Nanometre-range acoustic sensitivity in male and female mosquitoes

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Johnston's sensory organ at the base of the antenna serves as a movement sound detector in male mosquitoes, sensing antennal vibrations induced by the flight sounds of conspecific females. Simultaneous examination of acoustically elicited antennal vibrations and neural responses in the mosquito species *Toxorhynchites brevipalpis* has now demonstrated the exquisite acoustic and mechanical sensitivity of Johnston's organ in males and, surprisingly, also in females. The female Johnston's organ is less sensitive than that of males. Yet it responds to antennal deflections of ±0.0005° induced by ±11 nm air particle displacements in the sound field, thereby surpassing the other insect movement sound detectors in sensitivity. These findings strongly suggest that the reception of sounds plays a crucial role in the sensory ecology of both mosquito sexes.

**Keywords:** auditory mechanics; bioacoustics; insect antenna; Johnston's organ; mosquito hearing; sound perception

1. INTRODUCTION

In 1855, Johnston discovered a complex mechanosensory organ at the base of the antennae of mosquitoes. Based on sex-specific structural specializations, he predicted that this so-called Johnston's organ serves auditory functions in males allowing them to use female flight sounds as acoustic cues for mate detection. Since their discovery, Johnston's organs have been shown to constitute the key evolutionary innovation of the major of the two main insect subgroups, the eugnathes, which comprise more than 99% of all known insect species (Hennig 1981; Kristensen 1981; Westheide & Rieger 1996) (figure 1a). The antennae are characteristically composed of three major elements in eugnathic insects: two proximal segments, namely the scape and the pedicel, and a distal flagellum (figure 1b). The pedicel always comprises a Johnston's organ with the sensory cells connecting to the base of the flagellum and sensing flagellar deflections, which are usually caused by contact and wind (e.g. Heinzel & Gewecke 1987; Camhi & Johnson 1999).

Compared to the other eugnathes, it is the extraordinarily high complexity of Johnston's organ in mosquitoes which is striking. A total of around 15 000 sensory cells, almost as many as in the human cochlea (Retzius 1884; Hudspeth 1989), are found in each of the male's organs (Boo & Richards 1975a) and approximately half that number are present in females (Boo & Richards 1975b). In addition, mosquito antennae usually exhibit an outstanding sexual dimorphism in their flagellar structure (e.g. Risler 1955; see the most recent review by Clements 1999) (figure 1a). In males only, the flagellum is densely verticillate, bearing a large number of long hairs (figure 1b). Both the high complexity of the sensory organs and the dimorphic flagellar structure led Johnston (1855) to rightly assign an auditory function to the antennae of male *Culex pipiens*. Subsequent studies on several different species confirmed the prediction that the male mosquito Johnston's organs and antennae constitute a sound detector. It was also established that males are able to detect conspecific females by flight sounds, i.e. the sounds which are generated by the wing stroke during flight (Roth 1948; Tischner & Schief 1955; Wishart & Riordan 1959; Belton 1974; Clements 1999). However, a close examination of mosquito sound reception is only now possible, due to recent developments in computer-controlled laser vibrometry (Robert & Lewin 1998; Göpfert et al. 1999). When used in conjunction with neurophysiological and near-field acoustical measurements, this technique permits a stepwise evaluation of the acoustic sensitivity of mosquitoes, as demonstrated here for males and females of the species *Toxorhynchites brevipalpis*.

2. MATERIAL AND METHODS

The culicid *T. brevipalpis*, a large-sized, predatory mosquito species of special interest in mosquito control (Steffin & Evenhuis 1981), were taken from laboratory cultures. All experiments were performed on a vibration isolation table (TMC, type 78–442–1) at constant room temperature (24–26 °C).

The preparation procedures and general set-up used to examine sound-induced, antennal vibrations in mosquitoes are described in Göpfert et al. (1999). Here, the acoustic sensitivity was investigated by applying two different types of analysis. First, the general characteristics of the sound-induced flagellar vibrations, i.e. the deflection shapes and frequency characteristics, were examined by means of white-noise analysis using hand-limited random noise for acoustic stimulation (frequency range 100–1000 Hz). The stimuli were generated by a Stanford Research System network analyser (Stanford Research Systems, Sunnyvale, CA, USA; type SR780), the output of which was amplified and fed to a loudspeaker positioned at a distance of 0.97 m from the preparation (for details, see Göpfert et al. 1999). Two parameters were simultaneously measured, i.e. the particle displacement in the sound field (δp) and the flagellar displacement (δf). The particle displacement δp was assessed from measurements of the particle velocity in the sound field using a particle velocity microphone (Knowles Electronics, Inc, Itasca, IL, USA; model NR-3158) (for details about the use and calibration of particle velocity microphones see Bennet-Clark et al. 2000).
Johnston’s organ. We recorded the compound neural response of the organ using electrolytically sharpened tungsten electrodes inserted proximal to the scape in the antennal nerve. The electrode did not affect the flagellar vibrations, as confirmed by control measurements before and after insertion of the electrode. As an additional control, flagellar vibrations were also monitored before and after the experiments in order to exclude any effect due to desiccation of the animals. The microphone, laser and electrode signals were sampled together with the trigger pulses on a computer at a rate of 8 kHz for off-line evaluation. The recorded signals were averaged per stimulus cycle (average response to 400 cycles per intensity) and their magnitudes were calculated either as path lengths (electrode signal) or peak amplitudes (laser and microphone signals). The latter values were converted to the corresponding vibration velocity and displacement amplitudes, whereas the magnitudes of the neural responses were normalized to 100. To determine the neural thresholds, intensity–response plots were computed for each animal. Logarithmic curves were fitted to the logarithmic part of the plots where resolution of measurements was high. As a conservative estimate, the threshold intensity was measured at the intersection between the fitted curve and the intensity axis.

3. RESULTS

The first distinct step in mosquito hearing is the transmission of the kinetic energy from particle oscillations in the sound field to mechanical vibrations of the antennal flagellum which acts as the sound receiver. Experimentally, this process was examined by means of an input–output analysis based on simultaneous measurements of both the particle displacement \( d_p \) and the flagellar displacement \( d_f \). Since the characterization of the flagellar displacement \( d_p \) requires knowledge of the general vibrational behaviour of the flagella in the sound field, we reconstructed the flagellar deflection shapes from the vibration magnitude and phase, which were systematically measured at various points by means of scanning laser vibrometry (Robert & Lewin 1998). As shown by deflection-shape analyses (figure 2(a)), the male and female flagella move almost like stiff rods rocking about their socket (the pedicel–flagellum joint) (figure 1b) when stimulated at biologically relevant frequencies. The flagella thus behave like simple, forced damped harmonic oscillators vibrating at the fundamental mode. Due to this pattern of vibration, routine measurements were made at the flagellar tip, which vibrates with maximum magnitude and reflects the overall mechanical response of the flagellum. As expected from a simple, forced damped harmonic oscillator, the frequency spectra of the relative vibration magnitude \( |d_f/d_p| \) show a resonance in the mechanical response of the male and female flagella (figure 2b). In addition, resonance was confirmed by considering the phase relationship between the flagellar and particle movements, revealing the expected 90° phase shift around the resonant frequency. Interestingly, the male and female flagella resonate at different frequencies. The resonant frequency of the male flagellum and the dominant fundamental frequency of the female flight sounds coincide (figure 2b and table 1). In contrast, males produce flight sounds at frequencies above the flagellar resonance of males and females.
Both sexes also differ in their mechanical sensitivity. Mechanical sensitivity can be quantified as the relative vibration magnitude. The ratio $d_l/d_p$ constitutes a transfer function which indicates the efficiency of coupling between the flagellar and particle movements and is commonly used in mechanical analysis and its application to movement sound detectors (Fletcher 1978; Tautz 1979; Humphrey et al. 1993). In the mosquito species studied, the mean mechanical sensitivity at the flagellar resonance is $d_l/d_p = 4.3$ in males and $d_l/d_p = 2.8$ in females (figure 2b and table 1). The sound of a given intensity therefore elicits flagellar displacements which are on average 1.5 times larger in males than in females (table 1). This higher mechanical sensitivity of the male flagellum presumably results from the verticillate flagellar structure, which is likely to enhance the sensitivity by increasing the effective surface of the sound receiver (Risler 1955; Gopfert et al. 1999).

The transformation of flagellar vibrations to neural activity was quantified as a second analytical step. Specifically, we examined the relationship between the flagellar tip displacement $d_l$ and the magnitude of the compound neural response of Johnston’s organ at the flagellar resonance. As shown by the intensity–response plots in figure 3, flagellar vibrations elicit responses of Johnston’s organ in both sexes. The response magnitude is positively correlated with the flagellar tip displacement $d_l$ produced by tethered flying males (black lines) and females (white lines) at a distance of 1.5 cm from the particle velocity microphone. The number of animals is indicated in table 1.

Table 1. Flight sounds, antennal mechanics and Johnston’s organ sensitivity

<table>
<thead>
<tr>
<th></th>
<th>mean ± 1.s.d.</th>
<th></th>
<th>sensitivity factor</th>
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<tbody>
<tr>
<td></td>
<td>males</td>
<td>females</td>
<td>factor</td>
</tr>
<tr>
<td>flight sound frequency (Hz)</td>
<td>525.0±27.0</td>
<td>415.0±33.0</td>
<td>—</td>
</tr>
<tr>
<td>flagellar resonant frequency (Hz)</td>
<td>420.0±5.0</td>
<td>244.0±11.0</td>
<td>—</td>
</tr>
<tr>
<td>mechanical sensitivity $d_l/d_p$</td>
<td>4.3±0.4</td>
<td>2.8±0.4</td>
<td>1.5</td>
</tr>
<tr>
<td>$d_l$ at the neural threshold (nm)</td>
<td>7.3±1.6</td>
<td>22.8±6.4</td>
<td>3.1</td>
</tr>
<tr>
<td>$d_p$ at the neural threshold (nm)</td>
<td>2.4±0.3</td>
<td>11.2±2.5</td>
<td>4.7</td>
</tr>
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the bending stiffness of the flagellum (figure 2a), the mechanical coupling between the flagellum and the sensory cells (Belton 1989) and the sensitivity of the sensory cells are the parameters which may contribute to the observed difference in mechanical sensitivity.

We simultaneously recorded the particle oscillations in the sound field along with the flagellar tip vibrations and neuronal responses. Thus, the relationship between the response magnitude of Johnston’s organ and the particle displacement in the sound field \( d_p \) can be evaluated independently (figure 3b). Such analysis reveals a mean particle displacement at the neuronal threshold of \( d_p = 2.4 \) nm in males and \( d_p = 11.2 \) nm in females (table 1). This means that, in comparison to males, a 4.7 times higher particle displacement is required to elicit responses in the female Johnston’s organ. This figure corroborates the stepwise mechanical analysis, for which the overall sensitivity difference between males and females was established to be \( 1.5 + 3.1 = 4.6 \) (table 1).

4. DISCUSSION

The present analysis clearly demonstrates that the antennae provide male mosquitoes with an elaborate acoustic sense which is suited to perceiving the sounds emitted by conspecific females in flight. In males, the efficient transmission of particle oscillations to flagellar tip vibrations on the one hand, and of flagellar tip vibrations to neuronal activity on the other, together result in an exquisitely high acoustic sensitivity. The male Johnston’s organ responds to sinusoidal flagellar tip movements as small as \( \pm 7 \) nm around the resting position. Given a flagellar length of \( ca. 3.3 \) mm in male \( T. oxorhynchites \), this is equivalent to an angular deflection of \( \pm 0.0001^\circ \). To borrow Hudspeth’s (1989) analogy, such deflections correspond to displacements of the pinnacle of the Eiffel Tower of no more than \( \pm 0.7 \) mm. This information about the threshold sensitivity offers the opportunity of estimating the distance at which males can detect female sounds, information which has long been elusive in mosquito research (Markl 1973; Clements 1999).

Female mosquitoes produce rather faint sounds. Measured at 1.5 cm distance in front of a tethered flying female, the particle displacement is only \( ca. 700 \) nm (figure 2). For bipolar sound sources such as mosquito wings, the particle displacement decreases by \( -18 \) dB per doubling distance as long as the distance is less than one-sixth of the wavelength (Bennet-Clark 1996). Due to this steep decrease in particle displacement with distance, a particle displacement of \( 2.4 \) nm (the minimum displacement eliciting responses in the male Johnston’s organ) already occurs at 9–10 cm from the female. According to this conservative estimate, neglecting possible asymmetries in sound radiation (Tischner & Schieß 1955), the use of flight sound as an acoustic cue for mate detection is only possible when the male is already close to a flying female, as has been surmised by behavioural observations in other mosquito species (Wishart & Riordan 1959; Downes 1969; Charlwood & Jones 1979). In an evolutionary sense, the low acoustic efficiency of female sound radiation apparently constitutes a constraint driving the males to high acoustic sensitivity.

The female Johnston’s organ is less acoustically sensitive than the male’s. However, counting some 7000 sensory cells and sensing flagellar deflections of \( 0.0005^\circ \) caused by \( \pm 11 \) nm particle displacements in the sound field, the female Johnston’s organ is among the most complex and acoustically sensitive mechanosensory organs in insects (McIver 1985; Bailey 1991). Its sensitivity surpasses the most sensitive arthropod movement detector known, i.e. the cerebral hair system of crickets (angular hair deflections at a neural threshold of as little as \( \pm 0.001^\circ \)) (Kumagai et al. 1998). This fact strongly suggests that Johnston’s organ serves an auditory function in females as well. Furthermore, the results of this study demonstrate that the specialized antennal structure found in males merely results in an improvement of the antenna’s acoustic properties rather than being an essential prerequisite for sound reception. Though lacking comparable flagellar specializations, females are almost as sensitive to sounds as males. Interestingly, despite the acoustic sensitivity of the female Johnston’s organ, nothing is to be found in the literature which reports behavioural evidence for female audition (Roth 1948; Tischner & Schieß 1955; Wishart & Riordan 1959; Belton 1974; Gibson 1985; Clements 1999). It may be that an entire aspect of female mosquito sensory biology has been
overlooked. Considering the relevance of male audition in mosquito reproduction, unravelling the enigma of the significance of female audition is expected to be an important step towards future understanding of the sensory and behavioural ecology of mosquitoes.

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