

THE DISTRIBUTION AND STRUCTURE OF THE LEG SPINES IN THE LARVAE OF SOME ANISOPTERAN DRAGONFLIES

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The distribution of the leg spines on the tibia and tarsi is described for the larvae of 3 anisopterans: *Anax imperator* Leach, *Aeshna cyanea* (Müll.) and *Libellula* sp. There are 2 rows of spines on the ventral surface of the legs and these rows broaden at the distal ends of the tibia and first 2 tarsal segments. There are 2 types of spine, single and tridentate. There are differences in their distribution between spp., but the greatest differences are between each of the 3 pairs of legs. The innervation of the spines of *Anax* is described and is typical of other insect mechanoreceptors. There is a single, bipolar sensory neuron, the dendrite of which gives rise to a ciliary structure. This ends distally in a tubular body at the base of the spine. The sensory apparatus is surrounded by three enveloping cells. The definition of tubular bodies and the optimal stimulus are discussed.

INTRODUCTION

The ultrastructure of tactile hair sensilla has been studied fairly extensively in arthropods (cf. LEWIS, 1970; SLIFER, 1970; McIVER, 1975), but the distribution of the different types has received comparatively little attention, at least in recent years.

The sensory cells which innervate these sensilla are always bipolar. The dendrite contains a large number of microtubules. Part of the way along its length there is a slight constriction dividing it into an inner (proximal) segment and an outer (distal) segment. The latter ends in a dilation, the tubular body, which is juxtaposed to the base of the hair and contains electron-dense material laid down around a matrix of microtubules.

There are normally three accessory cells associated with each sensillum. The innermost cell (inner enveloping or dendritic sheath cell) forms the dendritic sheath, the second (middle enveloping or trichogen cell) forms the

hair shaft, and the third (outer enveloping or tormogen cell) produces the membranous socket. This pattern has been described for several arthropod mechanoreceptors (SCHMIDT, 1969, 1973; BARTH, 1971; SCHMIDT & GNATZY, 1971). The distal end of the outer dendritic segment, surrounded by the dendritic sheath, passes through an extracellular space formed by the withdrawal of the trichogen and tormogen cells after moulting (ERNST, 1972).

The number of bipolar sensory cells innervating a single tactile hair varies. In most insect and crustacean sensilla the innervation is single (THURM, 1965a; GNATZY & SCHMIDT, 1971, 1972; DUMPERT & GNATZY, 1977; ROSSIGNOL & McIVER, 1977), although some receptors of the locust (BERNAYS, COOK & PADGHAM, 1976) are dually innervated, as also are some tactile setae on the thorax of decapod crustaceans (MELLON, 1963), and the tactile hairs in mites (CHU-WANG & AXTELL, 1973). In spiders the tactile hairs are innervated typically by three sensory cells (FOELIX & CHU-WANG, 1973a; HARRIS & MILL, 1977).

The leg sensilla of dragonfly larvae include both single and forked spines. In Zygoptera an apical comb occurs on the distal region of each tibia. This consists mainly of bidentate spines in Lestidae and tridentate spines in Platycnemididae and Coenagrionidae (GARDNER, 1954). However, the detailed distribution and structure of the leg spines appears not to have received attention.

MATERIAL AND METHODS

Scanning electronmicroscopy was carried out on exuviae and detached legs of late instar larvae of *Anax imperator* Leach, *Aeshna cyanea* (Müller) and *Libellula* sp. For transmission electronmicroscopy, recently moulted larvae of *A. imperator* were used.

Light microscopy. — Photographs were taken of the legs of *Anax* using a Pentax Spotmatic camera attached to a Zeiss Tessovar Macrosystem.

Scanning electronmicroscopy. — Legs from air dried exuviae were mounted on a specimen stub, coated with a thin layer of gold and examined in a Cambridge Stereoscan electronmicroscope.

Transmission electronmicroscopy. — Pieces of leg were fixed overnight in 2.5% glutaraldehyde (SABATINI, BENSCH & BARNETT, 1963). The preparation was washed in cacodylate buffer, post-fixed for at least one hour in 1% osmium tetroxide in veronal buffer (PALADE, 1952) and washed in veronal buffer. This was followed by dehydration in a series of alcohols before gradual introduction (over three days) into the embedding resin (i.e. using increasing proportions of resin in the resin: propylene oxide mixture).

Sections were cut with glass knives using a Cambridge ultramicrotome.

The sections were collected on carbon-coated 100 mesh copper grids and stained with uranyl acetate followed by lead citrate (REYNOLDS, 1963), before examination in an AEI EM6B electronmicroscope.

RESULTS

Spines occur on the ventral surface of the distal tibia and tarsi of all the legs of the larvae of the three species studied. Some of the spines are single, others are tridentate (Figs. 1, 2). In *Anax* and *Aeshna* both forms range in length between $40\text{ }\mu\text{m}$ and $200\text{ }\mu\text{m}$, but in *Libellula* the single spines tend to be longer ($140\text{--}260\text{ }\mu\text{m}$) than the tridentate ones. The single spines are cylindrical and tapered, while the tridentate spines are divided into three prongs with the central one usually rather longer than the lateral ones.

There are 300–400 spines on each leg. They all point distally and are arranged in a distinct pattern consisting of two rows, which increase in width at the distal end of all segments except the third tarsal segment. The two forms have fairly precise distributions which differ on each of the three pairs of legs, and also to some extent between the three species (Fig. 1). On the prothoracic legs of *Anax imperator* and *Aeshna cyanea* most of the spines are tridentate,

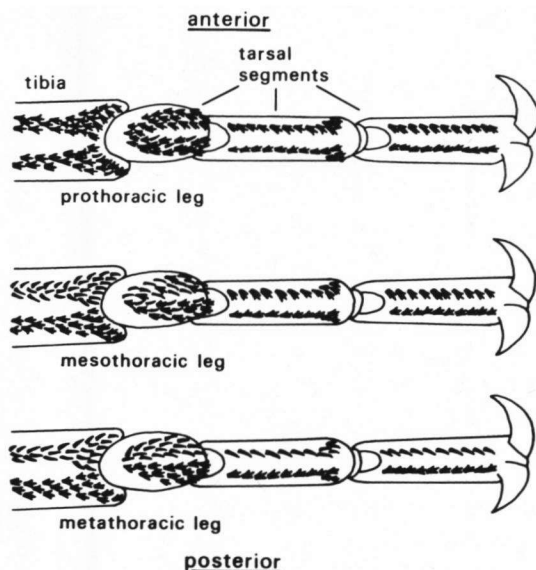


Fig. 1. Diagrams to show the distribution of the single and tridentate ventral spines on the tarsal segments and distal parts of the tibia of the left prothoracic, mesothoracic and metathoracic legs of *Anax imperator*.

but *Libellula* has some single spines in the anterior row on the tibia and on the second and third tarsal segments (Fig. 2c). In all three species both rows on the second and third tarsal segments of the mesothoracic legs consist of tridentate spines, but on the anterior side of the tibia and first tarsal segment single spines predominate. On the metathoracic legs single spines occur on the anterior side, tridentate spines on the posterior side, of the tibia and tarsal segments of *Anax* and *Libellula* (Fig. 2a, b). However, in *Aeshna* there are some long slender tridentate spines on

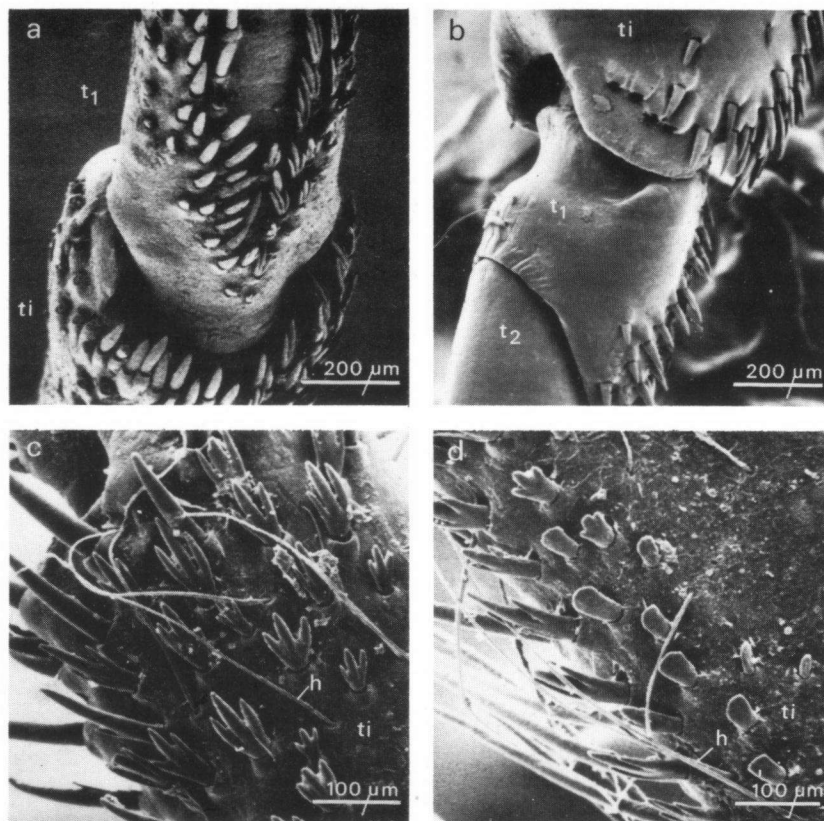


Fig. 2. (a) Ventral view of left tibia and first tarsal segment of metathoracic leg of *Anax imperator* to show the distinct demarcation between anterior single and posterior tridentate spines. — (b) Anterior view of right tibia and first tarsal segment of metathoracic leg of *Anax imperator* to show the single spines. — (c) Right tibia of prothoracic leg of *Libellula* to show the tridentate spines and scattered large single spines. (d) Right tibia of prothoracic leg of *Libellula* to show the development of the tridentate spines. The spines all point distally. h, hair; t₁, first tarsal segment; t₂, second tarsal segment; ti, tibia.

the anterior side. Figure 2d shows various stages in the development of tridentate spines. In the aeshnids the dorsal surface of the leg is almost devoid of cuticular sensilla except for a few hairs, but in *Libellula* long hairs occur on both the tibia and the femur (Fig. 2c, d).

ULTRASTRUCTURE

Both types of spine appear to be innervated in the same way. The dendrite of the single bipolar sensory neuron tapers from about $7\mu\text{m}$ near the cell body

to about $1.5\mu\text{m}$ at the tip and is divided by a ciliary constriction into an inner (proximal) segment and an outer (distal) segment (Figs. 3, 4). The inner dendritic segment contains mitochondria and microtubules, the former being particularly abundant just proximal to the ciliary constriction (Fig. 4a). The outer dendritic segment contains single longitudinally orientated microtubules (Fig. 4b, c). Towards its distal end, where it enters the base of the spine, there are a number of invaginations in the dendritic wall and the microtubules are particularly prominent around these invaginations (Fig. 4d). This region is called the tubular body. The extracellular dendritic sheath surrounds the distal half of the outer segment and is particularly thick around the tubular body, where it fills the invaginations in the dendritic wall (Fig. 4c, d). Dendritic sheath material is also laid down around the microvilli at the distal end of the inner enveloping cell (Figs. 3, 4c).

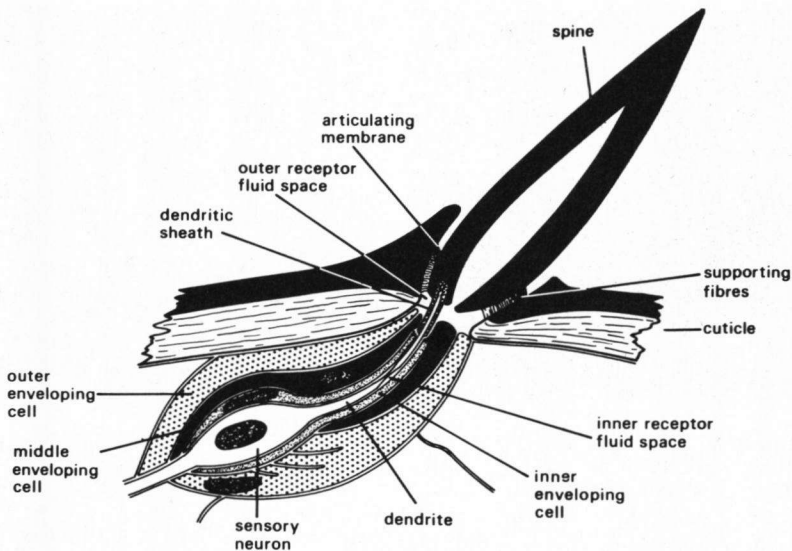


Fig. 3. Diagram to show the innervation of a leg spine.

There are three enveloping (accessory) cells associated with the sensory structure. The inner enveloping cell (dendritic sheath cell) is the smallest and it surrounds the sensory cell body and the dendrite as far as the proximal end of the dendritic sheath, which it is thought to secrete. At its outer end it bears several microvilli (Figs. 3, 4c). The cell cytoplasm contains mitochondria, ribosomes and microtubules. Near the ciliary constriction, electron dense structures occur around, and often associated with, the surface membrane adjacent to the dendrite (Fig. 4b). The middle enveloping cell (trichogen cell), which presumably secretes the hair shaft, surrounds the inner enveloping cell and

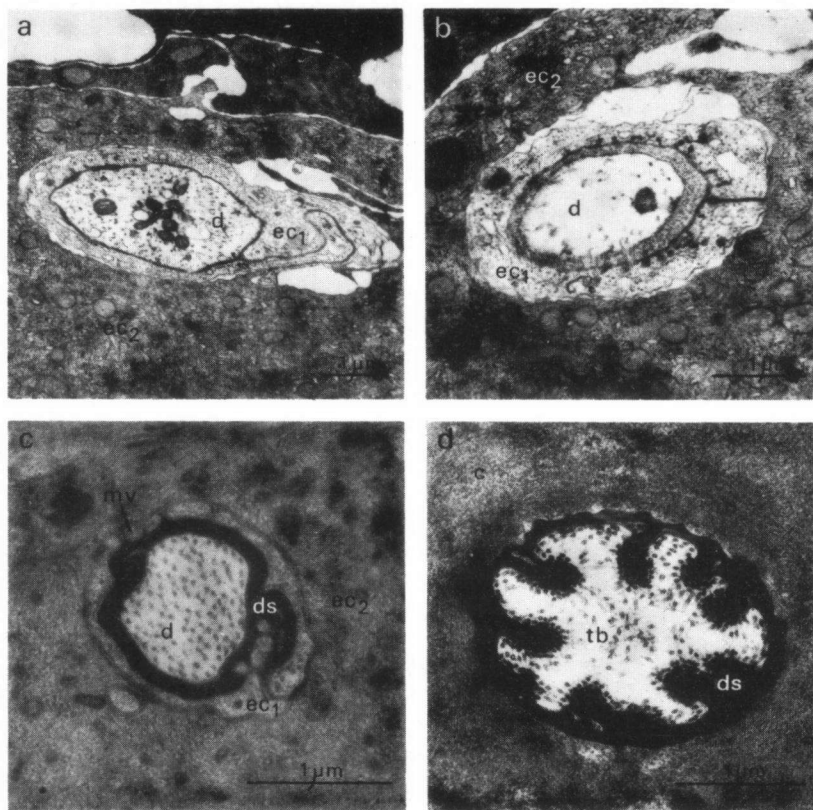


Fig. 4. Transmission electronmicrographs of transverse sections through the dendrite and surrounding structures of a spine of *Anax imperator*. Through: (a) inner dendritic segment; — (b) outer dendritic segment in the region of the inner receptor fluid space; — (c) outer dendritic segment in the region of the dendritic sheath; — (d) tubular body. (c, cuticle; — d, dendrite; — ds, dendritic sheath; — ec₁, inner enveloping cell; — ec₂, middle enveloping cell; — is, inner receptor fluid space; — mv, microvillus; — tb, tubular body).

the proximal part of the dendritic sheath (Fig. 4a-c). It is attached to the inner enveloping cell by septate desmosomes. Its cytoplasm contains large amounts of rough endoplasmic reticulum and ribosomes as well as numerous mitochondria, and overall it is of a greater electron density than the inner enveloping cell (Fig. 4a, b). The outer enveloping cell (tormogen cell) surrounds the middle enveloping cell and is thought to secrete the hair socket.

The inner receptor fluid space is very small and lies between the dendrite and the inner enveloping cell in the region of the ciliary constriction (Figs. 3, 4b). The outer receptor fluid space is bounded proximally by the distal ends of the middle and outer enveloping cells (Fig. 3).

DISCUSSION

The need for morphological and distributional differences in the leg spines is far from clear. They increase in density through successive instars, but are absent in the adult (unpublished observations). In zygopterans the spines at the distal end of the tibia have been referred to as a 'comb' (GARDNER, 1954) which implies a grooming function for this particular group. Alternatively they may serve as flow receptors, which from their position seems unlikely; or they may help the larva to cling to vegetation, especially during metamorphosis. However, they most certainly have a mechanoreceptive function (unpublished observation).

The tubular body is the dendritic region where transduction of the sensory stimulus is thought to occur. Since THURM's (1964) original description, the tubular body has been redefined to incorporate the wide range of structural variations which has been observed since then. Thus FOELIX & CHU-WANG (1973a) proposed that the term should be used for "any dense arrangement of microtubules which is restricted to a dendritic terminal, regardless of the amount of electron-dense substance present". They differentiated between 'dense' and 'loose' tubular bodies. Dense tubular bodies contain a fairly regular array of numerous, closely-packed microtubules, interspersed with electron-dense material (the original type as described by THURM (1964) and many authors since then). In loose tubular bodies there are fewer microtubules, which tend to be juxtaposed in chains, and less electron-dense material. Nevertheless, they still have a fairly dense arrangement of microtubules (FOELIX & CHU-WANG, 1973a, 1973b). RICE, GALUN & FINLAYSON (1973) described a mechanoreceptive ending in the LR7 sensillum of the tsetse fly, in which there are very few microtubules (arranged in a ring), together with a comparatively small amount of electron-dense material. McIVER (1975) suggested that the definition of a tubular body should be extended so as to include this type of ending, thereby accepting that tubular bodies may vary widely in both the density of microtubules and the amount of electron-dense material present. Following McIVER's definition, the dendritic ending described in this paper should definitely be called a tubular body. There are numerous microtubules, mainly aggregated around the periphery, but a comparatively small amount of electron-dense material.

THURM (1964, 1965a, 1965b) suggested that compression of the tubular body is the optimal stimulus for mechanosensitive neurons with the dense type of tubular body, while RICE, GALUN & FINLAYSON (1973) argued that a stretch mechanism may operate in the tubular body of the LR7 sensillum. In the dragonfly leg spine the tubular body is firmly anchored into the base of the spine via the dendritic sheath and, while compression can not be ruled out entirely, the arrangement of this junction seems more

commensurate with a stretch-sensitive mechanism.

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