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THE FUNCTION AND FINE STRUCTURE OF THE CEPHALIC AIRFLOW RECEPTOR IN *SCHISTOCERCA GREGARIA*

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SUMMARY

Electron micrographs of parts of the sense organ showed that the dendritic axis consisted of a large and a small envelope containing microtubules as their main inclusion. The envelopes are supported by a thick-walled tube believed to be part of the 1st-tier sheath cells. The small envelope is segregated from the large envelope near its apex by a fold of the tube wall. The packing of the neurotubular array within the small envelope is both more dense and more regular than within the large envelope. The tube is separated by an extracellular space from the trichogen–tormogen cell. Sections through the apex of the dendrite reveal a homogeneous cap unlikely to be part of a structure continued into the upper region of the hair shaft. No ciliary structures were visible within the dendrite, whose microtubules pass into the neuron cell body proximally. Sections through the neuron cell body reveal branched mitochondria, and numerous microtubules.

Rates of discharge in sensory axons from these hair organs produced by deflexion of the hair shaft were found to be within the range 300–100 impulses/sec. There is an initial phase of rapid adaptation which gives place to a steady rate.

It is suggested that the fine structure of the receptor may indicate mechano-electrical transduction at a more proximal level than is believed to be the case in some other types of receptor.

The diaphragms that support the hair shaft laterally can be seen to be composed of fine cuticular strands.

INTRODUCTION

The fields of trichoid sensilla on the head of the locust, involved in flight, were first described by Weis-Fogh (1956). Haskell (1958) published a brief description of their physiological characteristics. Wilson (1961, 1963), Wilson & Wyman (1965), Gettrup (1962) and Neville (1963) have published material dealing with other parts of the nervous system involved in flight. Guthrie (1964) described the structure of the head hairs as seen with the light microscope, and attempted to show their connexions with the flight motor centres in the thoracic ganglia. The present communication describes research into the fine structure of the hair sense organ, and some observations on its function.

METHODS

Considerable difficulty was experienced in obtaining reasonably thin, complete and well-preserved sections of the receptor apparatus. This was due largely to the thick cuticle that surrounds it. Longitudinal sections were almost impossible to prepare due

to the hardness of the horizontal elements or Balken. Therefore, it was not possible to study serial sections in the electron microscope, and the isolated sections obtained have had to be interpreted by comparison with serial sections studied with the light microscope.

Glutaraldehyde fixation followed by post-fixation in osmium tetroxide (Sabatini, Bensch & Barrnett, 1963) was more effective than Palade-type fixation alone, in preserving the tissues, and embedding in a hard Epon mix gave sufficient support to the tissues. A diamond knife was tried at one stage for sectioning, but the results were not notably better than those obtained with glass knives. Sections were examined with an AEI EM6B electron microscope.

Recordings were made using a Grass P5 preamplifier and conventional display equipment.

RESULTS

The fine structure of the dendrite

The gross structure of the sensillum and its associated cells is figured in a previous paper (Guthrie, 1964). In the middle region the dendrite appears as a tube with 4 dense regions within it. At this point the dendrite occupies a position near the centre of the cuticular cavity. The electron micrograph fits this description quite well as can be seen in Fig. 3. The tube is about 1·5 μ in diameter with a thick wall (300–800 Å). The material of the tube appears to be of high electron density, but there are less dense zones within, suggesting the presence of a bilamellar structure, the lamellae being connected by struts or denser zones of similar material. The striking folds of the tube wall can be equated with the 4 dark areas seen in the light microscope. Within the tube a large (*le*) and a small membranous envelope (*se*) can be seen, and within these again are small circular profiles 160–200 Å in diameter, resembling neurotubules. In the small envelope the tubules are mostly 300–350 Å apart and there are about 280/μ^2, while in the large envelope the tubules are much less regularly arranged (100–2400 Å apart) and the density is about 100/μ^2. There is no reasonable doubt that these are neurotubules or, as they are referred to by Porter (1965), microtubules, and similar elements are to be seen in cells adjacent to the neuron, such as the trichogen–tormogen cell. The suggestion that they may have some mechanical role in cells, as suggested by Porter, may receive additional support from their presence as the only subcellular component of these mechanoreceptor dendrites. Their structure will be described in more detail at the end of this section.

Surrounding the dendritic tube can be seen an irregular double fold of membrane, corresponding to the material described as the column in an earlier paper (Guthrie, 1964). The folds come together at one side of the neuron tube as in a mesaxon (Fig. 3, *f*). The areas of low density in the light-microscope picture of this region (Guthrie, 1964, fig. 2) may correspond to the large vesicular bodies visible in the electron micrograph (Fig. 3, *vb*), some of which resemble lysosomes. Continuity of this glia-like cell with the second tier of sheath cells surrounding the neuron cell body observed in the light microscope cannot be conclusively demonstrated by electron micrographs, but trans-

verse sections at different levels suggest that they are the same. Parts of the 2nd-tier sheath cells can be seen in Fig. 15 (*2s*), which also shows the first-tier sheath cells. The latter are distinguishable by their strikingly dense appearance (Fig. 15, *1s*). If the first tier of cells are within the second tier, at the level of the neuron cell body, then they may remain in this position further distally (Fig. 9, *1s*). It follows that they must form the neuron tube wall at the level of the section illustrated in Fig. 3. At this level both 1st and 2nd tier cells have the appearance of single cells, even though further proximally overlapping folds of membrane (Fig. 15) suggest this may not be so, and this apparent contradiction is supported by light-microscope observations.

In Fig. 3 the space (*es*) surrounding the 2nd-tier sheath cell can be seen to contain a few villi, some poorly defined material, and a few large, irregular vesicles. The position of this space within the trichogen–tormogen cell is also shown in Fig. 10 (*es*), numerous villi forming the inner border of the cell and identifying the space as an extracellular one. The trichogen–tormogen cell contains many microtubules, mitochondria, subspherical globules and a few tracheae. That this cell is divided asymmetrically by the neuron dendrite is suggested by the junction between the two sides of the cell seen in Fig. 10 (*j*) and in more detail in Fig. 11 (*j*). A palisade-like formation bridges this point of apposition between the two folds of membrane.

The distal insertion of the dendrite complex is clearly of greatest interest and a few sections were fortunately secured through the apex. Fig. 4 shows a section through the hair shaft within the socket (see also Fig. 1). This is the part of the shaft connected to the walls of the socket by circular diaphragms (compare with Fig. 1). These diaphragms can be seen to be composed of fine cuticular threads (Fig. 4, *ct*). The tip of the tube can be seen making contact with one side of the hair shaft (Fig. 4, *ad*), as had previously been demonstrated by light-microscopic examination. In this electron micrograph the outer part of the dendritic apex still preserves the outer dense layer believed to be derived from 1st-tier sheath cells. The material within the sheath layer in this apical region is ill-defined, although some local accretions can be seen. Occupying the remaining area of the shaft lumen is a cytoplasmic reticulum presumably derived from the trichogen–tormogen cell (an interpretation is shown in Fig. 1). No sections could be obtained of the more distal region of the hair shaft, so that the question of the identity of the material within the upper part of the shaft visible in some light-microscope preparations could not be resolved, though it seems unlikely to be derived from the neuron. Thurm (1964) illustrates a continuation of the dendritic apex (tubular body) into the hair lumen in the honey-bee mechanoreceptor.

Sections displaying the structure of the tube at levels between those shown in Figs. 3, 4 are illustrated in Figs. 6–8. In this order, these are taken from levels proceeding from near the level of Fig. 3 to just below the apical zone. Fig. 9 was representative of a more proximal level, nearer the neuron cell body.

In the most proximal of these sections (Fig. 6) the small envelope is well defined and segregated from contact with the large envelope by a fold of the tube wall. The microtubules appear rather closely packed within the large envelope as compared with Fig. 3. Further distally this separation remains, but the tube profile is more circular, and the tube wall has many short processes. Fig. 8 displays a much greater develop-

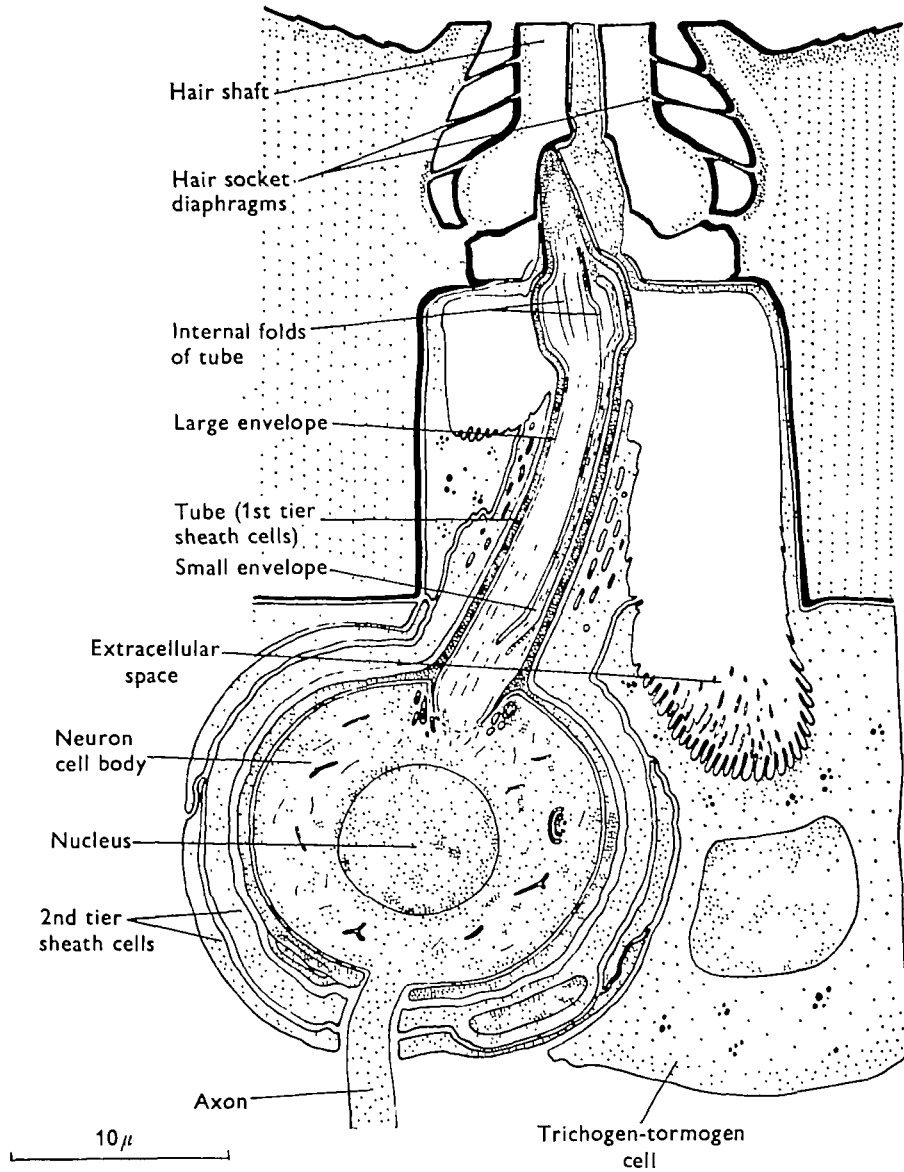

Fig. 1. Diagram to illustrate the structures observed in electron micrographs as they would appear in longitudinal section. The breadth of the tube has been slightly exaggerated. × about 4000.

ment of these processes or folds. All these sections show the tube surrounded by extracellular material, without any indication of sheath-cell membranes being present, and thus they are representative of regions distal to the column. The segregation of the two envelopes in the distal part of the tube suggests that they have separate distal insertions, yet sections of the dendritic apex give no support to this view. The section shown in Fig. 9 represents a much more proximal region of the tube. A cursory

examination reveals a considerable similarity between this region and the tube as shown in Fig. 8, especially as regards the infoldings of the tube wall, but it is evident that in Fig. 9 the tube is surrounded by cellular material (this was identified in the whole section as being formed by 2nd-tier cells). The double layer of the sheath-cell wall forming the tube can be seen irregularly developed in this latter illustration. Most striking is the absence of clearly defined small and large envelopes at this level (Fig. 9). Adjoining sections, not reproduced here, show the small envelope in a reduced form, or with its tubular contents no longer clearly defined (Fig. 12), and still further proximally the outline of the large envelope is obscure (Fig. 13). The small profile shown in Fig. 13 partly surrounded by the 1st-tier sheath cell may be in part small envelope. The appearance of the cytoplasm of the surrounding cell is similar to that of the neuron illustrated in Fig. 15, but, if the enclosing cell is the neuron, the 2nd-tier cells should be seen, and the cytoplasm is therefore probably part of a 2nd-tier sheath cell. In Fig. 13 the section is oblique and the proximal region is towards the bottom left-hand corner.

Fig. 5 shows parts of the nucleus, dictyosomes, ribosomes, branched mitochondria, microtubules and investing sheath cells characteristic of the neuron cell body. In the previous section (Fig. 13) microtubules can be seen traversing breaks in the tube wall, and some of these may pass into the neuron cell body.

No evidence for the existence of a ciliary body at any point in the dendrite could be found, but it is possible that a very short one, like that figured by Thurm (1964), does exist. The origin of the small envelope is particularly obscure. Light-microscope examination suggests that a lateral diverticulum of the tube exists at the proximal level at which it is seen to disappear in the electron micrographs, but the latter showed no aperture in the wall of the tube. The large envelope is almost certainly the major part of the neuron dendrite and is continuous with the cell-body cytoplasm, although the tube (1st-tier sheath cell) can be seen to invest in its most proximal regions. An interpretation of the major structures of the hair sensillum is shown in Fig. 1.

The neurotubules, or microtubules, are the main cytoplasmic component of the dendritic envelopes. In oblique sections some degree of periodicity may be apparent in their structure, while in transverse sections adjacent neurotubules may differ markedly in density. In one section through a region of the neuron cell body containing numerous neurotubules, a few of these appear to have become partly dissociated, revealing a helicoid structure. Their position and lack of taper suggest that these structures are not tracheoles (Fig. 14).

The electrical function of the hair sensillum

Haskell (1958) described the sensillum as showing slow, incomplete adaptation, when a maintained pressure is applied to a single hair, recordings being made from the dorsal tegumentary nerve. Impulse frequencies declined from 50/sec in the first $\frac{1}{4}$ sec, to 20/sec after 6 sec.

Following a similar recording procedure responses were obtained from single sensilla, but discharge rates were as follows. In the first 20 msec the impulse frequency was about 300/sec, but it fell sharply at the end of this time to 150/sec, at which level

it remained for several seconds. Thus a degree of rapid adaptation occurs initially at the onset of stimulation (Fig. 2), but there is little decline after this. This rather high discharge rate is found also in some other mechanoreceptors (Pumphrey, 1936; Pringle, 1938).

DISCUSSION

The first point that arises is whether the structures that have been described throw any light on the response mechanism of the sensillum. Bullock (Bullock & Horridge, 1965) remarks that the transduction process is not well understood in any single receptor, and it is clear that the small dimensions of the structures involved offer one major obstacle. Nevertheless, while the details of transduction remain obscure (with the possible exception of photo-reception), some general correlations emerge which may be relevant to the study of the locust hair organ.

In mechanoreceptors of the type termed stretch receptors, many dendrites extend into the muscle or connective tissue undergoing deformation, and these dendrites tend to be rich in mitochondria and poor in oriented inclusions other than microtubules (neurotubules); see Katz (1960), Whitear (1965), Osborne (1963), Osborne & Finlayson (1965). There is some evidence (Edwards & Ottoson, 1958; Katz, 1960) that in these receptors non-propagating generator potentials originate peripherally, and are linked with the more central appearance of propagated spike potentials. Other types of mechanoreceptor exist in which there is a complex assembly of axial structures; a detailed example has been provided by Gray (1960) in the case of the auditory neuron of the locust. Unfortunately the regional electrophysiology of this sensillum is not known, and Gray was careful to restrict his speculations as to the function of individual components.

Nevertheless, it is difficult to believe that primary electrical changes occur at the apex of the single dendrite of the locust auditory cell. No mitochondria appear to be present in this region, which has a degree of continuity with the cell body through the cilium and rootlet system. The rootlet system with its collagen-like structure strongly suggests a mechanical function, even though, as Gray points out, the upper part of the dendrite is not directly connected to the attachment cell. It is suggested that the tympanal displacement probably affects the more proximal regions of the dendrite or the cell body by the transmission of tension differences in the structurally discrete units observed within or surrounding the dendrite.

In the third type of system, to which the receptors described by Thurm (1964) and the locust head hair may belong, the dendrite contains no complex structures, at least in its distal portion, and this includes mitochondria. Thurm's hymenopteran hair organ has, it is true, a rather reduced ciliary apparatus, and an apical tubular structure whose components are about the same diameter (150–200 Å) as the microtubules of the locust head sensillum, but the outer segment has the appearance of a tubular envelope containing only dispersed microtubules. In the locust sensillum no ciliary structure of any kind was discovered, and the microtubules lost their identity apically, but much of the dendrite consists of a region of low optical density containing microtubules, surrounded by the neuron-cell membrane, and a thick wall formed by accessory cells.

A thick outer wall appears also in Thurm's illustration. The nature of the tubule wall strongly suggests a structure resistant to pressure changes, and together with the absence of mitochondria, and the delicacy and uniform distribution of the microtubular inclusions, support the idea that fluid pressure changes could be involved in the transmission of forces impressed on the dendritic apex by movements of the hair shaft.

If receptor function depends on a fluid transmission system the proximal areas of the tube wall might be expected to be more delicate than those more distal. This can be seen to be so in Fig. 13; indeed the wall appears incomplete at several points.

The contribution of the microtubules to such a system is not easy to estimate, and is likely to be impossible of solution other than by rather indirect means. The apparent spiral nature of these structures has already been mentioned, as has their different aggregation in the large and small envelopes, and the conclusion that they play an important mechanical role in the activity of the dendrite is difficult to avoid. Their coiling suggests that they may be capable of small changes in length, a property that might be involved in pressure-induced differences in the proximal limits of the dendritic envelopes. The mechanical responsiveness of the two envelopes may be differentiated on the basis of the packing density of microtubules and the diameter of the envelopes, although it must be noted that the envelopes are segregated from one another only near the dendritic apex (Figs. 6, 7).

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REFERENCES

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*. London and San Francisco: Freeman.

EDWARDS, C. & OTTOSON, D. (1958). The site of impulse initiation in a nerve cell of a crustacean stretch receptor. *J. Physiol., Lond.* **143**, 138–148.

GETTRUP, E. (1962). Thoracic proprioceptors in the flight system of locusts. *Nature, Lond.* **193**, 498–499.

GRAY, E. G. (1960). The fine structure of the insect ear. *Phil. Trans. R. Soc.* B **243**, 75–92.

GUTHRIE, D. M. (1964). Observations on the nervous system of the flight apparatus in the locust *Schistocerca gregaria*. *Q. Jl microsc. Sci.* **105**, 183–201.

HASKELL, P. T. (1958). The physiology of some wind sensitive receptors of the desert locust (*Schistocerca gregaria*). *XVth Int. Congr. Zool.* pp. 960–961.

KATZ, B. (1960). The terminations of the afferent nerve fibre on the muscle spindle of the frog. *Phil. Trans. R. Soc.* B **243**, 221–240.

NEVILLE, A. C. (1963). Motor unit distribution of the dorsal longitudinal flight muscles in locusts. *J. exp. Biol.* **40**, 123–136.

OSBORNE, M. P. (1963). An electron microscope study of an abdominal stretch receptor of the cockroach. *J. Insect Physiol.* **9**, 237–245.

OSBORNE, M. P. & FINLAYSON, L. H. (1965). An electron microscope study of the stretch receptor of *Antheraea pernyi*. *J. Insect Physiol.* **11**, 703–710.

PORTER, K. (1965). Illustrations of cell fine structure. In *Ideas in Modern Biology*, chapter 3*a*. *XVIth Int. Congr. Zool.*

PRINGLE, J. W. S. (1938). Proprioception in insects. III. The function of the hair sensilla at the joints. *J. exp. Biol.* **15**, 467–473.

PUMPHREY, R. J. (1936). Adaptation in a tactile receptor of the cockroach. *J. Physiol., Lond.* **87**, 15–19.

SABATINI, D., BENSCH, K. & BARRNETT, R. (1963). Cytochemistry and electron microscopy. *J. Cell Biol.* **17**, 19–21.

THURM, U. (1964). Mechanoreceptors in the cuticle of the honey bee. *Science, N.Y.* **145**, 1063–1066.

WEIS-FOGH, T. (1956). The biology and physics of locust flight. IV. Sensory factors in the control of flight. *Phil. Trans. R. Soc.* B **239**, 553–561.

WHITEAR, M. (1965). The fine structure of the crustacean proprioceptor. II. The thoracico-coxal organs in *Carcinus*, *Pagurus* and *Astacus*. *Phil. Trans. R. Soc.* B **248**, 437–456.

WILSON, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471–490.

WILSON, D. M. (1963). The relative refractoriness and patterned discharge of locust flight motor neurones. *J. exp. Biol.* **41**, 191–205.

WILSON, D. M. & WYMAN, R. J. (1965). Motor output patterns during random and rhythmic stimulation of locust thoracic ganglia. *Biophys. J.* **5**, 121–143.

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Fig. 2. The electrical response of a single sense organ to deflexion of the hair shaft. The calibration stripe placed near the onset of stimulation is equivalent to 50 msec.

Fig. 3. Transverse section through the dendritic region. The large envelope (*le*), and the small envelope (*se*) can be seen, surrounded by the dense tube (*t*). The 2nd-tier sheath cells enclose the dense tube so that membrane folds (*f*) are formed. Vesicular bodies (*vb*) are visible within these sheath cells, which are surrounded by extracellular space (*es*). × 60000.

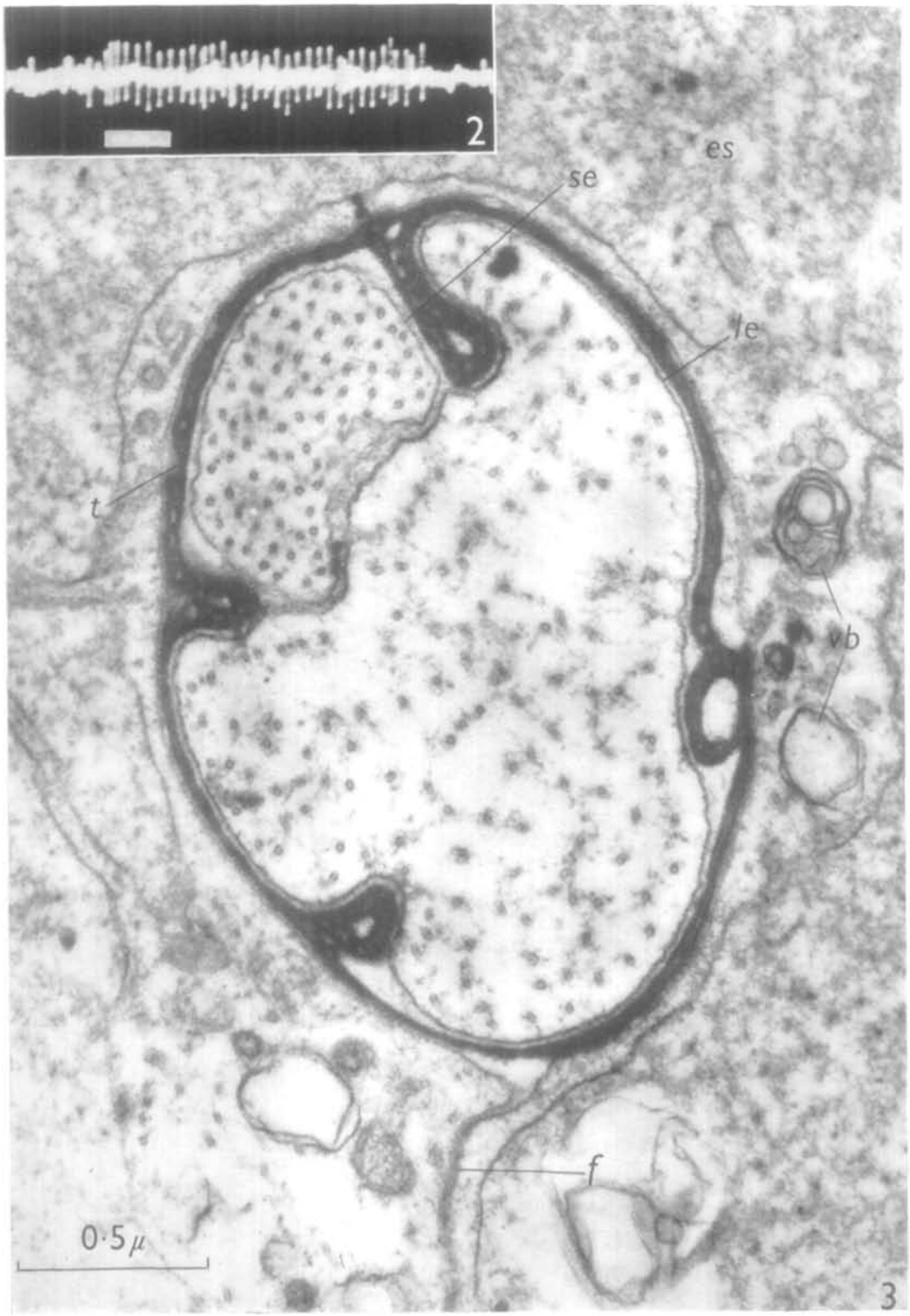

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Fig. 4. Transverse section through the apex of the dendritic axis (*ad*) at its point of insertion within the base of the hair shaft (*hs*). The hair socket diaphragms (see Fig. 1) can be seen to consist of radial cuticular threads (*ct*). × 12000.

Fig. 5. Transverse section proximal to that shown in Fig. 4 displaying the head of the dendrite in a more expanded form. × 27000.

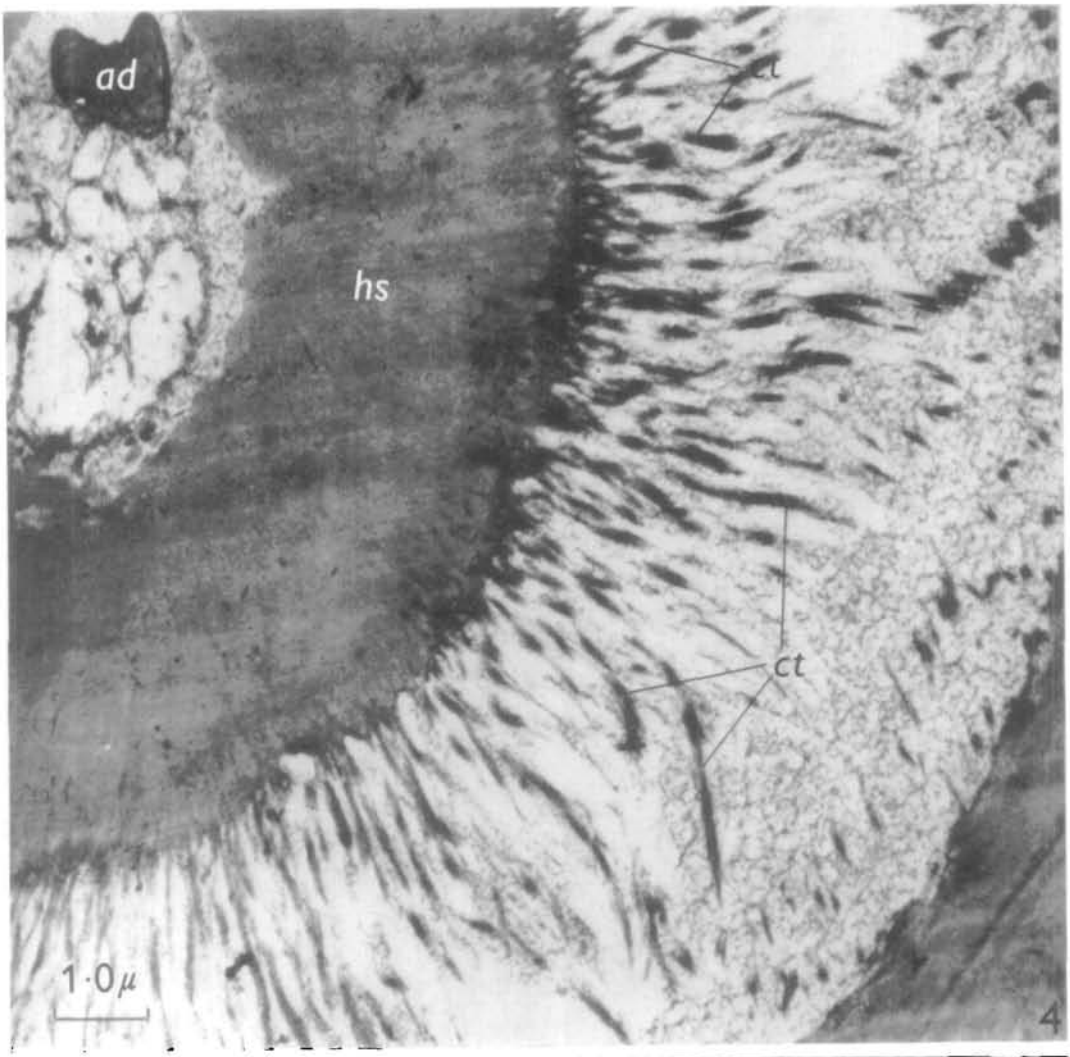
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Fig. 6. Transverse section through the dense tube (*t*), and the large (*le*) and small envelopes (*se*). Proximal region. × 35000.

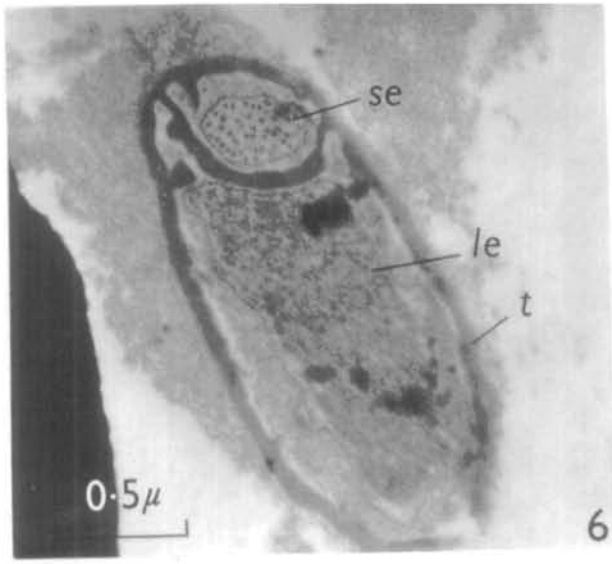
Fig. 7. Transverse section through the dendritic axis at a more proximal level than shown in Fig. 6. Dense tube, *t*; large envelope, *le*; small envelope, *se*. × 20000.

Fig. 8. Transverse section through the dendritic axis. The level illustrated lies between those of the sections shown in Figs. 5 and 7. Note the processes of the tube wall (*pt*), and the large envelope (*le*). × 25000.

Fig. 9. Transverse section through the dendritic axis at a distal level, near the cell body of the neuron. The tube wall is very irregular, and has much more the appearance of the 1st tier of sheath cells (*1s*). × 12000.

Fig. 10. Transverse section through the cuticular channel enclosing the sense organ. At about the same level as shown in Fig. 3. The dense tube (*t*), can be seen surrounded by the 2nd-tier sheath cells (*2s*), the extracellular space (*es*), and the trichogen–tormogen cell (*ttc*), and finally the cuticle (*c*). The dendritic structures enter the trichogen–tormogen cell through a point of cleavage marked by a membrane junction (*j*). × 10000.

Fig. 11. Detail of the membrane junction (*j*) illustrated in the previous figure. Note the palisade-like structure. × 62000.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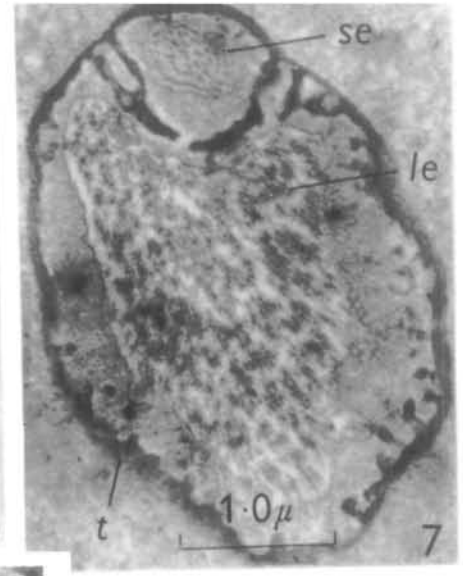
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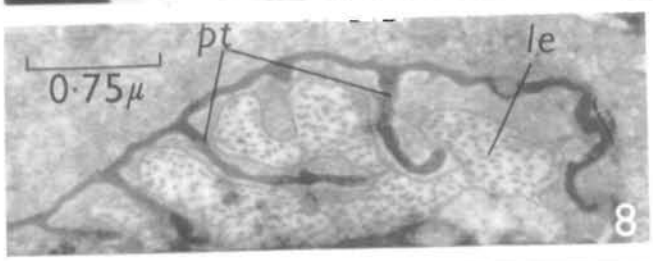
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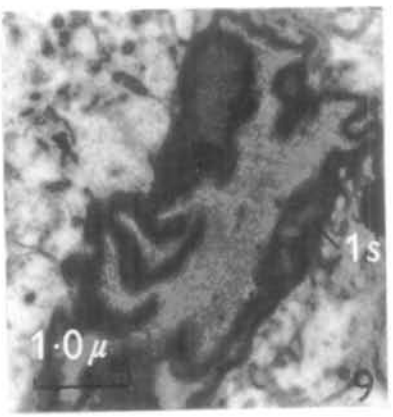
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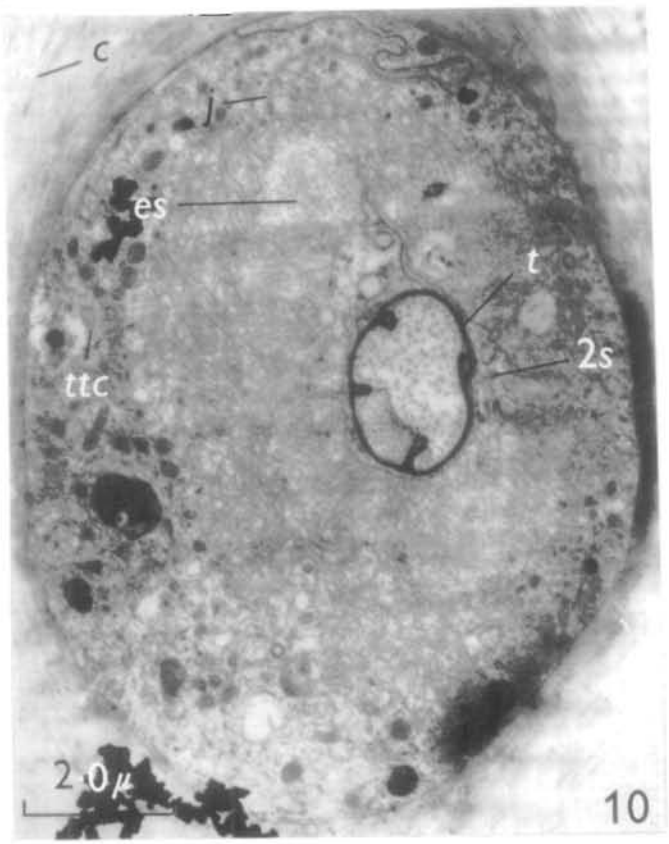
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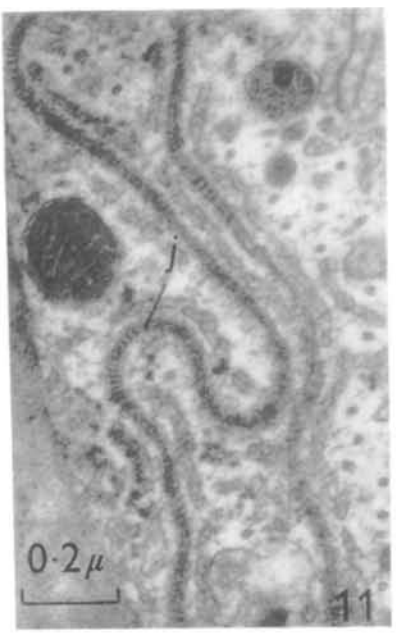
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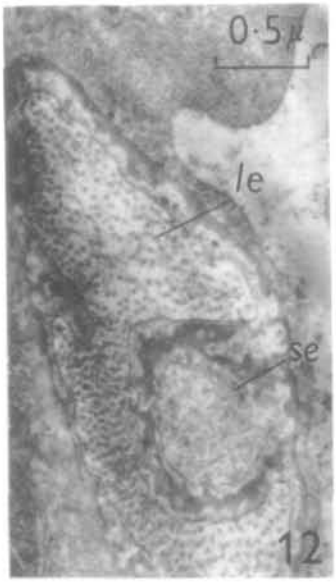
Fig. 12. Transverse section through dendritic structures at a distal level. The large envelope (*le*) partly surrounds the small envelope (*se*). × 27000.

Fig. 13. Oblique section through the dendritic region at a distal level at the periphery of the neuron cell body. The neurotubules are still enclosed by an indistinct tube wall, which at this level can be equated with the 1st-tier sheath cells (*1s*). × 25000.

Fig. 14. Section through the neuron cell-body cytoplasm to show structures believed to be neurotubules (*nt*). Note spiral appearance. × 150000.

Fig. 15. Transverse section through the neuron cell body. Dictyosome, *d*; mitochondria, *m*; nucleus, *n*; neurotubule, *nt*; 1st-tier sheath cell, *1s*; 2nd-tier sheath cell, *2s*. × 22000.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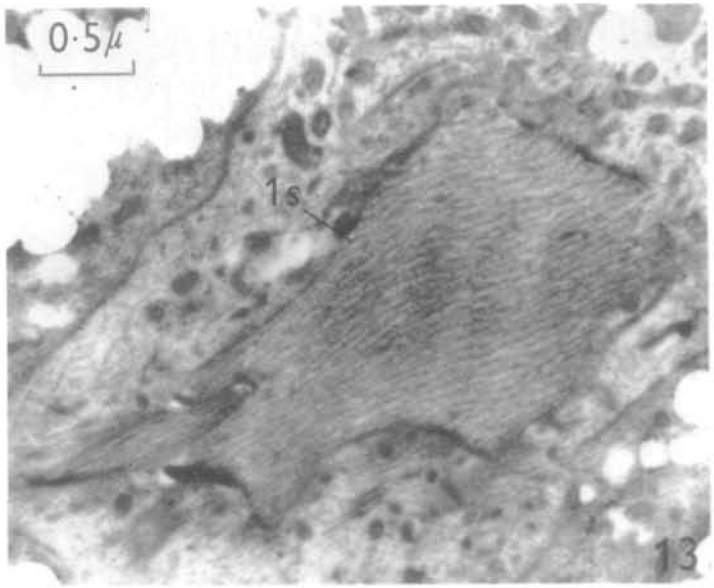
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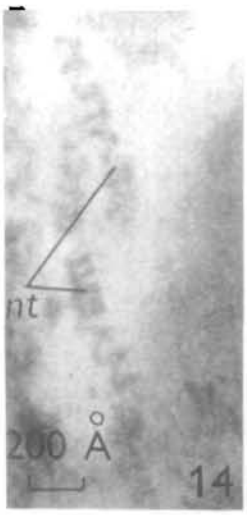
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