to the back of the tympana (Fig. 1) (5). Because sound waves are both amplified and slowed down while traversing the ear canal, the internal tracheal input provides more sound pressure to the (middle ear) tympana than sound arriving from the outside, thereby constituting the system's main energy input (6).

The tympanal vibrations are transmitted to a linear array of mechanosensory receptor cells, in this

case scolopidial sensilla, called the crista acustica (CA). This organ is located along the proximal–distal length of the dorsal wall (DW) and consists of a row of neurons with their sensory dendrites covered in cap cells (CCs), which are linked with an overlying tectorial membrane (TM), covered by a fluid-filled channel (Fig. 1) (3). Analogous to the mammalian inner ear, the morphology of the CA changes gradually along its length, exhibiting, amongst others, decreasing CC sizes and DW and TM width from proximal start to distal end (7). These size variations cause changes in mass, stiffness, and resonance of the CA and are the basis for a tonotopical activation of the sensory neurons along the CA via mechanical traveling waves (3, 7–9). This generates a frequency-place map, with the location of neurons activated by an acoustic signal depending on the signal's frequency (10, 11).

While various morphological, structural, and neuronal mechanisms influencing the tuning and tonotopy of the individual sensory cells have been identified in both mammalian and insect ears (e.g., 1, 7–9, 12, 13), many questions are still unanswered. Often, this has been due to the inaccessibility of the inner ear components, especially in its living and intact state. In ref. 4, the authors used optical coherence tomography (OCT) to overcome this problem to investigate the simultaneous motion in response to sound of the tympana and the internal components of the CA in an intact bushcricket ear.

In OCT, near-infrared light is used to noninvasively perform imaging and vibrometry measurements within soft biological tissues. Tissue penetration can be down to several millimeters with micrometer image resolution, allowing for motion measurements in the nanometer range (14). This technique has allowed for investigating the mechanics of mammalian hearing in vivo in greater detail than possible before (14), and Vavakou et al. (4) employ it here to great advantage in *Mecopoda elongata*, a bushcricket using broadband sonic and ultrasonic songs (up to 70 kHz) for communication.

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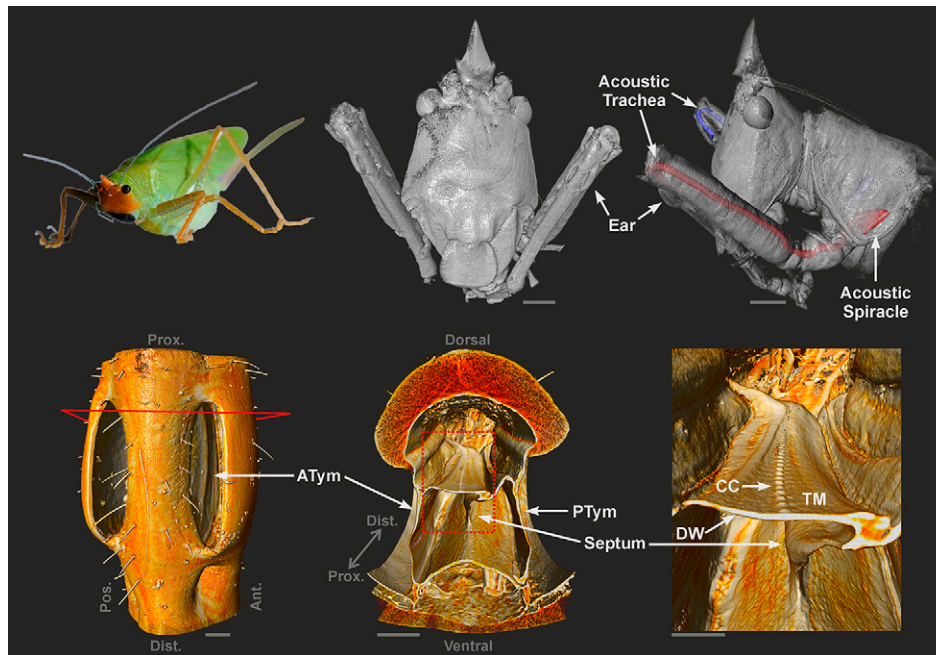


Fig. 1. The bushcricket hearing organ. (Upper) A female *Copiphora gorgonensis* (Left) and three-dimensional models from micro-computed tomography recordings. Frontal (Center) and semitransparent lateral views with acoustic tracheae in red and blue (Right). (Scale bars: 2 mm.) (Lower) A false color model of a right ear (Left) and a cross-sectional view at the level of the red line (Center; outer flaps removed). (Right) Magnified CA (red dotted rectangle). Ant., anterior; Dist., distal; Pos., posterior; Prox., proximal. (Scale bar: 200 μm .)

Using OCT, the authors first investigate the motion in response to sound stimulations of ATym and PTym and the septum dividing the acoustic trachea simultaneously. The results show that, in *M. elongata*, ATym and PTym move in antiphase (180° phase difference) relative to each other. The tympana both move inward or outward at the same time, reacting to the pressure difference between sound pressure waves arriving from the (low-amplitude) external and (higher-amplitude) internal inputs. These results corroborate earlier, albeit indirect, findings in other species (9, 15, 16), where ATym and PTym were measured separately. Importantly, it is now also shown that the septum moves in phase with the PTym and that both membranes describe a hinged motion around their dorsal ends (figure 2G in ref. 4). Interestingly, the antiphase motion of ATym and septum could impose periodically changing stresses on the CA by stretching and compressing the DW, thereby at least partly driving the activation of the sensory neurons. Additionally, Vavakou et al. (4) also remark on the striking similarities of the hinged tympana motion with the vertebrate impedance matching mechanism (via the lever-like movement of the ossicles), but more details are needed here to disentangle the simultaneous motion profiles of the structures involved.

After describing the anterior–posterior movements of the middle ear structures, the authors then meticulously analyze the dorsal–ventral motion of the inner ear, namely the DW and the CCs of the CA (Fig. 1). For the CC, results from earlier experiments using laser Doppler vibrometry on the exposed CA are broadly confirmed (7, 8). The CCs are tuned tonotopically, with frequencies of peak vibration magnitude gradually decreasing toward the proximal end of the CA. Furthermore, the authors show that the tuning of the DW directly underlying the CC is also tonotopically organized, resembling the CC tuning. However and most crucially, the tuning curves of CC and

DW are not identical, with DW tuning expressing broader and slightly more proximal peaks compared with the overlying CC. These systematic differences in mechanical tuning of the dorsal and ventral boundaries of the CA are shown along its whole length. This leads to the conclusions that the sensory neurons are sandwiched between two structures with “parallel but shifted place-frequency maps” (4) and that the relative motion of DW and CC exhibits a sharper tuning than either structure alone.

This important finding has multiple implications for the biomechanics of both insect and vertebrate hearing. For bushcrickets, it represents a possible explanation for the tuning mismatch that has been found between mechanical tuning of the CC and the neuronal tuning curves of individual CA neurons, with the former being much broader than the latter (17). As is pointed out, the differential tuning of DW and CC could introduce a (potentially quite complicated) shearing motion of the sensory dendrites resulting in activation of the neurons with a sharper overall tuning than expected from the local DW and CC tuning alone. Combining OCT with electrophysiological recordings would be the next logical step toward a more complete understanding of the mechanics of this particular transduction process. Furthermore, the process shown here, by which differential mechanical tuning of two structures is employed to result in a differential motion with sharper tuning properties, would also be interesting in relation to the complex interactions of structures within the mammalian organ of Corti. In many ways, the bushcricket DW–CC system described here is analogous to the basilar membranes and TMs in the mammalian cochlea, where discrepancies in mechanical and neuronal tuning have been reported as well (12, 18). How exactly the mechanical behaviors of the TM and other organ of Corti structures influence and shape the auditory response in mammals and humans is still

largely unknown, so that, to quote ref. 4, “any progress in the field of insect hearing may advance our understanding of mammalian hearing and vice versa.”

The introduction of OCT into insect auditory research is certainly an important step into this direction, as it has now been demonstrated that the investigation of complex mechanics simultaneously at work inside these miniature ears is feasible and can be carried out without the need to open and potentially alter the system (although the authors also present data here suggesting that the impact of surgically opening the ear has only minor effects on the parameters quantified, incidentally at least defusing, if not resolving, a long-standing argument about the consequences of opening such a delicate system). This methodology, therefore, presents ways to tackle important questions in insect hearing. As mentioned above, OCT combined with single-cell recordings could be used to shed light on the relation of tympanal and septum vibrations to CC motion and CA neuron activation. As suggested, applying OCT from various different angles will also allow for the characterization of

the DW and CC motion vectors, as it is not clear at the moment if DW and CC move along the dorsal–ventral axis or describe more complicated motion patterns (figure 6 in ref. 4). Intriguingly, one could also imagine measuring the tympanic vibrations of both the left and right legs simultaneously in response to acoustic stimulation from various incident angles. Such an experiment could provide important insight into the peripheral processing of directional sound stimuli and the biomechanics of directional hearing.

By uncovering further similarities that these miniature hearing organs share with our ears (but also, by finding the differences), we will ultimately be able to understand how evolution shaped and enabled such—on first sight—vastly different hearing organs to perform, at least on warm summer nights, the same task: listening to insect love songs.

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