Navigation organs of the oriental hornet
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CHAPTER 4

Ciliary hair cells and cuticular photoreceptor of the hornet Vespa orientalis as components of a gravity detecting system: a SEM/TEM investigation

Abstract

This chapter describes three types of hair cell configurations with stereo- and kinocilia in the head of the hornet; these were encountered at the vertex and frons regions adjacent to the three ocelli and are assumed to be part of the hornet’s gravity detecting system, together with cuticular photoreceptors.

The first and most common type of hair cell configuration (type A) was a cell surrounded by a septum, having a diameter of 30-50 µm. Aggregates of over 20 of such hair cell groups together formed a larger unit, 130-300 µm in diameter, which was also enclosed by a septum. Many of these larger round units were, in turn, arranged in either angular or leaflike clusters. The hair cells bore numerous cilia of 4.5-6.0 µm long, and were themselves composed of smaller subunits of about 7-8 µm in diameter, which were not enclosed by a septum. The second type of hair cell configuration (type B) was discrete cells with a diameter of 12.5-14 µm. Type B cells are located in the vicinity of the pore canal outlet of the peripheral photoreceptor. These single hair cells were either devoid of or only partially enclosed by a septum. Their cilia were 4.5-6.0 µm long, however with a diameter of only 150-160 nm. On the exterior of each cilium a tubular system could be detected. Furthermore, the tips of adjacent cilia were interconnected by a kind of fibre bearing a spherical body in its middle. The third type of hair cell (type C), present in the neighbourhood of the second type of hair cell (type B), was chaliceshaped and showed interconnecting fibrils comparable to those found at type B.

We believe that these three types of hair cell configurations along with the ganglion cells interconnecting their bases are all components of the gravity organ of the hornet (the Ishay Organ) and together with the cuticular photoreceptors function in the navigation system of the hornet. We further conjecture that the described structures are engulfed by endolymph signals generated by each unit are conducted through neural fibres to the hornet central nervous system.

Keywords: Cilia, hornet, navigation, photoreceptors, SEM/TEM, noncoating

Introduction

Social wasps, including hornets (subfamily Vespinae) are insects that build their combs underground, in dim light or complete darkness [1-6]. Comb construction in species of Vespina is directed towards the gravitational pull of the earth [7,8]. In biological studies it was found that the Oriental hornet is sensitive to a slope of 1.5° and to a delta of radial acceleration as low as 0.1 g [9,10].

Suspecting the need of hornets for a gravity sensing apparatus, we have undertaken this study to ascertain the presence and mode of
action of such an organ that would enable these insects to sense the direction of the gravitational force during comb building [11]. Indeed we found that on the inner side of the frons plate in social wasps there is a complicated structure composed of static and dynamic nerve fibres, some of which connect between the frons plate and the central nervous system. Encouraged by this finding we conjectured that the interaction between the fibres and the various head structures to which they are attached, is probably part of the proprioceptive system of hornets and is responsible as well for gravity perception. The apparatus involved was accordingly named the “Ishay Organ” and in the same context the presence of cilia on the inner side of the frons plate was also reported [12].

The Oriental hornet *Vespa orientalis* (Vespinae Hymenoptera) is prevalent in the Middle East, North Africa and West Asia up to India [13]. This hornet’s cuticle is predominantly of a brown colour, but two segments of the gaster and several plates on the frons are of a yellow colour due to the presence of symbionts [14]. The frons plate, which bears the gravity organs, is yellow and is built to function as a photovoltaic system [15]. Indeed its upper portion contains parallel transparent plates, similar to these of a solar cell [16]. Additionally there are throughout the cuticle, dispersed several microns apart, distinct pores which are the outlets of peripheral photoreceptors [17]. These peripheral photoreceptors are comprised predominantly of a pore canal and a wide cell underneath the cuticle connected to the nervous system, that are important in hornet orientation and navigation [18].

In the past, several investigators have studied the ciliary structures of different insects mainly by transmission electron microscopy, whereas we have studied hair cells mainly by scanning electron microscopy to obtain a complete 3-dimensional image of the distinct hair cell components involved. Here, we review briefly the related findings:

- The fine structure of the sensory cilium of the auditory receptor of the Australian cicada (*Cyclochila australasiae*) has been studied by Young [19]. The chordotonal sensilla is comprised of one or more bipolar sensory neurons with the tip of the dendrite enclosed in a specialized structure, the scalopale. The distal ciliary shaft appears round and bears a ciliary dilatation. A ring of nine doublet fibrils creates a rod and a tube, the rod bearing a pair of arms which project towards the neighbouring doublet. The expanded central area is filled with moderately electron-dense amorphous material.
- Heimann [20] found that the first antennae of *Conchaecia spinirostris* (Ostracoda Crustacea) display sensory tubes which are arranged either as four tubes with one seta or as two tubes with three setae. These tubes divide into four regions, with each tube containing 40-60 ciliated dendrites, some hypodermal and nonneural cells, and a specialized cuticle. Each dendrite within the tube gives rise to approximately 25 cilia in a $9 \times 2 + 0$ pattern. Each cilium splits up into nine branches which extend to the tip in a partly helical course and form a ring in the distal part beneath the cuticle. The tubes are covered by a filamentous surface coat. The ciliary branches probably represent the receptive apparatus, while the sensory tubes are most likely chemoreceptors.

The coelocapitular sensillum and the antennal hygro and thermoreceptive sensillum of a number of different insects have been extensively studied [21-23]. The cuticular apparatus of the sensillum on the honey bee (*Apis mellifera*) has a mushroom shaped protrusion devoid of pores and set in a narrow cylindrical pit, positioned in the centre of a shallow depression on the cuticle. Often three or four receptor cells are encountered, three of them bearing unbranched sensory cilia of the $9 \times 2 + 0$ type and containing densely packed microtubules extending distally into the cuticular apparatus and completely filling its cavity. If there is a fourth
receptor it has a thin sensory cilium which terminates beneath the cuticular apparatus, its connecting cilium endowed with armed outer doublets. An outer cavity formed by their enveloping cells is sealed off.

The purpose of this scanning electron microscopic investigation was to analyse the 3-dimensional organization of hair cell structures and to provide a detailed classification of the distinct types of hair cells, that form part of the gravity organ of the hornet under investigation.

**Materials and methods**

**Pre-preparation of the hornets.** Hornets, one or two day old, collected from nests in the open field, were anesthetized with ether and killed thereafter. Subsequently, the heads were rinsed for 1 min in 0.1 M sodium cacodylate buffer solution and then fixed in a mixture of 2% GA (glutaraldehyde) and 2% PF (paraformaldehyde) and 0.2% acrolein in 0.1 M sodium cacodylate buffer (pH 7.4, 20 °C, for 24 h) [24].

**Field emissionscanning electron microscopy.** Specimens were prepared according to the TAO (tannic acid/arginine/osmium tetroxide) noncoating technique, which involved immersion of the samples in a mixture of arginine HCl, glycine, sucrose and sodium glutamate [2%, 16 h, 20 °C], rinsing (3x) in distilled water and immersion in a mixture of tannic acid and guanidine HCl [2%, 8 h, 20 °C], after which the samples were carefully rinsed (3x) in distilled water. Finally tissues were fixed by immersion in an OsO₄ solution in distilled water [2%, 8 h, 20 °C], followed by rinsing (3x) in distilled water, as described previously [25,26]. Dehydration with ethanol was followed by critical point drying in liquid CO₂ , observations were carried out with a JEOL FE-SEM, type 6301F, operated at 2-3 kV.

**Transmission electron microscopy (TEM).** Small portions of previously observed FE-SEM samples, prepared according to the GA/PF/Acrolein prefixation method and the TAO postfixation procedure, were carefully orientated, embedded in Epon and ultrathin sectioned. Sections were poststained with uranyl acetate/lead citrate and observed in a Philips TEM, type CM100, operated at 60 kV.

**Results**

Plate I. An overview of the exterior of the head of the hornet *V. orientalis* is shown in Fig. 1; where the left (L) and right (R) compound eyes as well as the triangular snout bearing the frons plate (fp), coronal suture (cs) and vertex (vt) are clearly discernible. The ocelli two lateral (a,b) and one medial (c) are positioned to fit a triangle, with its base orientated upwards; the two antennae (an) are also observable. A part of the interior of the head is shown in Fig. 2. Here the crossfractured cuticle (cu) and particularly the large groups of muscle fibres (mf) are dominant. In the central part of the figure, groups of ganglion cells (gc) are discernible besides ‘brain’ tissue (br), covered on one side by a thin otholitic membrane-like layer (om), presumably a kind of tectorial membrane, damaged at preparation. A large number of nerve fibres (nf), possibly axons, extend from this area proceeding towards the layer of hair cells (hc), located on the inside of the cuticle (cu).

In a more detailed image, Fig. 3, closely packed ganglion cells (gc) are found adjacent to groups of hair cells with cilia. These hair cells represent the type which later is referred to as hair cell configuration type A, see Figs. 9 and 10.

Nerve fibres (nf) extending from the ganglion cells (gc) penetrate the otholitic membranelike layer (om), originally covering the hair cells (hc). They interconnect between the ganglion cells (gc) and the bases of the hair cells as shown at the left side of figure 3.

Another view of the ganglion cells is offered in Fig. 4. Here, nerve fibres (nf) connect ganglion cells (gc) to the photoreceptors (pr) in
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side the cuticle by penetrating through the enveloping otholitic membranelike layer (om). The small pores (arrows) on the inside of the cuticle (cu) represent the entrance of the pore canals, each housing a photoreceptor at the endocuticular region. The axons are directed towards these pores; in the bottom left of the figure, part of such a photoreceptor (pr) is observable as a spherical body. A more detailed view of this photoreceptor (pr) is seen in the inset, where it appears as a woven basket, connected to the top of a kind of lamellar ribbon (ri). It is obvious that this photoreceptor cell has been damaged, due to forces exerted at fracturing. The image, nevertheless demonstrates some of the fibrillar structure of the photoreceptor cell. The substratum (ma) covering the inside of the cuticle appears as a highly fibrous layer; greater detail of that layer is shown further on in Fig. 19.

Another view of the inside of the head is provided by Fig. 5. On the right of the figure a
lateral (a) and a median (c) ocellus (the second lateral ocellus is obscured by the muscle fibres) can be observed close to the frons plate (fp) and viewed from the rear. The frons plate is covered with compact groups of hair cells, which at this magnification are barely visible; in the bottom left of this figure groups of muscle fibres (mf) can be seen. The somewhat elliptic openings (*) at the bottom left of the figure represent the bases of the two antennae.

Both the vertex area adjacent to the bases of the compound eyes (ce) as well as the part close to the three ocelli (a, b, c) are covered with hair cells, as observable in Fig. 6. In more distal parts, the density of hair cells is lower than in the area of the frons plate (fp) and around the compound eyes (ce). The hair cells are arranged in circular groups that are very dense in the area adjacent to the compound eyes but are less dense further up the figure where they aggregate in leafshaped groups, somewhat reminiscent of ‘branches’.

**Plate II**

In the distal area, close to the compound eyes, each more or less circular group of hair cells (hc) is separated from the adjacent group by a septum (sp), as shown in Fig. 7. The average diameter of such a group varies between 130 and 300 µm, depending on the location in the head. These groups are similar to the circular aggregates seen in Fig. 6; however, the density of these groups within the various areas differs considerably. There are over 20 sub-units within the circular groups, each probably comprising one hair cell unit, with an average diameter of 30-50 µm.

On the right side of the figure remnants of the layer covering the hair cells can be seen, particularly at the single hair cell to the right (+). It seems that the covering layer and the septae have a similar gross morphology. A more detailed image of a few hair cells (hc), here indicated as hair cell type A, is shown in Fig. 8. This type of hair cell (hc) is surrounded by a thick and somewhat granular septum (sp) and a large number of stereocilia to form its characteristic configuration.

These stereocilia appear to differ in length and are grouped together in smaller units, mostly lacking a septum, as observable in Figs. 9 and 10. More detailed images of the stereocilia occurring in these units of hair cells are presented in Figs 11, 12 and 13 (FE-SEM) and in Fig. 14 (TEM image).

A single stereocilium (st) is comprised of a number of tubular structures coated with glycocalyx (*). The average diameter of these tubular structures is appr. 0.5-0.6µm. Figure 13, likewise shows units of (stere)ocilia (st), coated with glycocalyx (*). Many of them (~15-20) are arranged in a bundle or unit; a nerve fibre (nf) is seen alongside the cilia (st). This type A hair cell is similar to the one shown in Fig. 3 adjacent to the ganglion cells. Remnants of glycocalyx or haemolymph obscure the view, yet the entire aggregate is clearly a large cluster of stereocilia whose diameter ranges between 7 and 8 µm. Figure 14 represents a TEM image of an ultrathin section of a few stereocilia (st) with an average diameter of 500-600 nm, comparable to what has been shown in Fig. 12. Stereocilium type A is composed of nine tubular structures, separated from one another by a rather thin layer of electron dense material, probably glycocalyx (*), heavily stained as result of the tannic acid/arginin/osmium tetroxide (TAO) noncoating method. At the bottom of the picture a sidelink (sl), bridging between two adjacent stereocilia, is observable. In a few other places sidelinke or places where they were present before the sectioning are observable, although less clearly.

**Plate III**

Particularly in the vicinity of a peripheral photoreceptor pore canal (po) outlet, cilia can be seen which are arranged in groups forming a type of hair cell configuration, further on indicated as hair cell type B. However, these hair cells are not or are only partially...
Legends of Plate II.

(Fig. 7) Larger unit with circular groups of hair cells (hc) type A with stereocilia, separated from one another by septae (sp). At the right, one hair cell (+) still is covered with an otholic membranelike layer. Bar = 100 µm. (Fig. 8), A few circularly shaped units of hair cells (he), separated by a septum (sp), with clusters of stereocilia. Bar = 10 µm. (Figs. 9, 10) Subdivision of part of one hair cell of type A with stereocilia (st) of different length, no septum present. Bar = 10 µm. (Figs. 11, 12) Detailed image of a stereocilium (st) of type A with neurofibrils (nf) inside, note glycocalyx (*) at in and outside stereocilia. Bar = 1 µm. (Fig. 13) Subdivision of a hair cell (hc) type A, consisting of a group of appr. 20 stereocilia (st) around a central area. Bar = 10 µm. (Fig. 14) TEM longitudinal section of a few stereocilia of type A hair cell, the middle one with a width of appr. 500 nm, consisting of about 9 closely packed tubular structures, interspaced by heavily stained glycocalyx (*). Note sidelin (sl) between two adjacent stereocilia. Bar = 500 nm.
Legends of Plate III.
(Fig. 15) Type B hair cells (hc) with cilia adjacent to pore-canal entrance (po) of photoreceptor cell, partly enclosed by a septum (sp); note matrix material (ma) covering the cuticle inside wall. Bar = 10 µm. (Fig. 16) Detail of type B hair cell (hc), with cilia (st) emerging from fibrillar matrix (ma), part of the structure is bordered by a septum (sp). Bar = 2 µm. (Fig. 17) Type B hair cell (hc) with cilia adjacent to chalice structure (ch) and otholithic membrane (om), part of type B and type C hair cells are bordered by a septum (sp). Bar = 10 µm. (Fig. 18) Detail of chalice type C hair cell (hc) with fibrillike connections (fi) with a globular body (gb) in the middle. Bar = 1 µm. (Fig. 19) Detail of fibrillar covering of inside of cuticle with tufts (tu). Bar = 1 µm. (Fig. 20) Detail of stereocilia (st) of type B hair cell with fibrillike connections (fi) bearing a globular body (gb) at the middle; note longitudinal lines at cilia and the presence of glycocalyx material (*). Bar = 1 µm. (Fig. 21) TEM section of stereocilia (st) of type B hair cell, note rather compact structure. Bar = 200 nm. (Fig. 22) Hair cells at distal part of vertex, covered with a slightly inferiorly turned lamellar layer (la), at some places showing part of the hair cell (hc) underneath. Bar = 10 µm
surrounded by a septum (sp), as can be observed in Fig. 15. The cilia seem to arise directly from a highly fibrillar matrix (ma) covering the multilayered cuticle (compare with Fig. 4). The average diameter of hair cell type B is appr. 13 µm, which is thus larger than the diameter of the ones seen in Fig. 12, although smaller than the diameter of those shown in Figs 8 and 9. Hair cell type B is shown in more detail in Fig. 16, which also shows the presence of a septum (sp) at one side and the occurrence of (stereo) cilia (st), emerging from the cuticular substratum (ma). The latter cilia are clearly longer and thinner (average diameter appr. 150 nm) than those shown previously.

Figure 17 represents a higher magnification of an area similar to that shown in Fig. 15. Hair cell type B seen here is adjacent to a structure resembling a chalice (ch), this is the third type of hair cell configuration, further on indicated with hair cell type C. Remnants of an otholithic membranelike covering layer (om) are seen at the right part of the figure. It could have a similar function as the tectorial membrane in mammals. A rather granular septum (sp), abutting on one side of hair cell type B, is also observable. Frequently hair cell type B and C are encountered in the very same unit as witnessed at the top left of this figure.

A detailed view of hair cell type C is provided in Figure 18. Here the granularity of the structure and the presence of very thin fibrils (fi), within the middle a globular body (gb), connecting the chalice (ch), are clearly seen.

A detailed image of the fibrillar substrate lining the inside of the cuticle, is provided in Figure 19, showing tufts (tu), which have a larger diameter at their base (~ 0.25 µm) at the top (~ 0.15 µm).

A higher magnification of the stereocilia of the of hair cell type B is shown in Figure 20. Here, individual cilia (st) display a certain pattern of lines along their length. The cilia are interconnected at their top by a thin fibril, protruding from the centre of the stereocilium at the top, bearing a globular extension (gb) in the middle.

In between the individual cilia a granular substance is found, which could represent glycocalyx or endolymph material. The individual stereocilium base has an average diameter of about 140-150 nm, while its length varies between 4.6 and 6.0 µm.

Figure 21 shows a TEM detail of a stereocilium (st) of hair cell type B. Comparing the stereocilia of a type B hair cell (fig. 21) with those of type A hair cell (Fig. 14), it is obvious that the diameter of type A is ~ 500-600 nm, while the diameter of type B is only ~ 120-130 nm, as calculated from TEM images. This is in agreement with the SEM results, as based on the visualization of stereocilia after tannic acid/arginin/osmium tetroxide treatment, which often produces larger complexes with osmium.

One stereocilium of type A hair cell consists of about 9 subunits held together by glycococalyx-like material, heavily stained by the TAO noncoating method. Each subunit of it is ~ 35-40 nm in width. A cilium of hair cell type B does not show much of a subdivision, it is heavily stained as result of the TAO treatment, giving it a somewhat coarse appearance. In the longitudinal section dark patches are seen; sometimes a thin enveloping heavily stained layer is seen, giving the impression of a rather compact unit embedded in glycocalyxlike material. The thin lines observable at the surface of the stereocilia type B (Fig. 20) suggest, together with Fig. 21, that the lining appears at the outside surface of the stereocilium.

Figure 22 presents hair cell units (hc) at the distal part of the vertex, each enclosed like a box by a thin striated lamellar layer (la). The layer has been somewhat damaged by the drying procedure used, causing shrinkage and resulting in deformation of the layer and of the caplike structure at the tip of each hair cell. At one point the presence of stereocilia underneath this layer is also discernible. It is possible that this layer represents the tectorial membrane-like structure seen before.
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Legends of Plate IV.
(Fig. 23) Longitudinal TEM section of photoreceptor cell (pr) with nucleus (nu) inside the multilayered cuticle (cu). The two compartments of the cell are partly filled with fibrillar material (fb) and enclosed by a folded outer rhabdomer membrane (rm) containing retinoides. Basal cuboidal cells (bc) extend along the inside of the cuticle, covered by the cell membrane (cm) of the photoreceptor cell (pr). A dendrite structure (de) entering the photoreceptor cell and typical haemolymph spaces (hs) can also be observed. Bar = 2 µm. (Fig. 24) Other TEM section of photoreceptor cell (pr), almost perpendicular to that of Fig. 23, is seen inside the cuticle (cu). A dendrite-like structure (de) is entering the cell close to the vitreous body (vb). A membrane(››), closing off the pore canal from the photoreceptor area, serves as an aperture to light from the outside. Bar = 2µm. (Fig. 25) Other TEM ultrathin section of a photoreceptor cell inside cuticle (cu), showing the vitreous body (vb), a dendrite (de) enclosed by various membranes (mb) and the spot where synaptic processes take place (Ÿ). Bar = 1 µm. (Fig. 26) FE-SEM image of a photoreceptor cell, cytoskeleton consist of a fine network of fibres with a spool-like appearance extending above cuticle (cu); image comparable with Fig. 4 (inset). Bar = 2 µm. (Fig. 27) FE-SEM image of inside area of the head covered by hair cells (hc) with cilia surrounding photoreceptor cells (pr) at pore canal openings. Bar = 50 µm. (Fig. 28) Detailed FE-SEM image of apical side of stereocilia of type B hair cell, note several kinocilia-like extensions at the top of cilium connected to other stereocilia. Bar = 200 nm.
Plate IV
Figure 23 shows an almost longitudinal section of a photoreceptor cell, located inside the cuticle (cu) extending to the left and the right. The cell nucleus (nu) and the basal membrane (cm) covering the inside of the cuticle with the basal cells (bc) are well observable, so are the spaces (hs) with endolymph at the top of the image. The photoreceptor cell seems to consist of two compartments containing fibrillar material (fb), separated by a membrane. The photoreceptor cell is enclosed by a rhabdomer membrane (rm) appearing as a folded membrane containing retinoids. A dendritic fibre (de) enters the photoreceptor cells at the left side of the cell close to the cuticle (cu). The thickness of the cuticular layers is clearly increasing towards the outside cuticle.

Figure 24 presents another view onto the photoreceptor cell (pr) enclosed by the cuticular layers (cu). It consists of different compartments, some filled with fibrillar material some are almost empty forming the vitreous body (vb) of the cell. A dendrite (de) leaving the photoreceptor cell and the location of the sinaps processes (m) can be observed. The image has been orientated in the same way as Fig. 23, at the bottom of the picture a membrane is found, closing off the photoreceptor area from the pore canal, serving as a kind of aperture for light coming in. The space below the membrane, the pore canal, is filled by resin as a result of the embedding procedure; the white spots are electron beam irradiation artefacts.

Figure 25 represents another section of the photoreceptor cell orientated almost perpendicular to that of Fig. 23, the presence of a vitreous body compartment (vb) and a dendrite (de) is well observable, obviously they are surrounded by membranous structures (mb).

Closely surrounded by the cuticular layers (cu), it appears that the cell is surrounded by a membrane (R) as observed in the TEM images before. The image shows some similarity with the inset of Fig. 4 and on the other hand with the fibrillar material found in the compartments observed in the TEM images of Figs. 23, 24 and 25. As shown previously (Fig. 15), pore canal outlets with photoreceptor cells inside are often surrounded by stereocilia of type B hair cells. The photoreceptor cell is closely surrounded by the cuticular layers (cu), it appears that the cell is surrounded by a membrane (R), as observed in the TEM images before. The image shows some similarity with the inset of Fig. 4 and on the other hand with the fibrillar material found in the compartments observed in the TEM images of Figs. 23, 24 and 25. As shown previously (Fig. 15), pore canal outlets with photoreceptor cells inside are often surrounded by stereocilia of type B hair cells.

In Figure 27, that part of the photoreceptor cells (pr) extending outside the cuticle, similar in Fig. 23 is visible, surrounded by hair cells (hc) with cilia. Obviously some drying artefacts have occurred, both at the photoreceptor cells and the hair cells, causing shrinkage and aggregation of cilia. Due to the location of the photoreceptor cell inside the cuticle, optimal preservation of the delicate structures is rather difficult, which accounts for the various appearances of the cell particularly in FE-SEM and to some extent also in TEM.

The variance in appearances in TEM though is mainly the result of different cutting faces obtained. Finally, Figure 28 represents a higher magnification of the apical side of a stereocilium of type B hair cell, at least three kinociliar extensions are found, which connect with other stereocilia; due to fixation and drying forces exerted, their shape might be altered slightly.

Discussion

The hair cell is a receptor cell in a sensory system. It performs an essential function, namely, of transducing a mechanical sensory
input into electrical signals [27]. It is thus a mechanoelectric transducer element which is vital to all functional properties of vestibular organs [26]. The name hair cell for the structures found, is chosen based on their resemblance to hair cell found in the organ of Corti as part of the cochlea of mammals, though, the origin of the cilia of the hair cells in the hornet are different from those in the organ of Corti. Those of the hornet seems to have their origin at the highly fibrous substrate covering the inside layer of the cuticle, nevertheless, the arrangement of various cilia form specific structures.

We conjecture that hair cells act as mechano receptors to gravity dependent accelerations. The hair cells are encountered in the head of the hornet covering almost all the area of the frons plate and vertex, i.e. the areas not occupied by muscle insertions, peripheral photoreceptor pores and the three ocelli. The overall covering with hair cells with a large number of cilia is enhancing the sensitivity to detect the smallest directional changes, in particular in the dark. The hair cells are connected to ganglion cells and to the nerve fibres in the Ishay Organ which ultimately enter the brain. Why have the described hair cells remained uninvestigated until now?

To our knowledge the ultrastructure of these cilia-bearing cells located in the interior of the head cuticle close to the frons and ocelli has never been studied extensively by SEM or TEM. There are a number of reasons for that. To begin with, standard GA/ PF prefixation and OsO₄ postfixation do not preserve these structures adequately, moreover, there is leaking out of certain components, the production of artefacts due to irradiation damage and inevitable shrinkage as a result of the dehydration/drying process. Secondly, the need for relatively high acceleration voltages (15-25 kV) used in the past, required the use of relatively thick (8-10 nm) conductive layers to avoid charging. These layers are often too thick and granular, obscuring surface details [29]. Furthermore, structures may be obscured or masked by the high ratio of secondary electrons generated within the investigated structures as compared to the secondary electrons generated at the very surface of the structures. Thirdly, a combination of SEM and TEM procedures on the same specimen, is virtually possible only on optimally preserved structures processed initially for SEM and subsequently embedded for TEM. [30-32]. The use of a GA/PF/acrolein prefixation followed by tannic acid/ arginin/ osmium tetroxide noncoating postfixiation provides optimal preservation of structures containing glycoproteins and mucopolysaccharides [33-35]. The use of a low accelerating voltage (23 kV) in FE-SEM offers high resolution and better surface topographic imaging, thus contributing to an improved image of ciliary structures. The low brightness of standard W or LaB6 electron sources is unsufficient to allow low kV SEM operation at an acceptable resolution.

Descriptive review of the encountered hair cells and photoreceptors. Three different types (A, B, C) of hair cells or hair cell-like structures could be distinguished within the head of V. orientalis.

Hair cell type A is found in the frons and vertex close to the ocelli and are arranged in more or less round clusters having a diameter of 180-300 µm, the clusters separated from one another by a septum (Fig. 6). These roughly round clusters were in turn organized in larger aggregates which assumed either an angular shape and high density when located closed to a compound eye or a leaflike shape and a lower density when located in the periphery. Within each such aggregate (Fig. 7) there were between 20 and 25 clusters of hair cells with cilia, each cluster averaging 30-50 µm in diameter and separated from one another by septae. In turn, such hair cell clusters subdivided into a large number of subclusters having a diameter of ~7-8 µm. The cilia in such a subunit vary in length as evident from the light charging of the tips of the individual cilia. These subclusters are discrete within the hair
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cell cluster. At higher magnification such a subcluster reveals a central area of app. 3-5 µm in diameter, surrounded by a ring of single stereocilia. Each stereocilium has an average width of 130-140 nm and seems slightly thicker at the base than at the top. The length of the single stereocilium in a given subcluster is app. 12.5 - 14 µm.

In mammals, the length of the stereocilia in the outermost row of hair cells in the organ of Corti is ~ 2.5- 3.8 µm, so they are considerably shorter than those of the hornet [37]. At this type of cilia, sidelinks have been observed, probably involved in the process of cilia activation and inhibition. Most of the exterior of the cilia is covered with a somewhat granular material deriving either from the glycocalyx or the endolymph, probably consisting of glycolipids, glycoproteins and other materials. The anionic sites of these compounds react with cations such as tannic acid and osmium.

In SEM imaging subunits of a single cilium can be discerned, namely, microtubules whose diameter is ~ 30-40 nm. This is in agreement with the data obtained from inspection of a section of a single cilium in TEM where the diameter was ~ 40 nm.

Hair cell type B possesses stereocilia which are close to the outlet of the photoreceptor at the cuticular pore canal and are arranged in groups, but lack a septum or have only a partial septum. The number of stereocilia within a type B hair cell is smaller than in type A and moreover, the diameter of the type B cell is ~ 4.6-6.5 µm as compared to a diameter of 20-50 µm for type A. The stereocilia of hair cell type B are not only different in length from type A, but are also much smaller in diameter (150-160 nm) as compared to 500-600 nm. In some places, the stereocilia just seem to be an extension of the fibrous matrix coating the inside of the cuticle. Such ‘single’ cilia are often connected at their very top to adjacent cilia by means of a thin fibril bearing a globular swelling in its middle.

Some of the stereocilia have more than one kinociliary extension. The length of these interconnecting fibrils is ~ 1 µm. It is possible that the globular swelling is connected to the base of the tectorial membrane covering the cilia, in which case it could be similar to the bulbs found on nonmotile kinocilia that are connected to the otolithic membrane in mammals [36]. They certainly differ from the tip and side links encountered on stereocilia of the hair cells of the outer rows in the organ of Corti in mammals, which contribute to mechanosensitivity of the hair cells [37-41]. In the organ of Corti of mammals [36-41] in the W-shaped stereocilia cross connections occur either between the stereocilia in the same row or between short and medium length stereocilia. Their function is to mitigate the effect of excessively strong vibrations on single stereocilia. Possibly the septae between groups of cilia in V. orientalis have a comparable function. The variation in both the density and the shape of the cilia may perhaps reflect on their particular physiological function in the gravitic system of the hornet.

Hair cell type C is a structure which until now has not yet been explained; they are the chaliceshaped units located close to the groups of cilia surrounded by a septum (conceivably representing the bases of muscles). These structures seem to be virtually empty shells, although transsected by very fine fibrils and bearing very small globular encrustations at their bottom. The material from which these structures are composed seems to be similar to that of the septae. The fact that these structures are often found devoid of content could be an artefact, that is, they may have originally been filled with endolymph which was leached out during the rinsing step in the preservation procedure. The original contents, if any, could perhaps play a role in the gravity system, conceivably slowing down excessively rapid movements. These peculiar structures are mostly located close to the type B hair cells, often separated from them by a septum. Hair cells of type B and the type C structure are frequently covered by the so called...
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tectorial membrane as is well demonstrated in Fig. 17. Photoreceptor cells are located in the multi-layered cuticle, very much enclosed in a pore canal formed by the cuticular layers, and are found in the head and in the abdomen at the yellow strip. The ones presented here are found in the head and are therefore rather difficult to reach for fixation mixtures, resulting in an uneven preservation of the various structures making up the photoreceptor cell. The comparison and interpretation of FE-SEM and TEM photoreceptor images for the same reason is rather difficult. The presence of fibrillar structures arranged in a kind of spoolshape seems obvious from both FE-SEM and TEM images. The FE-SEM specimens of photoreceptors could be slightly damaged, because they are obtained after fracturing the cuticle which often leads to the excertion of pulling forces.

The light enters the pore canal at the epicuticle side at a certain angle, due to the shape of the eaves at the outside of the cuticle, and is transported to the photoreceptor cell. A membranous structure acts as a kind of aperture to restrict the amount of light entering the photoreceptor cell. The light is transformed into a neural impulse which is transferred to the ganglion cells, the presence of a kind of nerve connection is acknowledged both in FE-SEM and TEM. Due to the special light entrance conditions, small directional changes of the body can be detected by the photoreceptor cell found in the cuticular layers of the head and the abdominal area at the yellow stripe. Clearly this is only possible in daylight; navigation in the dim is carried out most probably by the three ocelli.

A thin otholitic membrane-like sheet covers the cells and suggests a certain similarity with the tectorial membrane, covering the longest stereocilia of the outer hair cells of mammals. The membrane-like sheet comes in contact with the tips of the cilia, on this membrane some small otholites can be found, that can be compared with maculae of the vertebrate vestibular system. This analogy suggests that the role of the structure is in the sensation of linear accelerations. Over the tectorial-like membrane at the inner side, a large number of ganglion cells can be seen. Some dendrites connect these cells to the photoreceptors, others connect them to different types of ciliary cells interconnecting between ganglion cells. This structure suggests that the role of these ganglion cells is to integrate information coming from the ciliary cells of the total area of the head covered by these cells. This information is further integrated with information coming from the photoreceptors of the same area. Gravity direction and light are the cues for hornets navigation and so this is probably the ganglion of navigation.

Conclusions

We have encountered three types of structures in the head cuticle of the Oriental hornet, all of them naturally covered by an otholitic membrane-like sheet with small crystal-like structures, possibly otoliths. Not in all cases this sheet is well observable in the images shown, because some kind of disruption takes place as a result of drying forces. A similar process is seen at the tectorial membrane covering the rows of hair cells in the organ of Corti in mammals, where the tops of the longest stereocilia of the outer rows of hair cells separate from the tectorial membrane undersurface at appropriate fixation of the organ of Corti, if not the stereocilia can fracture at their base [31]. The mutual proximity of these three structures and of the common ganglion cells, suggest that they are closely related. The cilia cells of the frons probably are gravireceptors. The type A hair cells far outnumber the type B cells. The latter are mostly found close to the pore canal outlets of the peripheral photoreceptor. All these structures are located on plates in the anterior upper portion of the head, exterior to the nerve fibres of the Ishay Organ, but in close contact with them. Type A stereocilia are longer and
have a larger diameter than type B. In crossection type A stereocilia display tubular structures, particularly in TEM images, and also thin fibrils. Type B stereocilia do not show fibrils, although they have a tubular substructure visible from the outside. Long extensions act as a kind of kinocilium which interconnects adjacent stereocilia. The small spherical structure in the middle of such a kinocilium touches the covering otholitic membrane and is similar to the vestibular kinocilia in mammals. From the scanty endolymph still retained between the stereocilia, particularly of type B, it is reasonable to assume that the rinsing steps probably removed a great deal of the original amount. This endolymph or hemolymph probably acts in the same way as it does in mammals in the hair cells of the vestibular area. Both type A and type B cilia seem to originate from the highly fibrous substrate covering the cuticle on the inside. Indeed small tufts are seen to emerge from the fibrillar coat, probably indicating the incipience of stereo or kinocilia. Whether the chalice structure (type C), which sometimes is surrounded by cilia of type B hair cells, has a special function when filled with endolymph remains to be ascertained. The presence of all kinds of rather small spherical units and axonfibre connections in these shelllike structures suggests for them a role in hornet navigation. If this be true, then the slightest movement of endolymph within this structure could possibly generate a signal detectable by the fibrils connecting the various units within it.

An important question that remains to be answered is whether the cilia are stereo- or kinocilia. The cilia which are part of the hair cells in the organ of Corti in mammals are stereocilia, apart from their role as sensory cilia. Although the cilia under investigation bear a certain resemblance to the microvilli of the intestinal epithelium and to cilia in lung epithelium tissue, they have a central core which is ~ 3.5 µm in diameter and surrounded by 7-8 tubular structures. According to Ham and Cormack in their textbook on Histology [42], cilia are hairlike processes extending from free surfaces of uni-cellular organisms and cells in the body which are about 5-15 µm long and ~ 200 nm in diameter. They are developed from centrioles, whose wall is composed of nine longitudinally disposed and parallel bundles of microtubules, each bundle containing three microtubules (triplets). The triplets of a centriole are held in position by fibrillar material together forming the wall of a cylinder.

Cilia can undergo extensive modification, as occurs in the receptor cells of organs of special sense. These modified cilia become very important parts of the arrangement by which nerve impulses are initiated as result of exposure to certain forms of energy, such as the rods and cones of the eye, representing modified cilia which are receptive to light energy. Stereocilia are tufts of extremely long microvilli projecting towards the lumen from the free surface of the hair cell. Mostly no characteristic pinocytotic vacuoles are shown in the apical cytoplasm of those cells. The free surface of a hair cell has hairlike processes extending from it, mostly called stereocilia. Stereocilia are narrow towards the origin and widen towards their tip. Vibrations predominate in a given region of the organ of Corti vibrating at this particular frequency and is sensed by its hair cells as a result of their microvilli. They get displaced with respect to the tectorial membrane in which their tips are embedded. This causes the hair cells to alter the pattern of impulse activity in the afferent branch of the acoustic nerve they contact. Probably some large efferent nerve endings that contain synaptic vesicles are believed to pass impulses to the hair cells. From the above presented data it seems likely that the cilia under investigation are stereocilia.
References

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