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MICROMORPHOLOGY OF THE FIBERS BEHIND THE FRONS PLATE AND ITS ADJACENT REGIONS IN THE ORIENTAL HORNET (HYMENOPTERA, VESPINAE)

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Abstract

The present study describes the fibers occurring in the space extending behind the frons plate, or the region between the ocelli and the clypeal plate of the Oriental hornet. These fibers connect to the brain in the anterior part of the head in a zone which is demarcated by an imaginary line traversing the upper part of the two ommatidia. Static fibers are present in the upper part and in the center of the space. These static fibers do not move with acceleration of the structures attached to the head. They are perpendicular to the frons plate, are relatively numerous and usually short. In contrast, the fibers in the lower part are directed toward the organ with which they connect, are few in number, have triangular fastenings and are longer. All of the fibers are branched, especially near their point of attachment to the substrate. On the surface of these fibers, there are occasionally coin or bob-like protuberances or other dilations. As seen in serial sections, proceeding from the top down to the base of the space, the fibers are longer at the center of the frons and gradually shorten toward its margins. We propose that the interaction between the fibers and the various structures in the head to which they are attached, having a harp-like appearance, is responsible for the proprioceptive sensing in hornets including the detection of gravity in the course of comb building.

Key Words: Geotactic organ, comb building, statoliths, acoustic box, frons plate, Oriental hornet, laser irradiation.

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Introduction

Hornets and wasps are social insects that build their combs underground, in complete darkness (Ishay *et al.*, 1967; Wilson, 1971; Guiglia, 1972; Spradbery, 1973; Edwards, 1980; Matsuura and Yamane, 1990). Comb construction is guided by Earth's gravitational force with the orientation of the comb dependent on the direction of this force (Ishay and Sadeh, 1975). The present work presents our continuing efforts to elucidate the morphology and mode of action of the prime organ that enables these insects to sense the direction of the gravitational force during comb building. It follows our description of the external micromorphology of the frons plate and its adjacent areas in workers of the Oriental hornet (*Vespa (V.) orientalis*, Hymenoptera Vespinae), (Ishay and Ganor, 1992) and the internal micromorphology of the frons plate in females of this hornet species (Arcan and Ishay, 1993). To our knowledge, no investigators of hornet head structure (Kirmayer, 1909; Crampton, 1921; Buckhurst *et al.*, 1923; Schröder, 1925-1929; Bischoff, 1927; Snodgrass, 1928; Duncan, 1939; Short, 1952; Imms, 1960; Howse, 1972) have described the morphology or function of this organ. We reported on the acoustic box and the neural fibers associated with it (Ishay *et al.*, 1983a; Ishay and Shimony, 1986; Ishay *et al.*, 1986; Ishay and Ganor, 1992; Arcan and Ishay, 1993), but, those early publications dealt mainly with the structures located on both sides of the frons plate and especially with the conus that intrudes into the box from the center of the plate, i.e., in the lower side of the coronal suture. In the present study, however, we centered on the frons plate and the acoustic box with its contents (e.g., the various fibers) as an integral organ of orientation with respect to gravity. We named this geotactic organ "Ishay's organ." The structures on the frons are very complex and unique in that they include not only the basic components such as the setae, cuticle and various epithelia, but also layers containing specialized structures such as ciliated cells (stereocilia), weighted bobs, yellow granules and statoliths. The frons, however, is not adjacent to the brain but, rather,

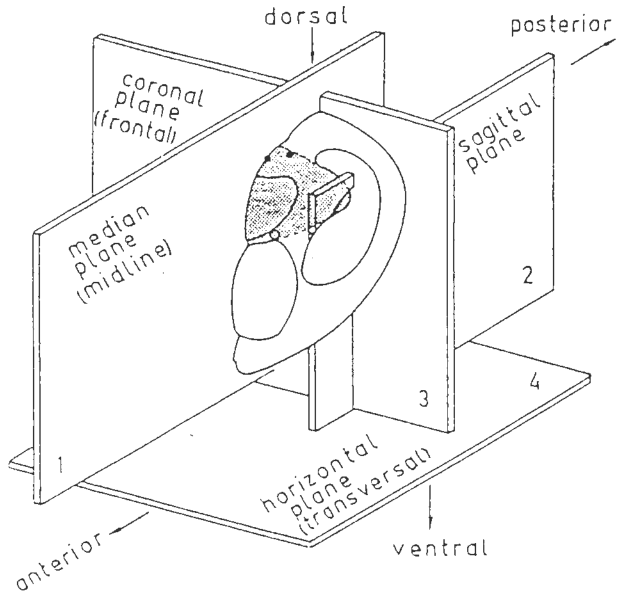


Figure 1. A scheme of the hornet (*Vespa orientalis*) head with indication of the various directions of sectioning used in the subsequent figures.

is at some distance from it, leaving a space (the acoustic box) that is crossed by bob-bearing nerve fibers and lined with a specialized epithelium. The nerve fibers that normally traverse the front part (the frons plate) to the hind part (the membrane over the brain) of the acoustic box afford Ishay's organ a tri-dimensional harp-like appearance. A glossary of relevant terms is provided in **Appendix 1**.

Materials and Methods

Oriental hornet (*Vespa orientalis*) females (queens and workers) and males were collected from fields around Tel-Aviv, as earlier described (Ishay, 1967, 1975) and either used after diethylether anesthesia or kept frozen at -20°C until use. The frons plates of 55 workers, 15 queens, and 6 males were observed. *Vespa* heads were cut in the frons area into sagittal, horizontal and frontal sections (Fig. 1), so as to expose the interior structure of the acoustic box and the various fibers traversing the cavity between the frons plate and the protocerebrum. After quench-freezing in liquid nitrogen to -150°C by insertion into a stainless steel cryostat (Ricor, Kibbutz Ein-Harod, Israel), the sections were freeze-dried for 24 hours at -80°C in a high vacuum device. For morphological examination, the specimens were attached to an aluminum stub with silver paint and coated with 20 nm of gold or carbon in a sputtering device and viewed in the secondary emission mode in a T300 JEOL (Tokyo, Japan) scanning electron microscope (SEM) operated at accelerating voltages of 10-20 kV.

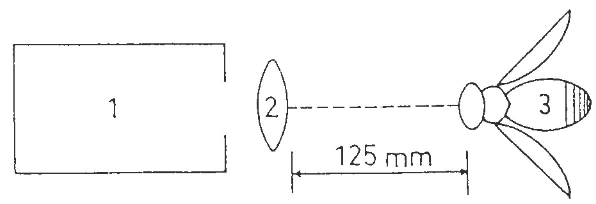


Figure 2. The manner by which the hornets (totaling 45 workers at 0-24 hours of age) were irradiated with a CO_2 laser beam. The beam (power = 1 watt; pulse duration = 0.1, 0.5 seconds) emerges from the CO_2 laser instrument (**1**); and proceeds towards the lens (**2**) which is comprised of ZnSe and undergoes amplification, and, at a distance of 125 mm, impinges upon the center of the hornet frons (**3**), which is fastened via a rubber band in order to prevent it from moving.

To determine the elemental composition of the specimens, energy-dispersive X-ray analysis was used. The specimens were secured to carbon planchettes with a thin carbon paint, irradiated with an electron beam, and then the resulting X-rays, produced by this electron-specimen interaction, were analyzed. The EDS (energy dispersive X-ray system) included an energy dispersive silicon/lithium detector connected to a multichannel analyzer and to a personal computer for processing. Elements of atomic mass less than that of sodium cannot be observed in the EDS spectra because of instrumental limitations. Thus, major elements of the cuticle, such as H, C, N, and O, remained undetected. Additionally, the chemical analysis was limited to a surface layer of about 5 μm of sample that the electrons could penetrate.

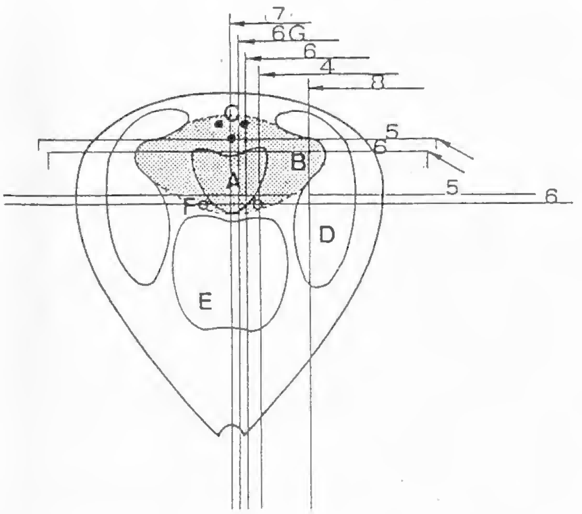
Acrylic casts of the acoustic box were prepared by direct injection of acrylic artist molding paste into freshly thawed female heads (7 queens, 26 workers). The injection site was at the frons plate superior to the antennal base. The specimens were then left to dry for 48 hours before removal of the cuticle.

Groups of hornets, 0-24 hours old, were irradiated with a CO_2 beam laser directed to the frons plate (1 mW, 1 milliseconds) (Fig. 2). Their comb building was compared with that of a control group. Ten hornets of the test group and 10 hornets of the control group were anesthetized after 14 days, and their heads were prepared for viewing by SEM, as described in Ishay and Ganor (1992).

Results

General planes

Figure 3A is a diagram indicating the location of the sections presented in the following figures.



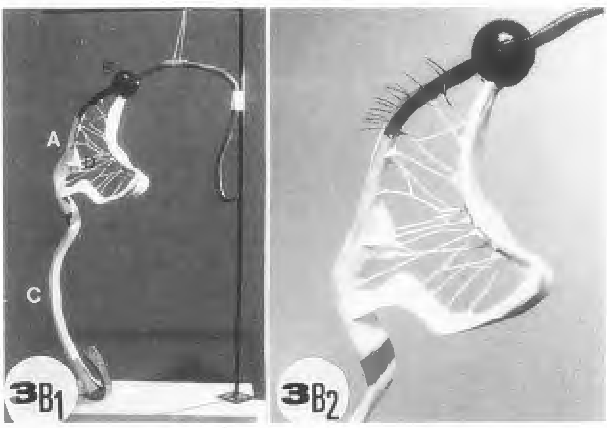


Figure 3A (top). Another scheme of hornet head pointing out the various external regions on the head whence some of the different micrographs were taken. The areas worth emphasizing are the following: A: the frons plate; B: the contour of the acoustic box; C: the ocelli (3 in all); D: the compound eyes; E: the clypeus; and F: the bases of the antennae.

Figure 3B (bottom). A model prepared to demonstrate the location of the acoustic box showing a section of the acoustic box through the median plane (**Fig. 3B1**; A: frons, B: median ocellus, C: clypeus, D: the conus.) and the location and dimensions of the acoustic box, behind the frons plate (**Fig. 3B2**).

Figure 3B is a model prepared for the purpose of demonstrating the exact location of the acoustic box and some of its details. Figure 3B1 is the model of a sliced section in the median plane through the head; Figure 3B2 is the exact proportion and location of the acoustic box, behind the frons plate.

In an anterior sagittal section near the coronal suture (Fig. 4A), proceeding from the left are the setae on the exterior of the frons plate (4A1), the cuticle of the frons plate (4A2), the conus (4A3) and the epithelial membranes protruding internally from the plate (4A4). From these membranes thread-like structures (nerve fibers and support fibers, 4A5), some torn, due to the technical procedures, extend posteriorly across a cavity (the acoustic box) to the protocerebral membranes. The acoustic box is triangular with the base of the triangle situated ventrally and the apex dorsally. A few fibers stretch ventrally from the frons to the base (4A6), while most stretch horizontally from the frons to the protocerebral membrane. At the level of the base of the acoustic box, the tubular stem (base) of the antenna (4A7) is seen close to two muscle protrusions associated with the pharynx (4A8). Perpendicular to the two protrusions extends the adductor mandibularis muscle that traverses the base of the box from the frons to the protocerebrum (4A10). Anterior to these protrusions, the muscle fans out to form a V-shaped insertion on the frons plate (4A9). Additional branches of this muscle extend ventrally toward the lower mouthparts and laterally towards the antennal bases where the internal and external antennal elevator muscles are located. The protrusions, the perpendicular muscle and its branches form a crossed muscular structure. A basal membrane (not seen here) covers the crossed muscular structure and adjacent area, delimiting the acoustic box ventrally. The anterior-posterior length of the base is close to 1.3 mm and its height (dorso-ventral length) is approximately 1.6 mm. The details of the muscles of the head of a wasp were earlier described by Duncan (1939).

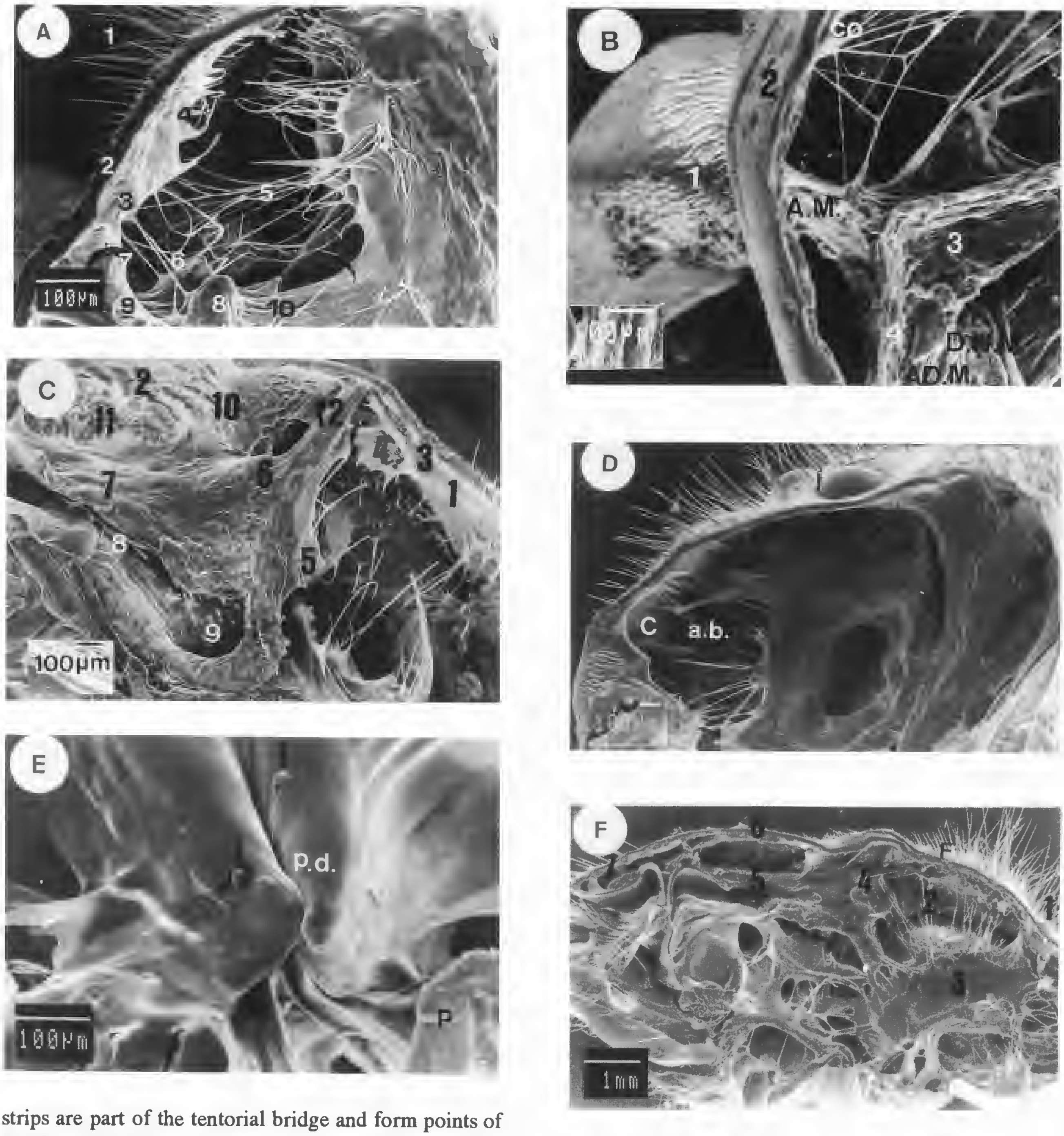
In a sagittal section lateral to the coronal suture (Fig. 4B), from left to right, the antennal base is observed (4B1) followed by a section of the frons plate with the coronal suture on its external surface (4B2). A few fibers stretch ventrally from the frons to the crossed muscular structure (Fig. 4A6). These fibers are of a dynamic type since, during mastication, the muscles repeatedly move up and down pulling and stretching the attached fibers with them. At the center is the crossed muscular structure with its associated protrusions (4B3) and its branch to the lower mouthparts, i.e., the previously mentioned adductor mandibularis muscle (4B4). This branch contains a dark brown supporting strip (not seen in figure) that is part of the tentorial bridge.

In an anterior sagittal section medial to the antennal base (Fig. 4C, viewed 90° counterclockwise), structures posterior to both the frons plate (4C1) and clypeus (4C2) are seen. Proceeding from the left to the right of the hornet's head, one may see the conus (4C3) with its covering epithelial membrane, remnants of the air sac (4C4) and a few fibers extending through the acoustic box from the frons to the protocerebral membrane. At the base of the box are muscular protrusions (4C5 and 4C6) with the associated V-shaped muscular insertion on the frons plate (4C12). The descending branch to the lower mouthparts (4C7) is the dilator pharynx muscle. To the right is a longitudinal section of the pharynx (4C8) extending upwards from the oral cavity and traversing under the base of the acoustic box to reach the opening of the esophageal entrance (4C9). Anterior to the pharynx and its associated muscles extends a pleated depression (4C10), indicating the junction between the frons plate and the clypeus. Inferior to this depression lies the anterior tentorial pit and clypeal salivary glands (4C11).

In a sagittal section in the region between the lateral ocellus and the compound eye (Fig. 4D), on the left, setae cover the frons and on the top are the three ocelli (4D1). At center left is the acoustic box (a.b.) close to its lateral termination, containing the previously described fibers. Dorsally below the ocelli, the frons plate contacts with the cerebral ganglion, thus forming the dorsal postero-lateral boundary of the acoustic box (4D2). At center is an oval space in the right cerebral ganglion. A similar space is present in the left cerebral ganglion and both contain hemolymph in live hornets. These are the sinus vessels of the protocerebrum. Figure 4E is a dorsal view of the two masticatory muscular protrusions (see Fig. 4A) with associated vibratory sensors on their surface (4E1). These protrusions are part of the pharyngeal diverticula and in addition provide points of attachment for the dynamic fibers traversing the acoustic box (Fig. 4B). Figure 4F (viewed 90° counterclockwise) is a sagittal section of a worker's head lateral to the median ocellus. This section provides a general view of the structures and their relative sizes. At the upper left corner one may see the setae, and the median ocellus (4F1) on the external surface of the frons plate. The cavity behind the plate is the acoustic box (4F2) and to the right is the cerebral ganglion (4F3). At the base of the box is the pharyngeal diverticulum (4F4) with the muscles to the lower mouthparts (4F5). The clypeal plate (4F6) forms the continuation of the frons plate and its ventral end is the labral fold (4F7).

In a dorsal view of a horizontal section at the level of the antennal base (Fig. 5A), at top center, the frons plate (5A1) is seen at a level inferior to the cone followed by the antennal base (5A2) and compound eye (5A3). Below the frons plate, is the crossed muscular structure (5A4), as described in Figures 4A8, 4A9, 4A10, with the branches to the antennal base (5A5) and lower mouthparts (not seen here). As mentioned before, the two branches to the lower mouthparts (vertical branches) contain cuticular supporting strips that extend dorsally to the muscular protrusions (Fig. 4A8). These

Figure 4 (on the facing page). **(A)** Median section through the frons plate showing the large volume of the "acoustic box." For details, see text. **(B)** A similar section to previous one but more sagittal and near the right antenna. The following features are discernible: the basis of the antenna, then the frons plate (1) up to its juncture with the frons (2); the muscles of the antenna (A.M.); the dorsal hypopharyngeal muscle (D.H.M.) and apparently also the adductor mandibularis (AD.M.). At the top right of the figure, one discerns fibers (nerve fibers) which fan out between the frons plate and the fibers that emerge from, and are perpendicular to, the muscles. These attached muscle fibers are motile. Noteworthy are the fastenings, occasionally of triangular shape, between the fibers extending from the frons and those originating from the muscles, which are perpendicular to them. The majority of fibers unite underneath the conus (co), which is behind the frons plate. Note also that from a point beneath the conus, there is a fanning out of fibers to two different distances, with most fibers extending to a nearby broad muscle, but some connecting to a more distanced broad fiber. **(C)** View (rotated 90° counterclockwise) of the frons plate (1) and its juncture with the clypeus (2), forming the epistomal suture (10). From the frons plate protrudes the anterior dilator pharynx (12) (according to Short, 1952) and farther on there is a space, occupying structure (at the top right) which is the pharyngeal cavity. At the top left, behind the frons, one can see several fibers which pass between the frons plate and the membranes above the cerebroganglion and apparently connect to the antennal lobe; this is the deutocerebrum. See text for a more complete description. **(D)** Sagittal section through the head capsule. At the left and center is the frons plate, and towards the right is the acoustic box (a.b.) with its numerous fibers. In the middle of the frons is the conus (C) and also fibers which pass from the frons plate in the direction of the facing cerebroganglion. At the center, a large cavity is seen in the brain. This cavity is the aorta. Above and outside this cavity are the two ocelli (paired). **(E)** At center of figure, the two pharyngeal diverticula (p.d.) are prominent, with their sensors (1) which are probably geared to detect vibrations (for higher magnification, see Fig. 8E). At the bottom right is a section through pharynx (P). At the top right are various muscles whose function is probably to dilate the various regions of the pharynx. **(F)** A sagittal section (rotated 90° counterclockwise) through the entire head intended to enable a general orientation of the described organ. At the top left are the setae (1) on the exterior of the frons (f) and below the frons is the cavity of the acoustic box (2) and the fibers which connect to the brain (3). See text for details of 4F4, 4F5, 4F6 and 4F7. Bars (A-E) = 100 μm; bar (F) = 1 mm.



strips are part of the tentorial bridge and form points of attachment to some of the support fibers. Centrally is the cerebral ganglion (5A6) with tentorial supporting strips (5A7) and the aorta (5A8).

An anterior view of a worker's head is presented in Figure 5B. The frons plate and the left ocular sinus plate have been removed to expose an acrylic cast of the acoustic box. The location of the box relative to the other structures is clearly seen; the anterior surface of the box faces both the frons (5B1) and ocular sinus plate (5B2). The lateral parts face the compound eyes (5B3) and follow their contour, forming two long, thin arms (not seen) that extend ventrally to terminate with the eyes at mid-clypeal level. The dorsal part (arrow) extends to the vertex up to the ocelli (not seen here). Ventrally and at center, the anterior surface is bounded by the V-shaped insertion (5B4) of the crossed muscular structure (Fig. 4A9) on the frons plate. On both sides of the muscular insertion lie the antennal sockets (5B5). The depression formed by the median ocellus (5B6) is at center with the coronal suture (arrow). At bottom is the clypeal plate (5B7) and the frons clypeal junction (5B8).

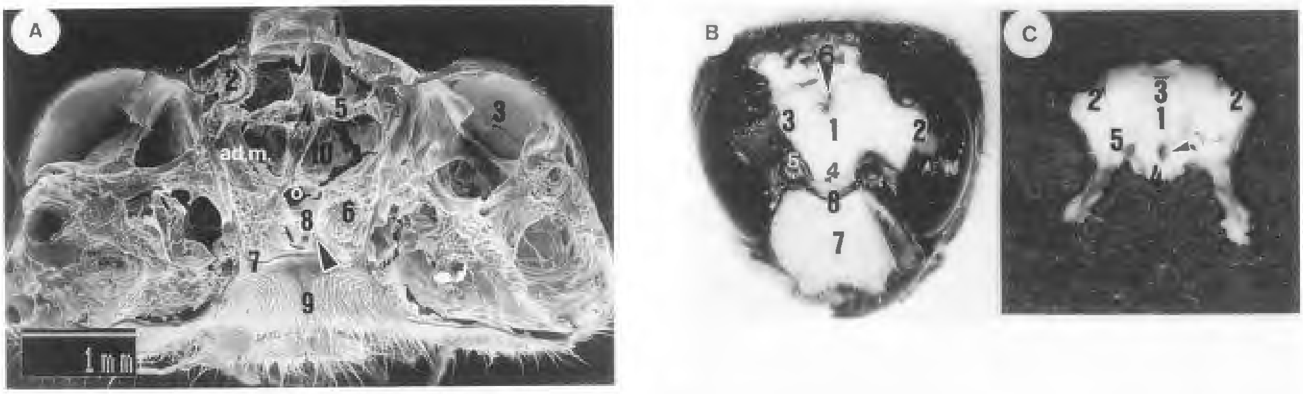


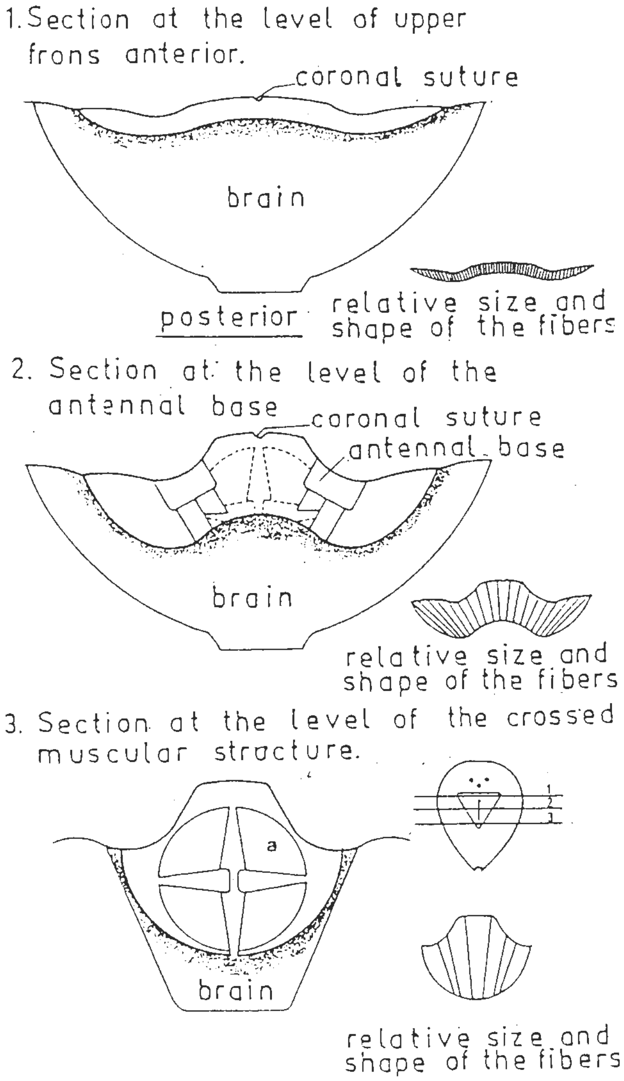
Figure 5. **(A)** Horizontal section through the base of the acoustic box viewed from below. The brain capsule is supported by the tentorial bridge (7) and toward the bottom one sees the postocciput (9). Above the tentorial bridge, at center, there is a round aperture, which is the salivary duct (arrowhead). Further upwards there is an expanded hollow, which is the dilatation of the esophagus within the occipital foramen (o). On both sides of the salivary duct are located the adductor mandibularis (ad.m.) muscles, and above them are criss-crossing muscles which interconnect the pharyngeal muscles. In the spaces between the two bundles of muscle fibers, there are vestiges of a membrane (10) which "seals" the acoustic box from below. In the upper part of the figure, one sees the "pocket" formed by broadening of the frons plate near its base (adjacent to the clypeus). On both sides of the frons there are vestiges of the antennal bases (5), and at upper margin of the figure on both sides, are the plates of the compound eyes (3). At bottom, the plate of the occiput is seen (9). See text for further details. **(B)** Injection of liquid acrylic into the cavity of the acoustic box through an aperture made in its top part results in the box filling up with a rapidly hardening material. Removal of part of the frons plate, as depicted in this figure, reveals the acrylic cast which fills the box. See text for further details. **(C)** Removing the acrylic cast in its entirety from the acoustic box reveals the complete spatial configuration of the acoustic box. One sees now that it has a wide upper part which narrows downward, terminating with two delicate extensions which represent the space between the antennae, compound eyes and the clypeus. In the pendant part of the main body, there is a depression representing the protrusion of the conus (arrowhead). At upper center, there is a rounded pit representing the site of the median ocellus {photo widths (P.W.) in B and C = 4 mm}.

Figure 5C shows an acrylic cast of a worker's acoustic box. The cast has been completely freed from the surrounding structures to reveal the anterior surface of the acoustic box. This surface is composed of two main regions: a central thick protrusion (5C1) that lies behind the frons plate and two lateral thin regions (5C2) that lie behind the ocular sinus plates and extend ventrally along the contour of the ommatidia to the mid-clypeal level. In the dorsal part of the central thick protrusion is the impression produced by the median ocellus (5C3) and proceeding in a straight line ventrally, a shallow impression produced by the conus (arrowhead). Inferior to the conical impression, an orifice (5C4) indicates the location of the crossed muscular structure (Figs. 4A8, 4A9 and 4A10). On both sides of this orifice are the impressions of the antennal bases with the orifice in their center (5C5). This orifice comprises a gap in the antennal muscles that extend towards the protocerebrum. The acoustic box is covered by a discontinuous epithelial membrane that allows hemolymph circulation.

Serial horizontal sections of the acoustic box (Fig. 5D) reveal that it is thin and long in its upper part (5D1), but broadens in its lower part down to its base (5D2), with the fibers lengthening and thickening accordingly. At the base (5D3), the crossed muscles are shown schematically. Above them is the passage-bearing membrane (5D3a) which delimits the acoustic box from the underlying organs. At the base of the acoustic box in both sagittal (Fig. 5EA) and horizontal sections (Fig. 5EB), an imaginary angle of 60° is formed.

The fibers

In Figure 6A, a sagittal section medial to the coronal suture, one may see nerve fibers attached midline and superior to the cerebroganglion, most probably to the central body. These fibers are of a static type and form an unusual fan-like attachment to this site. The horizontal fibers (Figs. 6B and 4A) traversing the acoustic box from frons to protocerebrum are of a static type, having no muscular attachments, and are mostly found in the dorsal narrower part of the acoustic box. These fibers (Fig. 6B) are covered by an epithelial membrane forming a characteristic bead-like appearance. Some are intertwined, and range in diameter from 8 to 15 μm. The majority of the fibers that are found in the acoustic box are of this type. The fibers (Fig. 6C) between the



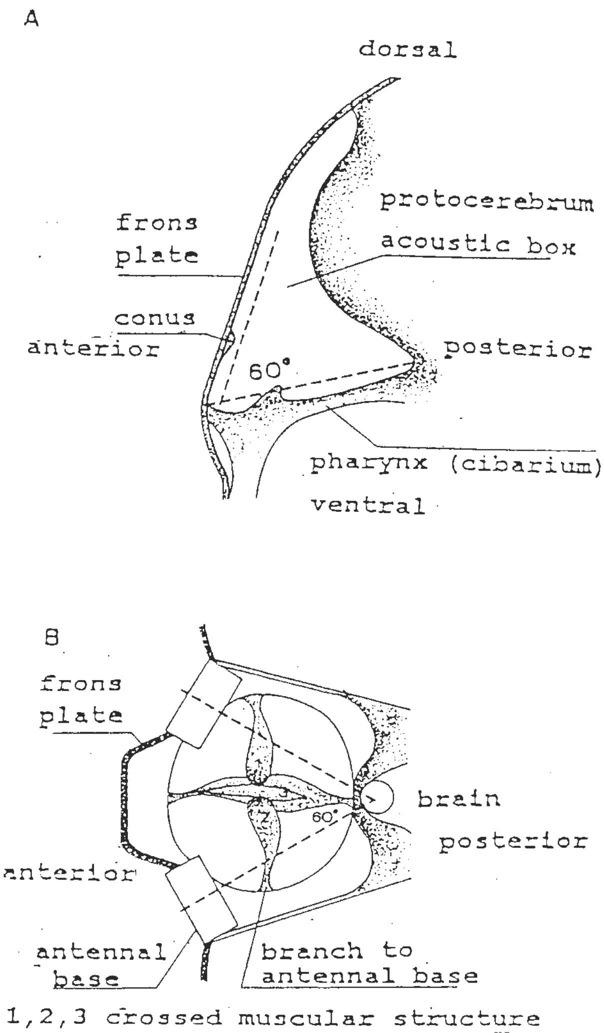


Figure 5D (at left). A diagram of successive horizontal sections through the frons plate intended to display the number and relative length of the fibers in the acoustic box. These sections represent the horizontal levels shown in the inset on the bottom right. Of note is the fiber length compared to the brain width at each level. In the upper part (1), the fibers are short and numerous; in the middle (2), they are longer; and at the base (3), the fibers are the longest and least numerous. Also see text.

Figure 5E (at right). (A) Schema of a sagittal section throughout the frons plate transversing the acoustic box. Note that in this direction the base of the box forms an angle of 60° with the frons plate, a feature which could possibly serve to measure angles in hornets engaged in building comb cells. (B) Schema of a horizontal section through the base of the acoustic box. From this aspect, as well, one can visualize a 60° angle formed by passing imaginary lines from the antennal bases to the brain.

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ocelli and the cerebral ganglion are approximately 62.5 μm in diameter. In a sagittal section, in the region where the frons plate enters the ommatidial fold (i.e., at the level of the ocular sinus) (Fig. 6D), the support strands of the fibers traversing the acoustic box attach to the epithelial membrane superior to the cerebral ganglion. In a sagittal section lateral to the median ocellus, (Fig. 6E), to the left, is the external surface of the frons plate (6E1) with the coronal suture (6E2). The conus (6E3) is visible on the internal surface of the frons with a few static fibers emanating from it. Inferior to the conus, extend several dynamic fibers (6E4) associated with the air sac. These fibers form a unique structure with triangular support strands and branches to both the basal and protocerebral membranes. At the base of the acoustic box (bottom) are the masticatory muscles (6E5)

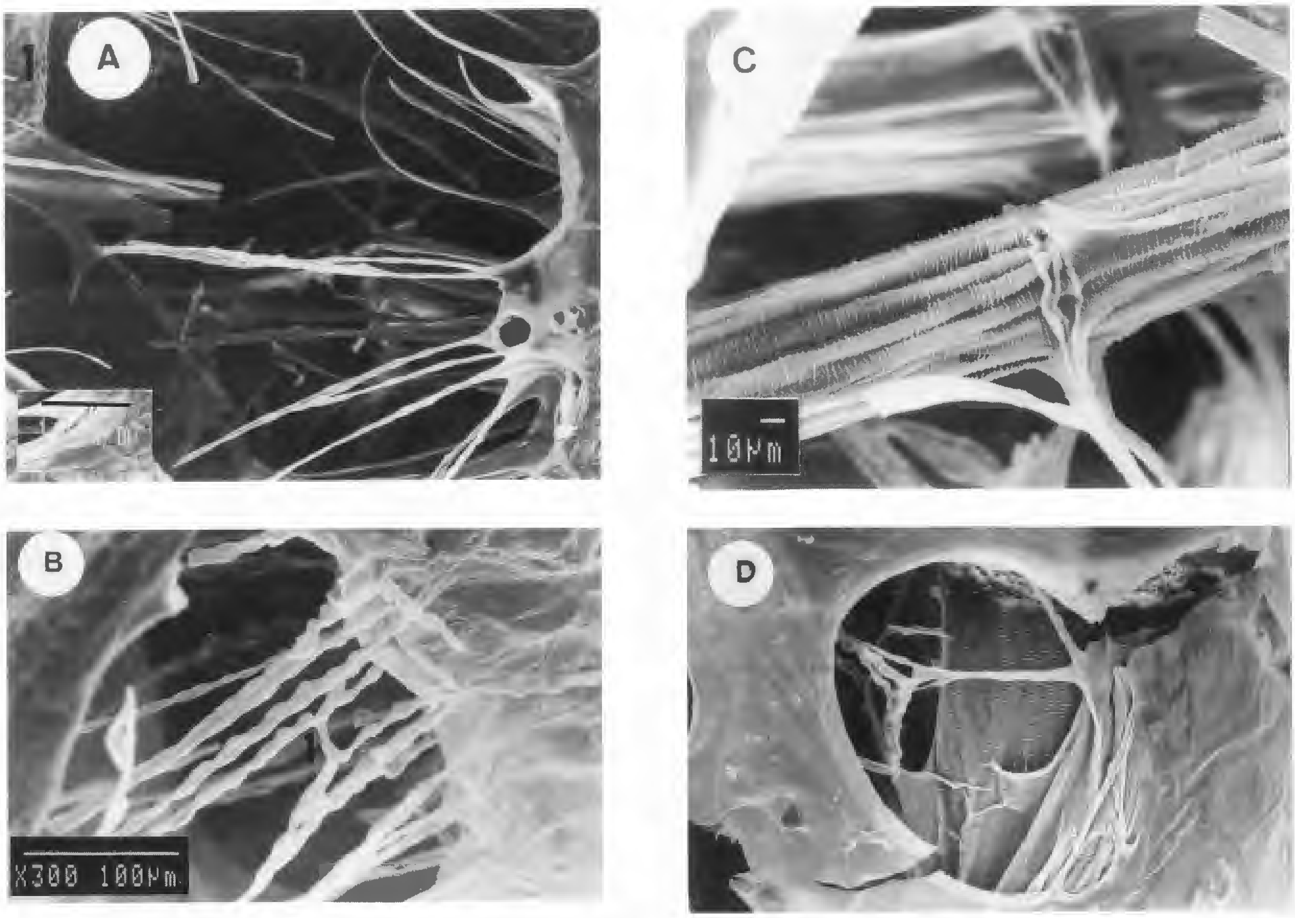

Figure 6. **(A)** A bundle of fibers originating from various regions inside the frons plate of *Vespa orientalis* including the region of the conus (1); this fiber confluence onto the opposing region, namely, the membrane that covers the protocerebrum, and the point of attachment of most of the fibers is most probably the central body. Bar = 100 μm. **(B)** An example of fibers possessing regular swellings as those in Figure 6A as well as regularly occurring spindle-shaped swellings. The fibers are reinforced by lateral (1) connections or are furcated at both ends, that is, both at the end attaching to the frons plate (left) as well as at the end attaching to the supracerebral membranes (right). **(C)** A bundle of nerve fibers originating from the upper part of the acoustic box and proceeding into the facing cerebroganglion. These are nerve fibers of one of the paired ocelli and their attachment is probably to the optic fibers (protocerebrum). Above this bundle one discerns another fiber bundle emerging from the second paired ocellus. Note the ring-like swellings along the length of each fiber. Each of the swellings is 1-1.5 μm in diameter, and there are about 3-4 of these along a length of 10 μm. The role of these swellings is possibly any or all of the following: (1) to strengthen the fibers; (2) to lend elasticity to the fibers, i.e., to enable them to stretch or contract as the frons plate undergoes changes under pressure or tension; (3) to render the fibers capable of sensing environmental changes, in that the matter of which the swellings are comprised has piezoelectric properties; (4) to shield or cover structures located below them, in the fiber proper; (5) to amplify the friction of the circumflowing hemolymph. The fibers proper are encased in epithelium, much the same as is the internal surface of the acoustic box. **(D)** The inner space containing fibers in one of the narrow extensions of the acoustic box (see Fig. 5C). In this transverse section, there are several features of note: (1) the space is oval, having a width of 200-250 μm and a height of 300-400 μm; (2) the fibers (few in number) are of ordinary appearance, that is, they appear the same as in the main region of the acoustic box, but (3) there are also layers of fibers intertwined with epithelium (bottom right) whose orientation is unclear as yet and (4) additionally, there are pockets which could serve as temporary reservoirs of hemolymph. P.W. = 4 mm.

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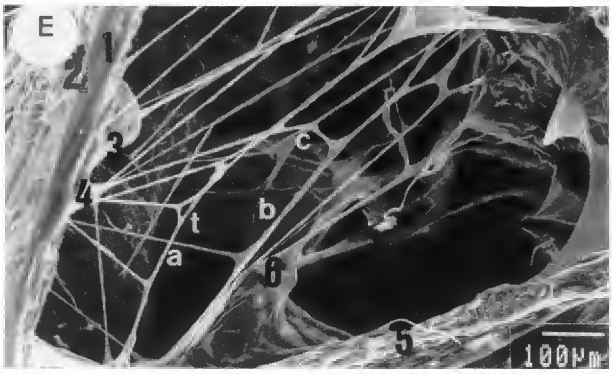
with protrusions (6E6). The salivary gland is based on the clypeal plate (Fig. 6F). The individual gland cells send branches of canaliculi that join to form a main duct (6F1). The nerve fibers (6F2) that stretch towards the acoustic box, as previously described (Fig. 4A), send branches to the base of the antennae as well as to the



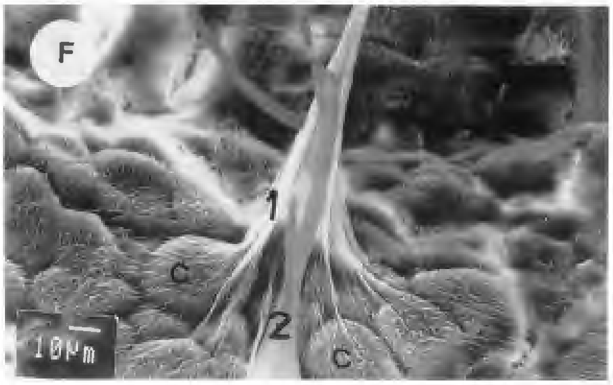


Figure 6. (E) Dynamic fibers in the lower part of the acoustic box are presented. One can see several fibers originating from the conus (3). Below these, there is a central point (4) from which at least 6 fibers emerge. These fibers attach to two transverse fibers (a, b), forming a unique complex. In the region adjacent to the brain membranes, there are several cross links (c) between the fibers in the complex. The linkage to the transverse fibers, at times, assumes the shape of a triangle (t) and is probably intended to reinforce the complex. The various muscles of the pharynx and the adductor mandibularis can be seen at the base of the acoustic box (5, 6). It is reasonable to assume that the transverse fibers attached to the masticatory muscles attach also to the tritocerebrum (see Gorb *et al.*, 1993). (F) The salivary gland is located on the clypeal plate and composed of singular gland cells (c). These cells send canaliculi that join to form a central duct (1). The duct opens into the mouth cavity, while the nerve fibers (2) from the gland cells, enter the acoustic box to contact antennal bases, conus and cerebral membranes.

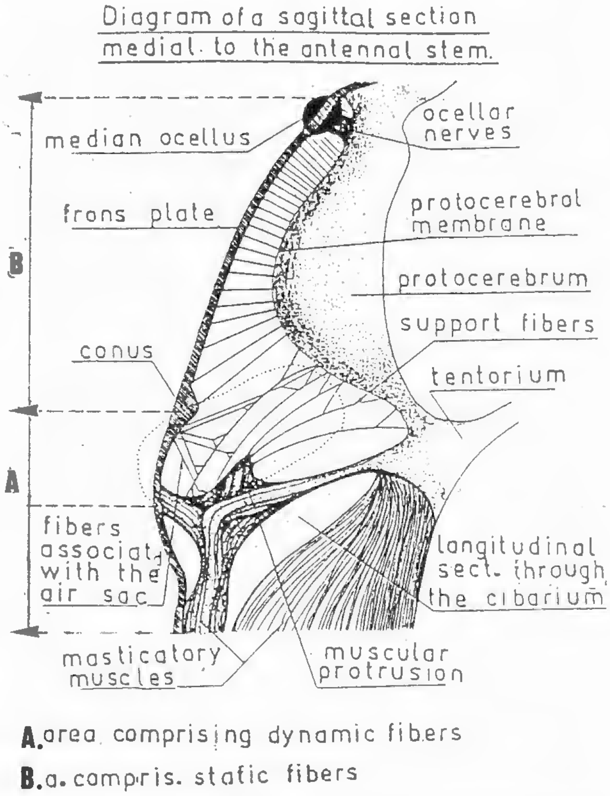


Figure 6. (G) Scheme illustrating the structure and interconnection of fibers in the acoustic box. See text for further details.

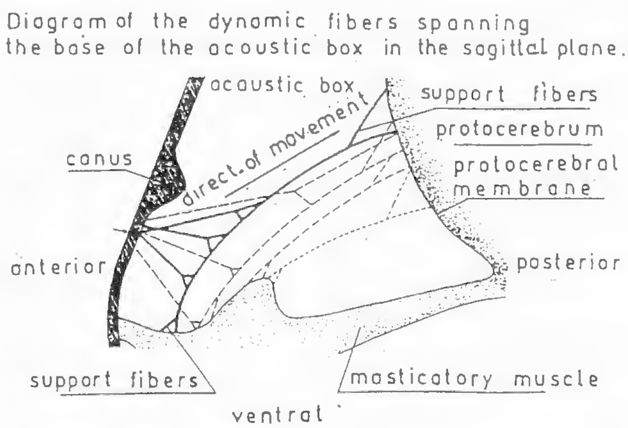


Figure 6. (H) Magnification of the dynamic fibers, the so-called fan-shaped fibers. For further details consult text.

conal tip and the protocerebral membrane (see also Figs. 4C11 and 4F6). The fibers are not smooth throughout but they display dilatations along their length which, most probably, are ganglia (see Ishay *et al.*, 1986). Figure 6G is a diagram of a sagittal section medial to the antennal stem. Several of the structures described above are depicted in this diagram. The encircled area at the base of the acoustic box (shown more clearly in Fig. 6H) depicts the location of the dynamic fibers (Fig. 4A6), and their various attachments and support fibers.

The conus in workers versus males

In a dorsal view of an anterior horizontal section through the coronal suture (of a worker) and superior to

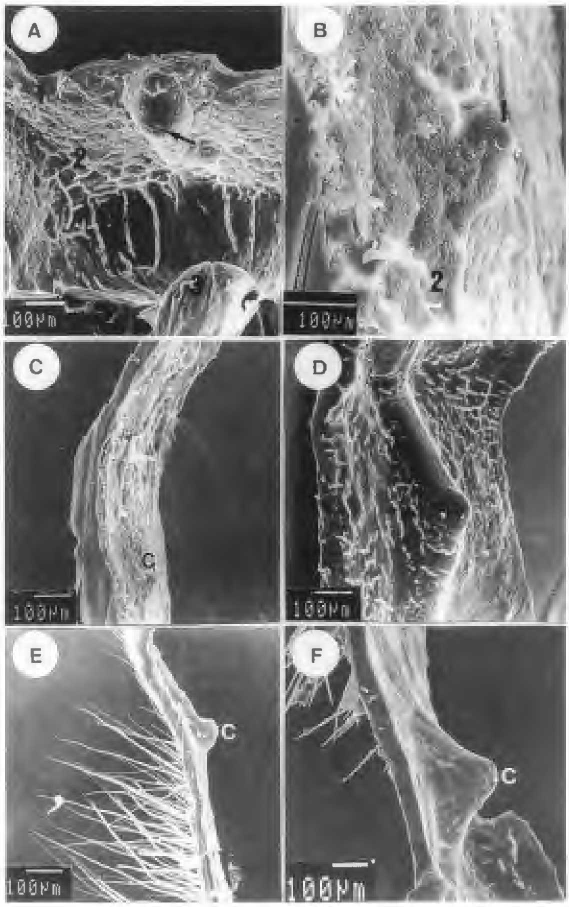


Figure 7. Comparison of the size of the conus in workers (females) and males (that possess a conus). **A**, **D** and **F** show the conus in workers and **B**, **C** and **E**, in males. Since the magnification is the same in most of these micrographs, it is quite evident that the conus in males is smaller than in workers, and in many instances, it is altogether absent (in males).

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the conus (Fig. 7A), proceeding from top to bottom, is the cuticle of the frons plate (7A1) and the conus with its blunt termination exhibiting a centrally located oval-shaped window (arrow). Most of the fibers are torn, revealing the epithelial membrane covering the internal frons plate (7A2). At the bottom are the two muscular protrusions (7A3) as described in Figure 4A8 and the anterior V-shaped insertion on the frons plate. A section through the frons of a male (Fig. 7B) is intended to show the shape of its conus. The domed structure of the conus as well as a portion of the ring (Fig. 7B1) encircling the dome is apparent. In the male (Fig. 7B), the conus proper is relatively small, circular, and lacking the prominent ridge found in the females (7D1). Towards the bottom, a protrusion is discernible which marks the air sac attachment site (7B2). A sagittal section through the frons plates of male hornets (Fig. 7C and 7E) is intended to demonstrate the smaller size of the conus (C) relative to that of the females (workers) in Figures 7D and 7F.

Special features in the acoustic box

There are in the head of the queen hornet (Fig. 8A) protuberances or a single protuberance measuring merely a few microns in size. These protuberances are located in the epithelium above the conus, in the inner portion of the frons plate. The epithelial membrane covering the internal surface of the acoustic box is presented in Figure 8B. At the bottom of the figure, the membrane is quite apparent with its characteristic grooved appearance and at the top right, the surface is seen immediately below this membrane. The surface consists of a single epithelial layer that comes into direct contact with the endocuticle; it is studded with pores (8B1), crossed with tracheae (8B2) and provides sites of attachment to the various types of fibers traversing the triangular cavity described in Figure 4A.

Figure 8C is a sagittal section through the cuticle in the dorsal region of the acoustic box close to the vertex. Proceeding from top to bottom, setae are seen on the external surface followed by several cuticular layers (approximately 40). The external layers most probably in the limits of the exocuticle are noticeably thicker than the internal ones, the endocuticle. The thickness of the cuticle in this region is approximately 38 μm.

When examining a sagittal section (Fig. 8D) through the frons of a worker, in the vicinity of the conus, the setae are visible (s, on the left), as well as the cuticle and the conus (c), with its extensions (nerves). This section is unique in that the air sacs (a.s.) on the conus and at its base have remained intact. The masticatory muscular protrusions showing the vibratory sensor are seen in Figure 8E (center). The length of the sensor is about 10 μm. An outstanding nerve fiber in the acoustic box of a worker is shown in Figure 8F. The bob-like swelling at its center measures about 8 μm x 8 μm.

Statoliths on nerve fibers

A very unusual appearance was discerned at the lower end of the conus (Figs. 9A and 9B). From the terminus of the masticatory muscles (9A1), a fiber arises which splits into three elements. Of the latter fibers, the sinistral one bears a coin or bob-like distension with a diameter of approximately 60 μm; the middle fiber bears several intermittent distensions while the dextral one is uniformly smooth, throughout almost all its length, barring a single distension. The series of micrographs in Figure 9 reveal that bobs may act as weight which is suspended from the fibers similar to the plumb of a stonemason. Some of these bobs appear as single distensions in the midst of an enlarged fiber (as in Figs. 9A

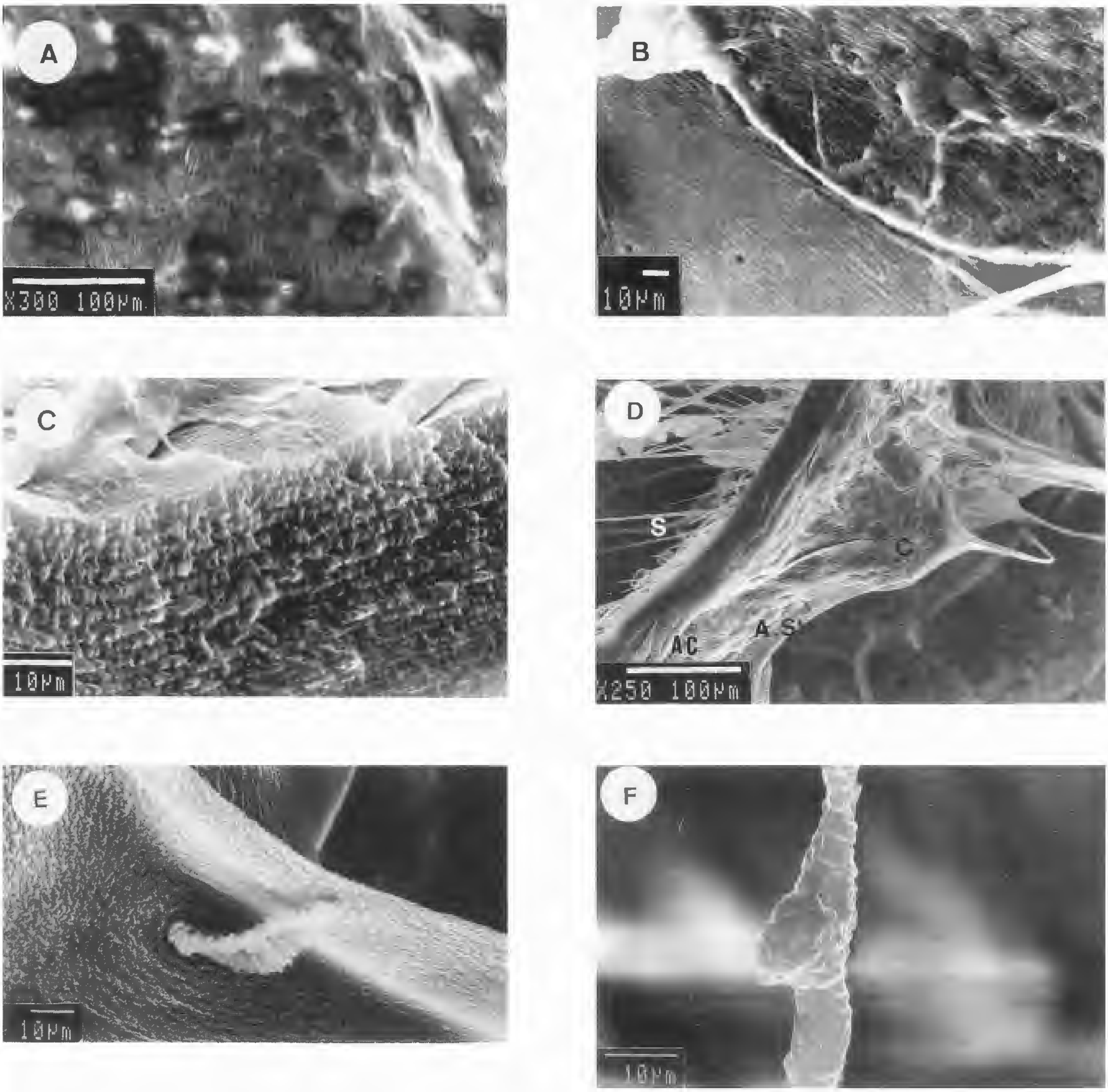

Figure 8. **(A)** Granular configurations in the epithelium of the acoustic box in a queen hornet. The function of these configurations is not clear. The composition of metallic elements in these configurations is not different than in their surroundings. **(B)** At bottom left, the acoustic box shows normal epithelium; when this is peeled off, (top right), a differently structured layer is revealed. This layer bears tubules, which are either trachea or nerves, numerous holes and a variety of morphologic structures. **(C)** Brown cuticle in sagittal section. At top is setae and the upper cuticular layer, i.e., the epicuticle, below it, about 15 layers thick, the exocuticle, followed by 15 (or more) thin layers of endocuticle (capacitor-like) down to the basement membrane (B.M.), which is the epithelium lining the interior of the acoustic box. **(D)** The epithelium lining structures in the interior of the acoustic box near and below the conus (c), following removal of the fibers. One sees an air sac (A.S.) which apparently extends into an air conduit (AC), i.e., a large trachea; s: satae. **(E)** A sensor projecting above the pharyngeal diverticulum, apparently for sensing vibrations. Note that the epithelium overlies the sensor as well as the diverticulum. **(F)** A dilatation on one of the short fibers in the acoustic box. This dilatation may accentuate vibratory motion and may also cause a downward stretching of the fiber.

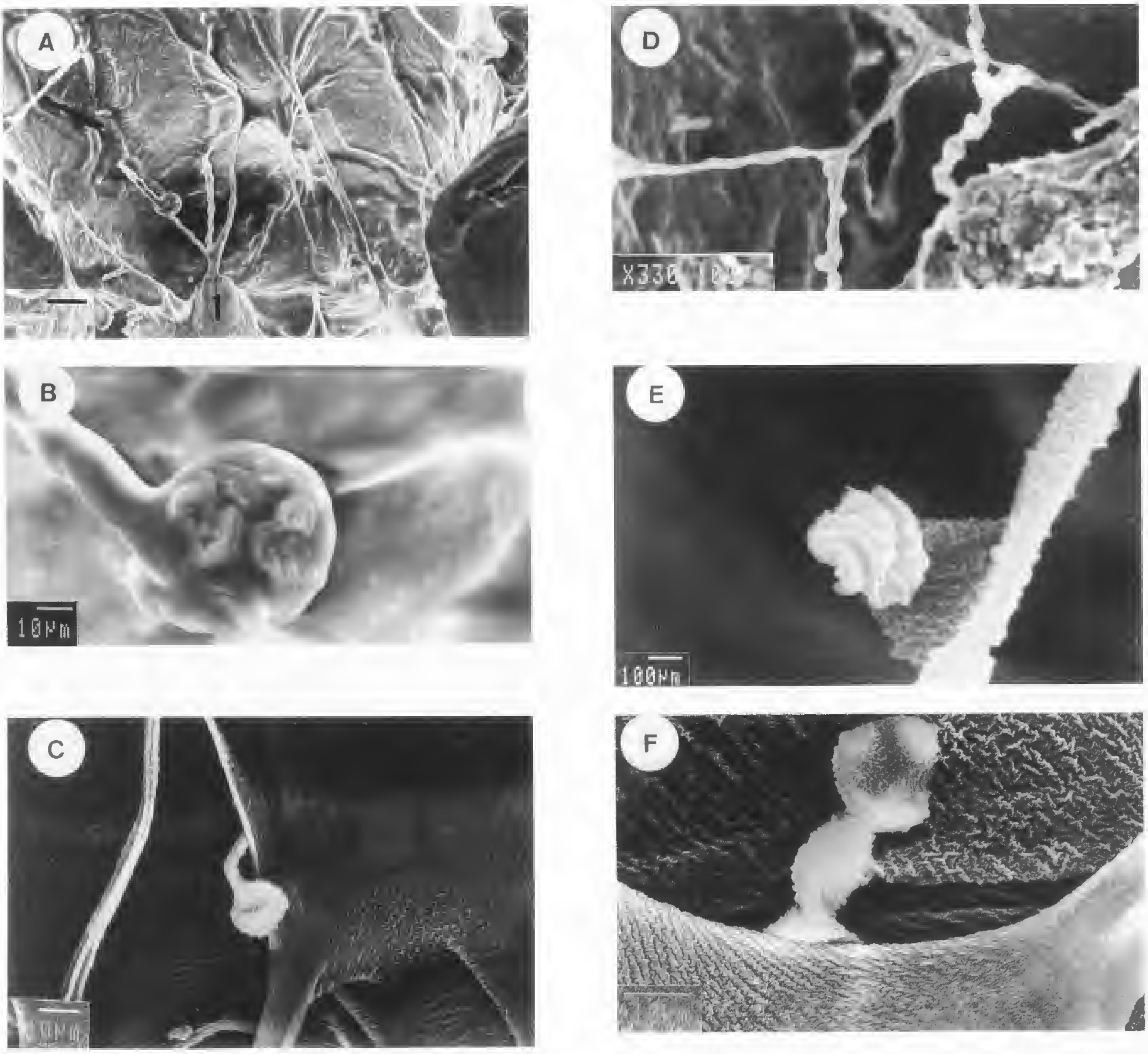

Figure 9. (**A** and **B**) Various "bobs" encountered within the acoustic box of the Oriental hornet *Vespa orientalis*. In Figure 9A, note the spatial orientation of three fibers containing bobs that attach through a common stem to the pharyngeal diverticulum (1). The fiber on the left is provided with a large ball-shaped bob, the central fiber shows intermittent dilatations, and the one on the right appears almost smooth throughout. In Figure 9B, the large ball-shaped bob is seen at a higher magnification. (**C**) A ring-like swelling on a short-fiber in the lower part of the acoustic box. On the left, a long fiber can be seen. (**D**) Branched fibers bearing bobs along their length. Bottom left is a fiber that bifurcates, with one branch proceeding to bottom left and the other to the right. (**E**) A bob with a short base arranged freely along a fiber. (**F**) A bob on a fiber with a relatively short winding stem emanating directly from the inner epithelium in the lower part of the acoustic box. Bars = 100 μm (A, D and E) and 10 μm (B, C and F).

and 9B) or a short fiber (Fig. 9C); others assume the form of knotted swellings on branched fibers (Fig. 9D), or single knotted swellings on a single fiber (Fig. 9E); and others, still, may appear as an enlarged, winding node (Fig. 9F).

X-ray microanalysis

Figure 10 presents the EDS analysis data on elements (of atomic number > 10, except those present in organic tissues, such as C, N, O) of various areas in the acoustic box. The elemental composition of the bob-like

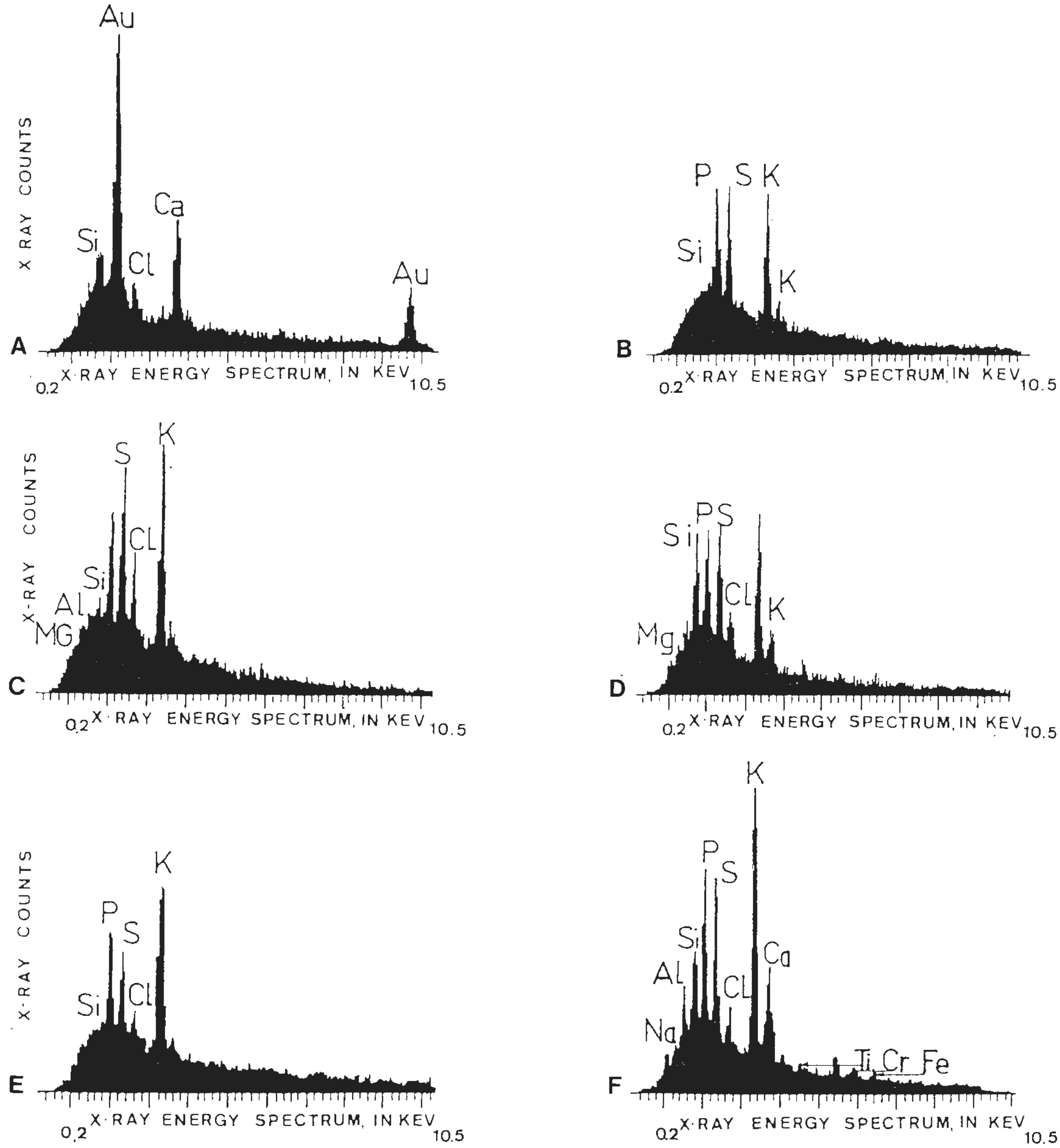

Figure 10. **(A)** EDS analysis data of the "bob" shown in Figures 9A and 9B. Calcium is present in a relatively large quantity. Also present are chlorine, silicon and potassium. The gold peak is a result of specimen coating and is therefore an artifact. **(B)** EDS analysis data of a base of a single nerve bundle in the acoustic box. The major elements are potassium, phosphorus and sulfur. **(C)** EDS analysis of the epithelium lining the inner surface of the acoustic box in the region opposite the frons plate. The major elements here are sulfur, phosphorus and potassium. **(D)** EDS analysis of the epithelium lining the inner surface of the acoustic box adjacent to the frons plate. The major elements detected are potassium, sulfur, and phosphorus. Also detected in relatively smaller quantities are silicon, sodium, chlorine and calcium. **(E)** EDS analysis of a fiber at its bifurcation in the acoustic box. The major elements are potassium, sulfur and phosphorus. Elements detected in relatively small quantities are chlorine and sodium. **(F)** EDS analysis of the epithelium lining the acoustic box adjacent to the conus. The major elements are potassium, sulfur and phosphorus. Elements detected in relatively smaller quantities are calcium, silicon, and sodium.

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distension depicted in Figures 9A and 9B is shown in Figure 10A. In this specimen, there was a higher concentration of calcium and silicon than in the adjacent parts of the fiber. This specimen was coated with gold (Au) unlike all others which were coated with carbon and, therefore, the Au peak in this figure is an artifact and not present naturally in the specimen. Figure 10B provides the elemental composition in the base of a single nerve bundle in the acoustic box. Figure 10C refers to the epithelium opposite the frons plate covering the brain. In this region, sulfur, phosphorus, chlorine and potassium are detected. Figure 10D refers to the epithelium adjacent to the frons plate, where silicon, phosphorous, sulfur, and potassium are present. Figure 10E focuses on the point of fiber bundle bifurcation, here phosphorous, sulfur and potassium are abundant. Figure 10F is from another area of epithelium close to the frons; here Fe, Ti, Cr, Ca, K, S, P, Si, and Al are evident.

Damage due to irradiation

Figures 11A to 11D present scanning electron micrographs of damaged epithelial membranes covering the internal surface of the frons plate; the damage was produced by direct irradiation of the frons plate with a CO_2 beam laser. The affected areas are seen as dark tracks imprinted in the epithelium, a feature not found in the control hornets (Fig. 8B). Figures 11E and 11F are two examples of combs: a normal balanced comb with a central stalk built by the control group of hornets (Fig. 11E) and an abnormal, asymmetric comb without a stalk and fewer cells (Fig. 11F). This abnormal comb was built by hornets irradiated with the CO_2 beam laser (Fig. 2). After two weeks of nest building, samples from the irradiated and control groups were prepared for viewing by SEM (displayed in Figs. 8B: controls; and 11A-11D: irradiated).

Discussion

The present work deals primarily with structures located between the frons plate and the anterior of the cerebroganglion, with the shape, size and density of the fibers and their junctions, with the borders that confine and delimit the space which we have named the acoustic box, and with the relative size of the conus which projects into the acoustic box from the frons plate. An attempt was made to depict the entire "organ" designated by us as Ishay's geotactic organ, dwelling on its varying proportions in different regions of the head.

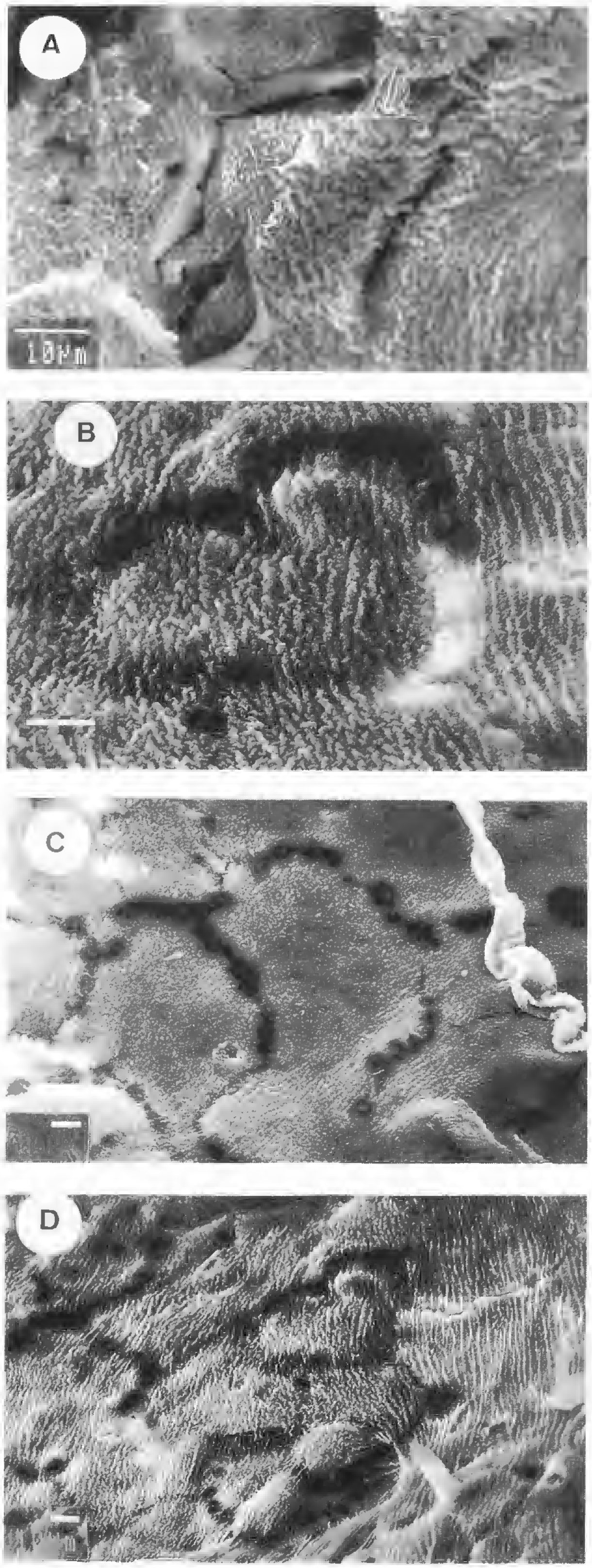
The "organ" is located in the space between the frons plate and the anterior part of the cerebroganglion. In this region are located, in addition (from the outside), the compound eyes (on both sides), the three ocelli (in the upper region) and a pair of antennae (in the lower region). In addition, the region is traversed at its base by nerves from the salivary glands and on both sides by muscles, the largest of which are the adductor mandibularis, the lateral pharyngealis, and the antennal muscles. For more details on hornet and wasp musculature, refer to Duncan (1939), and for structure and function of the nervous system in invertebrates refer to Bullock and Horridge (1965), for mechanoreception see Schwartzkopff (1974), Siegler and Burrows (1986) and Gorb *et al.* (1993). In young specimens, this entire space is immersed in hemolymph, but in mature specimens, it contains only a scant amount of hemolymph (Ishay, unpublished observation). Because it is difficult to obtain sections of the "organ" without severing fibers, we are not certain whether all the specimens possess the same exact number and arrangement of fibers but, in general, the observation is essentially the same in all specimens of females examined, i.e., workers or queens.

It is tempting to look for some analogy between the function of the pair of the semicircular canals in the inner ear of vertebrates and this single organ in wasps and hornets: here, it is bilateral and the distribution of the various strings may reflect sensitivity to up and down inclination: pitch, as well as sideways: yaw, and of course to roll, while the bobs hanging in the middle or bifurcation of fibers may act like plumb rules. On the basis of mobility of the fibers, the acoustic box can be divided into two portions: an upper portion which contains fibers that are static and a lower portion extending from the conus downwards where part of the fibers are dynamic, either folding or elastically contracting and stretching in harmony with the muscles of the organs they innervate (see in this context mainly Figs. 6E, 6G and 6H).

The conus (see Arcan and Ishay, 1993), with its associated structures (bobs, hair cells with cilia), is prominent and well-developed in female hornets (Figs. 7A, 7D and 7F), but small and hardly developed in the males (Figs. 7B, 7C and 7E). Of note is the fact that the male hornets do not engage in comb building, which is performed in the dark and in the direction of the gravitational force. This lends support to our contention that the detection of gravitation in the course of comb building is a main or one of the main functions of the acoustic box. Moreover, circumstantial support for this idea is provided by the fact that irradiated hornets with damaged epithelia in the acoustic box have built a non-symmetrical comb (see Fig. 11F).

The membrane which borders the acoustic box internally, is mostly uniform in appearance containing longitudinal strips and a few perforations (Fig. 8B, bottom left). If this membrane, the basal membrane, is peeled off (Fig. 8B, top right), the single layer of epithelium covering the endocuticle is revealed. This single layer is markedly different in structure, containing numerous fibers (nerves), and perforations (tracheae). The basal membrane overlies the structures in the acoustic box as can be seen clearly in Figures 4A-D.

The basal membrane occasionally bears protuberances, as in the region above the pharyngeal diverticula (Fig. 8E). The most protrusible here is a projection



Figures 11A-11D. Several views of the laser irradiated epithelium covering the internal surface of the frons plate. Bars = 10 μm.

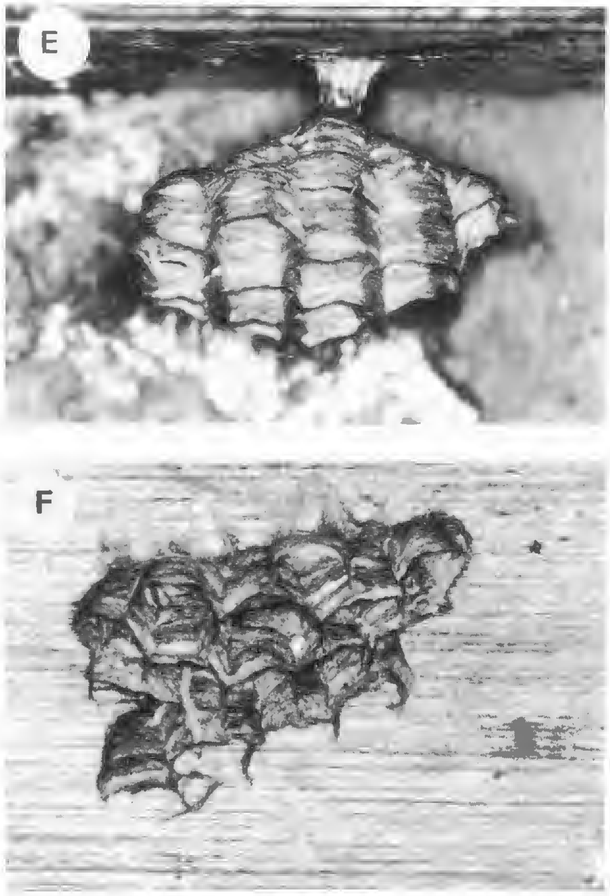


Figure 11. (E) A symmetric, balanced comb with a central stalk built by the non-irradiated group of hornets. P.W. = 8.1 cm. (F) An asymmetric, non-balanced comb built by hornets irradiated on the frons plate with a CO_2 beam laser. P.W. = 8.3 cm.

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about 50 μm long and 10 μm wide, which is probably a mechanical sensor (of vibrations) that protrudes into the space of the acoustic box. Along the fibers, as well, there are occasionally unusually large protrusions (Fig. 8F), measuring 10 μm x 10 μm, which resemble a bob suspended in mid-fiber. Such a bob probably vibrates when the hornet's body is in motion. These bobs are seen better in Figure 9A and B. This spherical bob bends downward, measures about 60 μm in diameter and contains a higher calcium concentration than is present in its surroundings. It is of interest to note that this bob is situated in the center of the fiber. This is also true for the ring-like bob, measuring up to 10 μm in diameter, seen in Figure 9C. Of special interest is the configuration, as depicted in Figure 9D, where the bobs appear in considerable number on the short-branched fibers, and singly (Fig. 9E), or doubly (Fig. 9F) on some of the bifurcated or trifurcated fibers. The latter fibers criss-cross the space of the acoustic box in all directions.

In the past, whenever we described the gravitationally-oriented comb building of hornets (and all the other social wasps-Vespinae), it was apparent to us that these hornets must rely on some organ(s) to determine the direction of the gravitational force (Ishay and Sadeh, 1975; Ishay, 1976; Ishay *et al.*, 1979; Ishay *et al.*, 1983b), and the perpetual question was what and where in hornets are the structures analogous to the plumb and level of human constructors? As we now know, statoliths occur in hornets in several places. First, in the center of the frons within the coronal suture, there are excretions that contain minerals such as Ca and Si, which are static and are, most probably, ultimately expelled (see Ishay and Ganor, 1992). Similarly, we have examined the mineral secretions from the glands on the frons, including those in the bases of the hairs (Ishay *et al.*, 1983a, 1986; Ishay and Shmuelson, 1994). On the other hand, while exploring the interior of the frons plate in hornets and focusing on the structure of the conus, which intrudes inward from the sutura coronalis, we detected in the various layers overlying one another, yellow granules, stereocilia, bobs, and disc-like plates, with the latter proceeding into the space of the acoustic box. The mentioned configurations are capable of some mobility and are thus not strictly statoliths but also kinetoliths. The disc plates are found to be immersed in hemolymph. Referring back to the various "swellings" or bobs depicted in Figures 8F and 9A-F, we have found that they are on brief fibers which attach at one or both ends to the substrate (the epithelium, the inner site of the basement membrane) or to a convoluted and branched network of other fibers.

We now propose that these bobs and other swellings fulfill several purposes:

(1) Due to their weight, they bend in the direction of gravitation when the hornet is stationary, but start vibrating in the direction of gravitation when the hornet becomes mobile. Contributory to this end is their greater mass, greater Ca content and the degree of freedom, especially of these bobs which function like a plumb.

(2) Concerning the plumb-like bobs (those discovered so far), they are found on fibers which are structured either to assist or to amplify the vibratory sensation initiated by them.

(3) The structures inside the acoustic box are coated with very specialized and sensitive epithelium (the basement membrane) (see Figs. 8E, 8F, 9C and 9F) which is apparently endowed with piezoelectric properties (see Arcan and Ishay, 1993).

(4) The entire cavity of the acoustic box is immersed in hemolymph which, in a very complex and specialized way, provides the "plumbs" with the necessary fluid, but, at the same time, also regulates the mobility of all of the fibers including those with attached bobs. In addition, the fact that the various vibrating nerve fibers are immersed in hemolymph (which most probably has a high relative viscosity) will not dampen the frequency of forcing oscillation, which contains the information, but will alter the amplitude of the whole fiber. In this manner, there is little or no interference between the information provided by the various adjacent fibers. It stands to reason that, at the maturation of the worker hornets, the amount of immersed hemolymph decreases. With diminution of the hemolymph, each bodily movement of the building workers will cause a part of the described structures to become submerged in hemolymph and another part to become exposed, thereby creating a sensory gradient. Also noteworthy is the fact that, prior to comb building, hornets invariably imbibe water or a nutrient fluid (Ishay, 1973, 1976).

In conclusion, the acoustic box is a very complex organ with weighted bobs of various configurations that may aid in gravity detection. The fibers and bobs are located within hemolymph and is bordered by epithelium that may be piezoelectric. In insects, the acoustic box may serve a similar function that mechanic receptors serve in other invertebrates. The two components that could detect gravity are: (a) the external sensors of the head and especially those on the frons plate which are dry and static, and (b) those inside the acoustic box that are static but are immersed in hemolymph. Finally, the fibers and bobs inside the box may also serve a mechano-receptive function that deserves further analysis.

Appendix I: Glossary

Acoustic box: the cavity behind the frontal plate, i.e., between the frontal plate and the protocerebrum (the brain).

Adductor mandibularis: the muscle that lifts the mandible.

Air sacs: a cavity, apparently an enlargement of a trachea.

Bob: a small terminal object such as a plumb line.

Cerebral ganglion: the insect brain.

Clypeus: the area of the facial wall of the insect's head between the frons and the labrum.

Conus: a protrusion into the acoustic box that starts at the lower end of the coronal suture.

Coronal suture: a depressed line in the center of the frontal plate of social hornets and wasps (Vespinae).

Cuticle: the integument of insects and other arthropodes.

Dilator pharynx: the muscle that dilates the pharynx.

Diverticulum: a blind, tubular sac, branching off from the pharynx.

Dynamic fibers: the fibers in the lower side of the acoustic box that move with the movement of some of the muscles that line the cavity.

Females: workers or queens in a Vespid nest.

Fibers: the nerve fibers that are connecting between the inner side of the frons plate and the membrane that covers the brain in the acoustic box.
Frons plate: the cuticular plate in the frons of the hornet.
Hemolymph: blood in insects and other invertebrates.
Males: the drones in a Vespid nest.
Mouthparts: the appendages associated with the mouth.
Ocellus (plural: Ocelli): one of the three simple eyes on the head of the insect.
Ommatidium (plural: Ommatidia): one of the elements composing a compound eye of the insect.
Penultimate: the one before the last one.
Perpendicular muscle: the muscle that is vertical to the other one.
Pharynx: the tube or cavity that connects the mouth with the esophagus.
Piezoelectric material: a semiconductor or a nonconductor that reacts to stress by producing electricity or electric polarity.
Pores: minute openings in the cuticle.
Protocerebral membrane: the membrane(s) that covers the brain.
Proto-, deuto- and tritocerebrum: first, second and third pair of enlarged cerebral ganglia that compose the brain of the insects (cerebrum).
Seta (plural: setae): a stiff hair, bristle or bristle-like standing up from the cuticle.
Static nerve fibers: nerve fibers that do not move together with a muscle (but vibrate).
Statolith: any of the granules made of some metals contained within a statocyst.
Supporting strip: stripes of cuticle to which muscles are attached for support.
Vibratory sensors: a sensor that reacts to acceleration by vibration.

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Discussion with Reviewers

C.D. Fermin: Why are standard histological or transmission electron microscopy (TEM) results on the bobs not being included to further illustrate their morphology and/or artifactual nature?
Authors: In the present paper, we present only the SEM results; we intend to continue TEM studies. However, a section through one of the strings, studied by TEM, has been published in Ishay *et al.* (1986), where we present, among other things, a cross-section of an axon bundle originating from the protocerebrum and entering the floor of the acoustic box. The individual axons were shown to penetrate the various strings. In the same paper, we provided additional data and results on the strings obtained by SEM.

Reviewer II: Which type of controls were performed to ensure that the bobs are not an artifact of fixation?
Authors: The (various) bobs are not an artifact of fixation because: (a) The bobs were encountered in 55 workers and 15 queens but not in the 6 males examined, despite the identical processing. (b) Only females (workers and queens), never males, engage in comb building. (c) The bobs are in the middle or end of a (nerve) fiber. Close scrutiny of the external surface of a bob reveals it to be continuous with the fiber. (d) The exact same preparations have been repeatedly examined by us and other researchers via SEM; in future, we plan to prepare bobs for TEM. (e) The bobs occurred invariably at the same site of the various specimens: always on short fibers, either static or dynamic.

V.C. Barber: How can the author be certain that artifact preparations will not affect the result described?
Authors: The results described were not affected by artifact preparation, because since 1972 different preparations and sections of the organ have been examined by numerous researchers without significant morphological changes. Some of the preparations were carbon-coated while others were gold-coated. Some were processed for SEM examination and others for TEM, some were prepared in Tel-Aviv University's electron microscopy laboratory (Life Sciences Faculty), and others in the Volcany Institute, The Beit Dagan Agricultural Research Center. Still others were prepared in Italy at the Department of Zoology of the University of Florence and the results obtained were identical. The late Prof. Leo Pardi, head of that department corroborated the existence of bobs and fibers and confirmed the complexity of the fibers (Ishay and Shimony, 1986).

C.D. Fermin: Please comment on the significance that direct contact with the endocuticle of a single epithelial layer may have in relation to the fibers described.
Authors: The obvious reason seems to be sensitivity of this membrane, which is in fact a single epithelial layer. Previously (Arcan and Ishay, 1993), we showed that any light touch of this membrane results in a sort of contraction and the formation of nodules, apparently temporary. The membrane in question displays piezoelectric properties and is apparently affected by the mechanical tension in the fibers that connect to it or pass through it.

C.D. Fermin: What is the significance of the presence and/or absence of minerals in the various components described?
Authors: The various mineral elements divide into two groups: those that occur in statoliths and those that occur in every organic tissue (including statoliths). Silicon is an example of an element that is likely to be found in statoliths mainly, and indeed Si is encountered in the

epithelium lining the acoustic box (Figs. 10D and 10F). On the other hand, in many places, we find a relatively high concentration of Ca, as in the bob, or near the cones. Interestingly, there are small amounts of Al, Ti, Cr and Fe also in the epithelium. Other elements: P, S, Cl, K and Na are also present in the tissue but their exact composition, i.e., either in ordinary form, or as part of complex molecules whose precise identity has yet to be ascertained, is not yet known. Even so, it is clear that these elements, including the heavy metals, are not an artifact of external contamination. Rather, their composition is similar to that we encountered and reported previously as occurring either on the exterior or interior of the frons plate. Their composition generally varies appreciably, percentage-wise, from the composition customarily found in living tissues of hornets in other regions of the body. We expect to be devoting closer and more in-depth attention to the elemental composition in the bobs and epithelium.

Reviewer IV: Do the fibers vibrate in live animals? If the fibers vibrate in live animals, how would the vibrations be sensed?
Authors: The fibers, consisting of nerves (axons and neurons) enwrapped in a vibration sensitive piezoelectric membrane, likely vibrate in live animals; mechano-receptors in the fibers transmit the stimuli to the brain.

Reviewer IV: Where is the transducer for the vibration and what would be the magnitude of the gravity vector?
Authors: The transducers of this vibration is likely located in any or all of the following: (a) the attachment point of the fibers to the epithelium lining the acoustic box (AB) around the frons plate and the cerebrum; (b) at the various bifurcations; (c) at the synapses of the various nerve junctions inside the fibers. The magnitude of the gravity vector of these vibrations should be 10-15% of the amplitude (or the degree of freedom) of the fibers inside the cavity of the AB (otherwise, there is a danger that the various fibers will interfere with one another). The main obstacle to such an interference is the hemolymph whose viscosity restricts the degree of freedom of the fibers and dampens their amplitude.

V.C. Barber: Can functional attributes be derived from purely morphological observations?
Authors: We provide evidence that hornets whose internal epithelial membranes and fibers are destroyed by laser-irradiation do not build in accordance with gravity; only females, which have a developed cone, build combs in the direction of the gravitational force, whereas males, which either lack or have a smaller cone, do not build combs at all. The cone and the acoustic box with the fibers and all its complex structures behind the frons plate are present only in the subfamily Vespinae which build combs in the direction of gravity while the whole structure is absent in the Polistinae which build combs by about 90° to the substrate (i.e., not directed towards the gravity pull of the earth) and always in the open (i.e., in light, and not like Vespinae, which build combs only in dim light, or even in complete darkness).

V.C. Barber: Why did the authors not consider other extensively cited papers on the statocyst?
Authors: Papers on the presence of statoliths were not considered here, but were considered in earlier papers (Ishay *et al.*, 1983a, 1986; Arcan and Ishay, 1993).

W. Thornton: Were the natural frequencies of the fibers left unchanged by viscous stamping?
Authors: The natural frequency of the fibers is not affected by the viscosity of the hemolymph but the amplitude of their vibrations is dampened by the hemolymph in order to: (a) obviate interference between adjacent fibers; and (b) reduce the duration of vibration to avoid interfering with a new stimulus which may arrive.

W. Thornton: Can the authors sort out (from "embarrassment of riches" and potential functions) the resonance and properties of the so-called sensors?
Authors: As for the natural frequency: the results have not yet been summarized, but it appears that the natural frequency is around 500 Hz in adult hornets and around 800 Hz in younger ones (Ishay, unpublished observations). This natural frequency appears to be influenced by physical factors such as temperature and is very important on the one hand for coordination of communication between the various colony members; on the other hand, for the building hornets who gauge with their antennae the physical quality of the building material (including strength, mixture, thickness, position, electrical properties and probably many others).

As for the various sensors, these are dispersed throughout the acoustic box, and it is very difficult at this point to provide details on their properties or functions.

W. Thornton: Would the presence of a statocyst eliminate the possibility of a redundant gravito-inertial sensory modality?
Authors: Functionally, the statocysts outside the frons plate and those inside the frons plate on the cone constitute an extension and reinforcement of the sensors present on the fibers, and are not redundant but, rather, complementary. The whole structure works as one unit and the fibers that are attached to the protocerebrum handle the various stimuli arriving through the antennae, statoliths and mouthparts.

W. Thornton: Are the fibers described in this paper for the wasp, similar or analogous to the hair on the mosquito antennae for acoustic tracking?
Authors: Perhaps the fibers in the acoustic box are somewhat analogous in their vibration to the hairs on the antennae of mosquitoes but, since hornets and mosquitoes belong to two different orders of insects, the fibers and hairs may be different in structure and function.

W. Thornton: Could the box membrane associated with the trachea in the ventral area serve as an acoustic function in a similar fashion analogous to the cricket?
Authors: The acoustic box has also a function associated with detection of vibrations and, in this respect, it is reminiscent of the cricket. However, it has several additional functions, such as detection of gravity while building a comb in the dark. This function, too, is initiated by vibrations but its purpose is different.

W. Thornton: Is the hemolymph in the acoustic box depleted with age?
Authors: It is well known that the amount of hemolymph in adult hornets is depleted in their body and, accordingly, also in the acoustic box.

W. Thornton: Do we have a system here that is analogous to the human cochlea in which the hair cells and the tectorial membrane are replaced by inherently frequencyselective sensors?
Authors: As to similarities with the Vertebrate cochlea, we have been looking for similarities between the two for a long time. In one of our previous papers (Arcan and Ishay, 1993), we described hair aggregates (stereocilia) on one of the inner-layers of the cone. The hairs in this case are arranged in groups and it was tempting to classify them as similar to those in the Vertebrate cochlea. For instance, we could not discriminate with certainty kinocilia from stereocilia. Work is now in progress to elucidate their precise structure and function.

W. Thornton: Is it possible that in the case of the hornet the antennae are used to transmit vibrations into the so-called Ishay's organ, which in turn might detect the amplitude and time profile by frequency selected sensors in the formal strength?
Authors: It is very likely that the antennae transmit vibrations into Ishay's organ. The linkage between nerve fibers and the acoustic box (AB) is evident from Figure 4B where one clearly sees interconnections between several dynamic fibers and the antennae, between the fibers and the tip of the cone on the inner side of the frons and between the fibers and the membrane covering the protocerebrum.

W. Thornton: Have you attempted to calculate or estimate the strength of resonance frequencies?
Authors: The resonance frequencies were around 500 Hz in adult hornets and much higher (around 800 Hz) in young ones (Ishay, unpublished observations).

W. Thornton: Are the sensory fibers used to shift the resonance of a large array of fibers?
Authors: It is too early to conclude with certainty that there is a "hierarchy" among the fibers, but judging by the number of interconnections on the fibers, it would seem that some of them are more active or "more involved" than others.

W. Thornton: Is it possible that the cyclops "pink hole" at the tip of the conus is an infrared sensor?
Authors: It appears that the "cyclops eye" is an infrared (IR) sensor. It is not easy to detect IR radiation on such a small area by the usual instrumentation available in the laboratory, but we certainly have tried and will continue to try to find an answer to this question.

W. Thornton: Will you comment on any plan follow-up investigations on this array or structure such as stimulus and response studies, selective ablation, frequency response of the structure, especially strings, evoked potential, etc.?
Authors: We think it works in the following way: the stimulus associated with change in the gravitational vector is of brief duration, hence there is need to organize a system that picks up and transmits all along the way, from the exterior of the frons to the interior of this plate, then to the tip of the cone, the various strings and then to the point where the strings connect to the protodeuto and tritocerebrum. From the cerebrum, we suppose that impulses emerge on their way to the subesophageal ganglion to activate the leg muscles, and likewise, the mandibles. On the other hand, there are probably impulses that emerge directly from the protocerebrum to the antennae. Elucidation of the system is a complex task that will probably require years to work out.

W. Thornton: Finally, what will be the behavior of the bobs at the end of the string at the microscopic level, with regard to weight versus surface tension?
Authors: At very high magnification (60,000x), the bobs seem to be structured like an enlarged string. Their specific weight is apparently greater than that of the hemolymph in which they are immersed, probably owing to their relatively high calcium content, inter alia. It is reasonable to assume that the bobs can move in a certain amplitude during changes in the posture of the head (and body) of the hornet, thereby briefly triggering the appropriate stimuli.

Note added in Proofs: The structure of Ishay's organ suggests some analogies to the Corti organ and vertebrate ear. This area in the hornet contains structures which may be transducers of sound or vibration and gravity inertial forces. Unlike the paired vertebrate transducer organs, Ishay's organ is single but appears to be bilaterally symmetrical.

While there is no direct evidence for functions at this time, the following is suggestive. The so called Ishay's organ may be structurally divided into: (a) the interior part, a peculiarly shaped cavity filled with endolymph and crossed by heavily innervated fibres with a regular distribution of fiber lengths; and (b) the envelope of the organ, extending from the basal membrane outwards, incorporating the frons plate, the conus with its various components including the ciliated epithelium and all the inner statoliths (in the conus), and the outer statoliths (in the frons) (Ishay and Ganor, 1992; Arcan and Ishay, 1993).

The interior part (a) has some 120 "strings," each a nerve and a sleeve, comprised by epithelium resembling a basal membrane and between sleeve and string (nerve) there is hemolymph (Ishay unpublished). There is a regular distribution of string lengths so suggestive of a harmonic structure that the array was dubbed a "harp" by some observers. Some of the longer strings have small masses of dense material (dubbes bobs) and the array is immersed in hemolymph. Frequency selectivity (resonance) of a string is a function of its length, tension and mass and its response time sensitivity or function of resistive damping, such as provided by a surrounding fluid. Physically, such an arrangement is compatible with a sound or vibration sensor comprised of an array of individual frequency selection transducers (string-nerve combination) with a fairly wide spectrum (based on string length). If this is the case, it appears to use mechanisms for frequency selectivity inherently different from the combination of location and transducer stiffness seen in vertebrate sound transducers.

This array is mechanically coupled to the frons plate, antennae and an air sac, any of which might couple sound or vibration to the array.

A possible second function of this insect organ is that of a gravity inertial sensor. The statolith and innervated epithelium especially of the conus would be compatible with such a function. There are also innervated cilia (stereocilia) on walls of this organ (see Arcan and Ishay, 1993) immersed in hemolymph which appears to be channeled into compartments. It is possible that acceleration might force a preferential flow of hemolymph over the cilia to provide acceleration signals. The bobs act to divide the frequency into shorter harmonic frequencies. In this respect, the cords are analogous to the cochlea in vertebrates where there is also a stereotactic division of the sensitivity to various frequencies. In vertebrates, the acoustic reception is affected via a tectorial membrane which moves following the picking up of sounds, whereas in Ishay's organ, it is the cords (fibers) that move. The hemolymph within the cavity of the acoustic box acts as an attenuator, so that motion of the cords terminates immediately after the sounds are reproduced. In this manner, there is an enhanced ability to discriminate between adjacent frequencies and also a faster reception of discrete sounds. The hemolymph thus becomes analogous to the perilymph which surrounds the organ of Corti in vertebrates.

The sensitivity to sounds is essential for intercommunication within the hornet's nest, and we can presume that there is also sensitivity to further sounds from the external environment of the nest. The special structure of the described sensory organ enables discrimination between various frequencies, and each frequency which is, of course, produced under different circumstances has different informational-communicative significance.

Numerous previous investigations have suggested that subgenual organs are the auditory organs of (some species of) insects. However, a subgenual organ lacks the structure suitable for discrimination between various frequencies, and consequently, even if it can serve as a primitive organ for mere sound detection, it certainly cannot function in more complex communication needs. We are still in the dark regarding the range of wavelengths the cords in the acoustic box are capable of responding to, but it may be presumed that the large number of fibers (at least 120) is suited for sensing a wide gamut of sounds.

In a previous description of sounds produced spontaneously by social wasps (Ishay and Sadeh, 1982), there is mention of frequencies of 118 and 130 Hz produced by the awakening dance of workers, and frequencies up to 600 and 637 Hz produced by workers facing the queen. Of course, it would be of equal interest to identify other wasp-related frequencies, like that of a sentinel worker at the entrance to the nest as it probes a foraging worker returning from the field or that of a building worker as it builds a new cell wall and examines the physical properties of the freshly deposited building material overlying the previous layer by tapping repeatedly, i.e., evoking harmonic sounds, concomitantly or consecutively with the tips of both antennae. In the latter instance, information is gleaned by the building worker through antennal vibration on both sides of the cell wall and can entail directional data (building in the direction of the gravitational force) or qualitative data (e.g., wall thickness, strength, electric conductivity, acoustic properties, etc.). Antennal tapping of a similar nature was observed when eating.

The envelope of the organ (**b**) is located between the basal membrane, which at least in its anterior part (i.e., the frons region) separates between the contents of the organ (the hemolymph in the acoustic box) and the ciliated epithelium which overlies the endocuticle.

As in vertebrates, the cilia, {called in Arcan and Ishay (1993) "rods"} are stereocilia; they are the mechano-electric transducer elements that are very important to the proper function of a vestibular organ. The role of the envelope is to **sense acceleration**. The space of the envelope is filled with hemolymph. The plates arising from the basal membrane towards the ciliated epithelium partition the envelope into different compartments. When the hornet is in motion, there is inertial movement of the hemolymph and the function of the partitions is probably to direct the flow of hemolymph so as to be maximal in regions where the direction of the plate (the plates at the tip of the conus, see Arcan and Ishay, 1993) is also the direction of flow, and minimal in regions where the plate is at a 90° angle to the direction of the flow. Hemolymph flow results in stimulation of the ciliated epithelium **through** the movement of the statoliths located on the epithelium. As described herein, the envelope of the organ can suitably serve for sensing linear acceleration in areas where there are no plates, and in such instances, it is analogous to the sacculus and utriculus of vertebrates. The sensing of linear acceleration enables the hornet (or wasp) to sense the direction of gravitation, and thereby, enables it also to direct or orient its comb building towards the gravitational force. The region in which plates are located can serve as a sensory organ for sensing radial acceleration, and thereby, is analogous to the semicircular canal of vertebrates. In turn, the hemolymph within the envelope becomes analogous to the endolymph in the vestibular organ of vertebrates. The cilia (rods) are in fact stereocilia that line the entire cavity of the acoustic box, over the frons plate.

Externally to the acoustic box, around the conus (on the frons plate), an air sac separates between part of the envelope and the anterior of the frons plate. This air sac can serve both for the **production of sounds** intended to stimulate the substrate or the colony as well as for the transmission of sound waves into the acoustic box. To the air sac attaches a trachea which is analogous to the eustachian tube of vertebrates and whose function is to enable air exchange within the air sac as well as **the production of sounds**. The air exchange and sound production are probably achieved by movement of the muscles which are attached to the sac, movement which results in changes both in the sac volume and the interior air pressure. Stretching of these sac muscles leads also to stretching of the **cords** within the acoustic box and probably also the rise in the pressure within the air sac, and thereby, augments the **stiffness** within the system while reducing its sensitivity conditions which are suitable for situations involving strong environmental noise (as prevail in the populated hornet nests). From this standpoint, the air-sac's muscles are analogous to the muscles in the vertebrate middle ear, namely, the stapedial muscle and the tensor tympani.

Studies are now under way to investigate the functional properties of this organ including theoretical calculation on observed physical properties and dimensions.

Additional Reference

Ishay JS, Sadeh D (1982) The sounds of honeybees and social wasps are always composed of a uniform frequency. J Acoust Soc Am **72**: 671-675.