

Organisation of sensory leg nerves in the spider *Zygiella x-notata* (Clerck) (Araneae, Araneidae)

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Summary

The two tarsal nerves in the different legs of the orb-weaver *Zygiella x-notata* (Clerck) were studied with the electron microscope. Each nerve consists of several hundred sensory axons and rather few glial cells. Tarsi of legs I-II contain up to 1500 sensory fibres; in legs III-IV this number is considerably lower (600-800). From axon counts at the coxal level it was determined that the total sensory input from the legs must be more than 40,000 nerve fibres.

A comparison of the number of tarsal sensory hairs and the number of tarsal axons showed that there were actually more nerve fibres than would be expected. This finding excludes any possible axon fusion. The "extra" fibres are attributed to tarsal sensory organs other than hairs, and to possible efferent fibres of central origin, which exhibit typical synapses onto surrounding sensory axons.

Introduction

In spiders and insects, specialised sense organs which perceive the various stimuli from the environment are concentrated on the appendages. However, in contrast to insects which bear their sense organs mainly on their antennae, spiders utilize their legs and palps to carry their sensory equipment.

Most of their sense organs are represented as cuticular hairs. They are especially concentrated on the tarsus, the distal segment of the legs and palps. Each sensory hair is innervated by several nerve cells (Foelix & Chu-Wang, 1973a, b), the axons of which are bundled together with those of other sensory hairs to form a sensory nerve. The spider tarsus contains two sensory nerves (Fig. 4), but no motor

nerves, since muscles are lacking beyond the tarsus/metatarsus joint. Thus the two sensory nerves should consist solely of the axons of the various sensory organs. All the tarsal sensory hairs can easily be counted under a microscope, and since the number of nerve cells per sensory hair varies only slightly, one should be able to predict fairly closely the number of axons in the tarsal sensory nerves. This, of course, holds true only under the now generally accepted assumption that the sensory fibres retain their individuality as they proceed toward the central nervous system (Steinbrecht, 1969).

The purpose of this study was first to clarify the general organisation of peripheral sensory nerves in a spider, and secondly, to show that the many sensory fibres do indeed *not* form syncytia. Furthermore, by counting the sensory nerve fibres of each leg just before entering the central nervous system, the total sensory input could be stated quantitatively.

Materials and Methods

The legs and palps of several female spiders (*Zygiella x-notata* (Clerck), Araneidae) were severed from the body and fixed immediately in cacodylate-buffered 5% glutaraldehyde. After postfixation in 1% OsO₄ and dehydration in an ethanol series the various leg segments were embedded separately in Epon resin. For orientation in the light microscope, 1-2 μm sections stained with Methylene Blue/Basic Fuchsin were used. After the proper orientation of cross-sections had been ascertained, thin sections (approx. 800 Å) were cut with a diamond knife. Practically all measurements (axon counts, fibre diameter) were taken from electron micrographs of cross-sectioned leg segments.

For counting the various sensory hairs, tarsi were mounted in an erect position on a movable metal block. They could then be viewed from all sides under a scanning electron microscope. The corresponding contralateral tarsus was used for transmission electron microscopy, e.g. for counting the number of sensory fibres at the level of the tarsus/metatarsus joint. At this level axon counts were performed for all appendages of several spiders. At the coxal level only a few legs could be used for axon counts, since nerve fibres were often cut obliquely, which prevented the identification of individual fibres. Therefore only

long, straight and pointed, or rather short, curved and blunt-tipped (Fig. 1). The first type are tactile hair sensilla. These are always innervated by three sensory neurons (Fig. 2). The other type are chemosensitive hair sensilla; they are usually associated with 21 neurons each, but there is some variation (Foelix & Chu-Wang, 1973a, b; Harris & Mill, 1973). In tactile hairs the three dendrites terminate at the hair base. In chemosensitive hairs the dendrites of the sensory cells (Fig. 3) enter the hair shaft and are then exposed to the stimulus through a small hole at the hair tip.

The axons of the bipolar sensory cells of both hair types bundle together, first between the hypodermis cells, later, at more proximal levels, as two discrete sensory nerves within the lumen of the leg (Fig. 4). The axons leaving the sensory cell somata are already encased by a glial cell. Within the sensory nerves several hundred axons lie directly adjacent to each other, and only such large bundles are wrapped in thin glial extensions (Fig. 5). Thus, the tarsal sensory nerve may consist of more than a thousand axons with rather few intervening glial processes. An extracellular "basement lamella" covers the surface of the nerve (Figs. 5, 6).

The diameter of the sensory fibres ranges from 0.1-2 μm , with a mean value of 0.4 μm . Aside from this large variation from fibre to fibre, the diameter can also vary within a single axon. The majority of sensory fibres measure less than 0.5 μm in diameter, whereas fibres of more than 1 μm are rare (Table 1).

b) Fine structure of axons and glial cells

The main cytological features of the axons are microtubules, occasional mitochondria and irregular profiles of smooth endoplasmic reticulum (Fig. 6). Some axons contain small synaptic vesicles or larger neurosecretory granules. Such fibres most likely do not represent sensory axons, but may be efferent (Fig. 7).

The axon membranes are usually separated from each other by a 200 Å gap; specialised cell contacts such as gap junctions are usually not observed between the axons – in contrast to the dendrites (Fig. 2). Typical synaptic contacts are rarely but consistently found in almost any cross-section of a tarsal nerve, particularly at the periphery (Figs. 8, 9). These synapses may represent interactions between sensory (afferent) fibres or between efferent and afferent

fibres.

The glial contribution to the sensory nerve is hardly visible in the light microscope, except for thin septae subdividing the nerve into bundles, and for elongated nuclei, which are located peripherally (Fig. 5). The glial cytoplasm may contain some microtubules, while other cell organelles are sparse. The entire sensory nerve is covered by a basement lamella of 0.2 μm thickness, this extracellular lamella often exhibits orderly arrays of collagen fibres. It is most likely a secretion product of the glial cells.

c) Axon number

The number of sensory fibres was counted from electron micrographs of cross-sections at the tarsus/metatarsus joint. The number of fibres is fairly

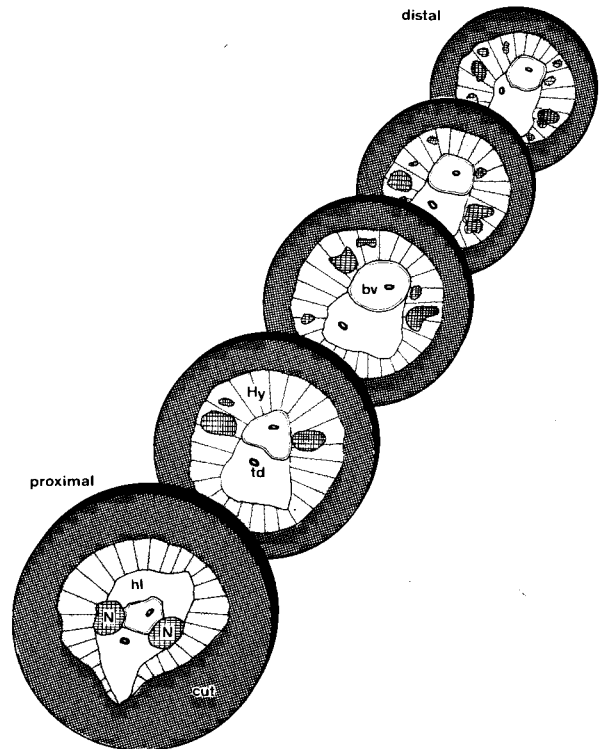


Fig. 4: Sequence of several tarsal cross-sections taken at 20 μm intervals. In the distal tarsus several smaller sensory nerves lie embedded within the hypodermis (*Hy*). At more proximal levels the smaller nerve bundles coalesce to form two nerves (*N*), which traverse the hemolymph space (*hl*). *bv* = blood vessel, *td* = tendon, *cut* = cuticle.

consistent for any given leg, but differs markedly between leg I and leg IV. Although there is a general decrease in the fibre number from front to rear legs, the most conspicuous difference lies between the forward directed legs I and II and the more backward directed legs III and IV. The tarsus of the palps contains a surprisingly high number of axons, almost as many as in the front legs (Table 2).

d) *Relation between axon number and number of sensory organs*

The number of tarsal sensory hairs could be counted fairly reliably under the scanning electron microscope, after the tarsus had been photographed

from two opposite sides (Fig. 1; see Materials and Methods). A minor difficulty was that in some cases the chemosensitive hairs could not be distinguished from tactile hairs. This was especially true for the palps and therefore no figures for the palpal tarsus are given in Tables 3 and 4. In the legs both hair types usually could be identified without difficulty, and any possible error was reduced by counting several legs of different spiders.

There is a definite decrease in the number of both tactile and chemosensitive hairs from the front legs toward the rear legs (Table 3).

The theoretical number of tarsal sensory fibres can be obtained by multiplying the number of hairs by

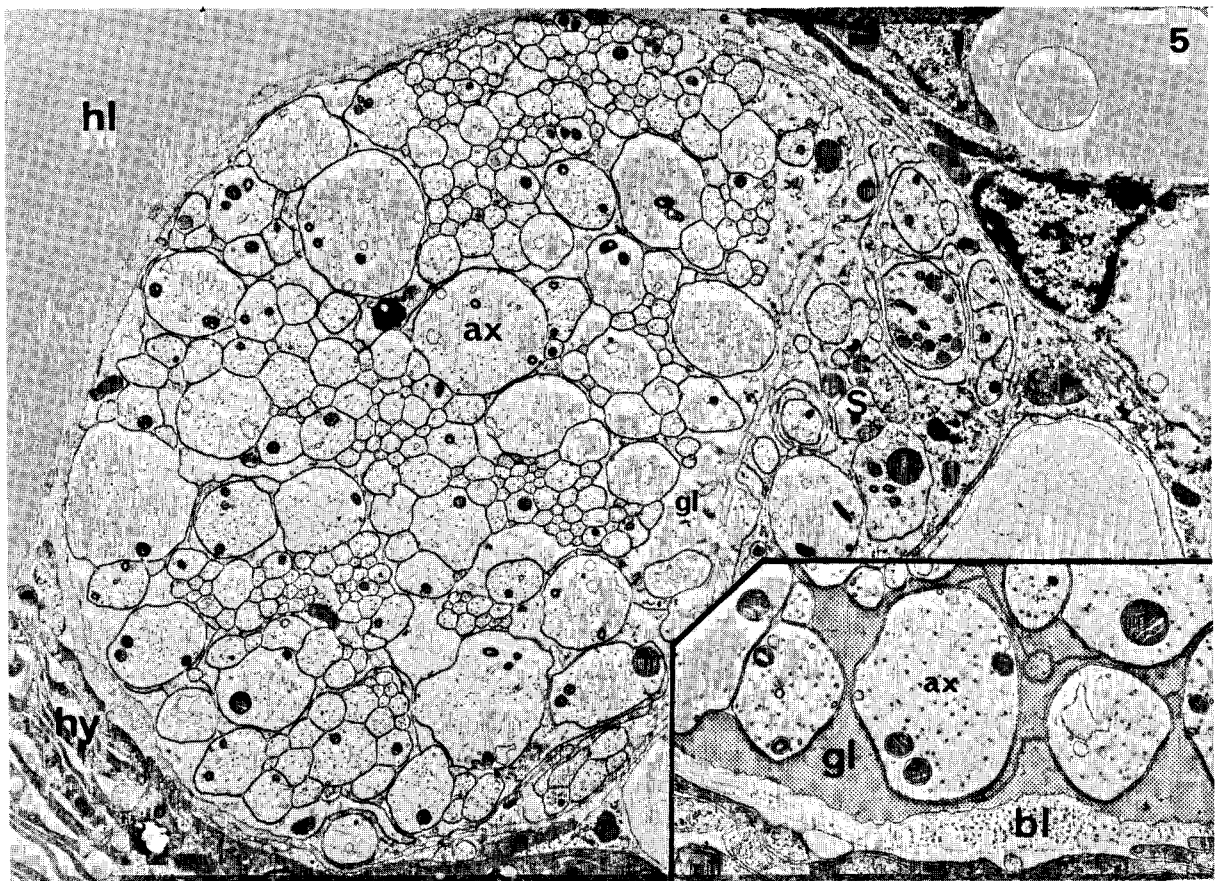


Fig. 5: Cross-section of one tarsal nerve of leg IV, partly embedded in the hypodermis (*hy*) and partly contacting the hemolymph space (*hl*). This particular nerve section contains 405 axons (*ax*) and relatively few intervening glia (*gl*). The area on the right is differently organised and exhibits synaptic contacts (*s*). x 7000. *Inset*: The stippled area represents the glial wrapping (*gl*) of some peripheral axons (*ax*). A basement lamella (*bl*) covers the nerve surface. x 20,000.

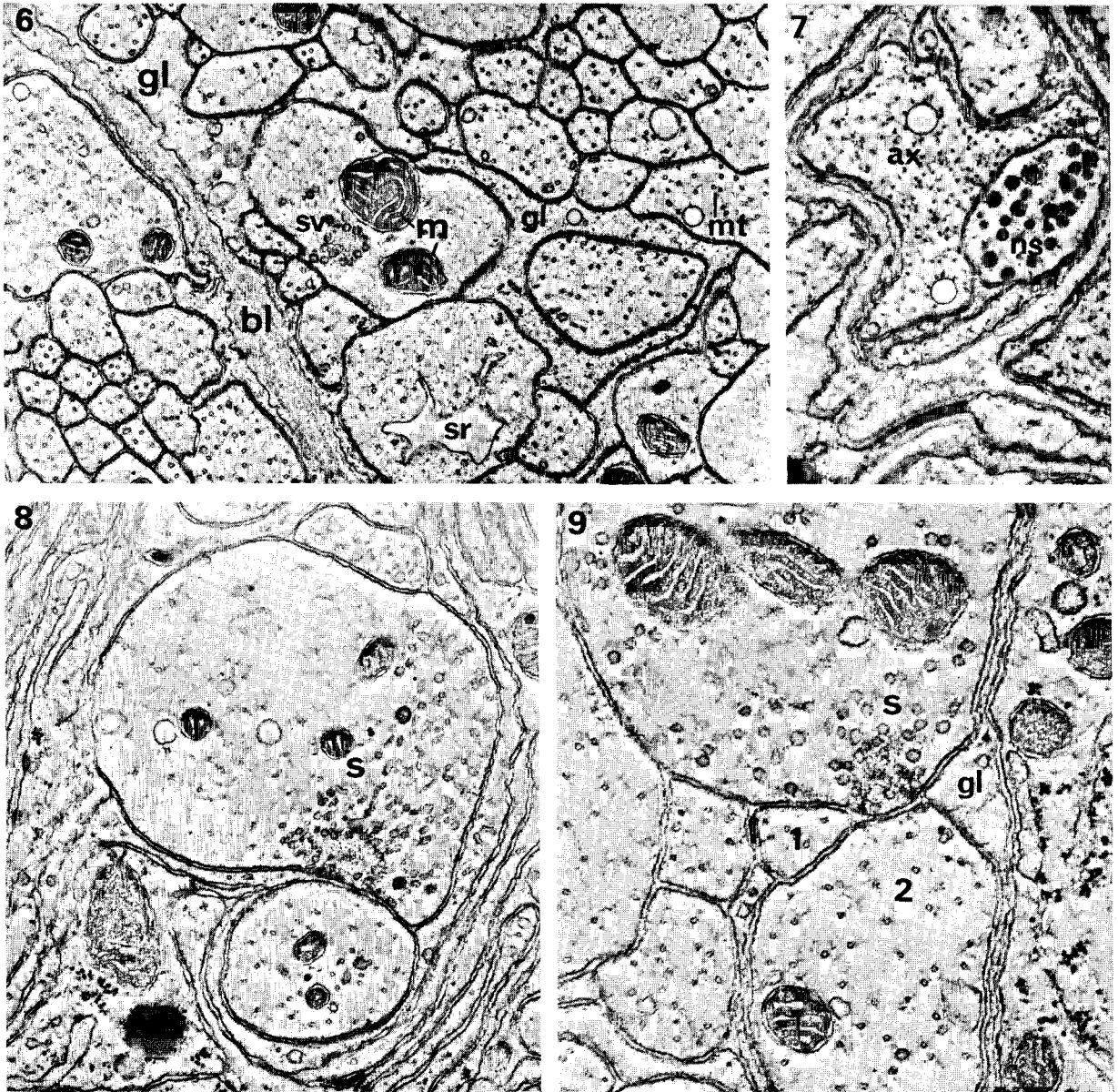


Fig. 6: Part of a cross-section of a tarsal nerve showing the large variation in shape and diameter of fibres. The axons always contain microtubules (*mt*) and often mitochondria (*m*), whereas smooth endoplasmic reticulum (*sr*) and synaptic vesicles (*sv*) are rarely seen. *gl* = glial cell processes; *bl* = basement lamella. x 26,000.

Fig. 7: Irregular profile of an axon (*ax*), filled with many microtubules, and adjacent neurosecretory fibre (*ns*). x 20,000.

Fig. 8: Large axon from the tarsal nerve making a synaptic contact (*s*) onto a smaller fibre. x 30,000.

Fig. 9: A double synapse (*s*) contacting two postsynaptic fibres (*1*, *2*) and a glial process (*gl*). x 45,000.

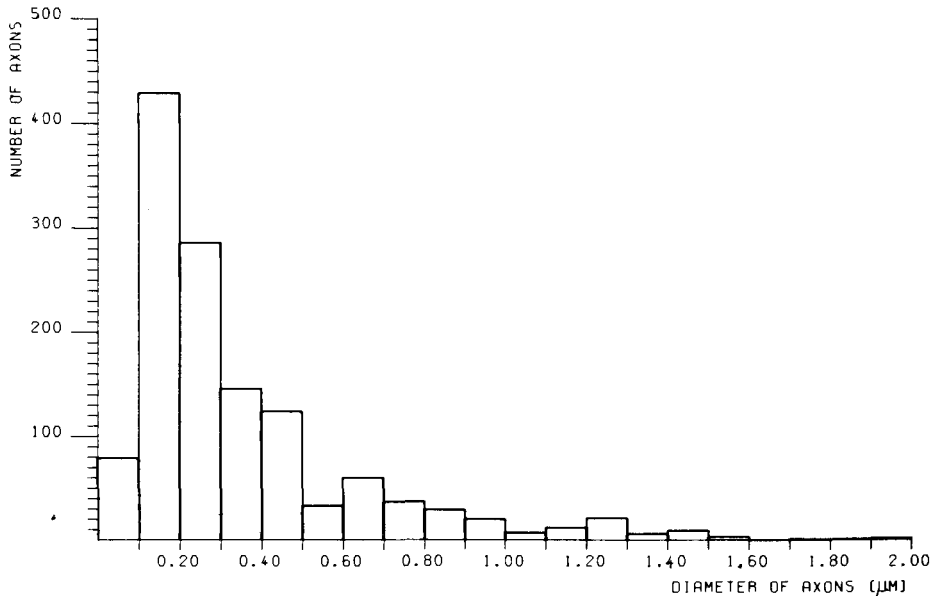


Table 1: Distribution of axon diameters in the tarsal sensory nerves of leg I (*Zygiella x-notata*). Mean diameter: 0.36 μm . Total number of axons: 1306.

the number of their respective sensory cells, i.e. three neurons per tactile hair and 21 per chemosensitive hair. When this extrapolated number is compared with the number of actually counted sensory axons of the contralateral leg, it becomes evident that these two figures are similar yet do not really match. In fact, the number of actually counted sensory fibres is always higher than that predicted from the number of hair sensilla (Table 4).

This discrepancy can be explained at least in part by the fact that each tarsus contains besides the hair sensilla other sense organs (e.g. slit sensilla, tarsal organ) which also contribute axons to the sensory nerves. Other possible explanations will be dealt with in the discussion.

Tarsus	Number of sensory axons
Palp	1289
Leg I	1554
II	1344
III	825
IV	628

Table 2: Number of tarsal sensory axons counted in the appendages of one female *Zygiella x-notata*.

Sensory leg nerves at further proximal levels

The two sensory nerves of the tarsus merge within the metatarsus into one large sensory nerve. In the following proximal leg segments three distinct nerves are found (Fig. 10): (1) the small leg nerve *A* (Parry, 1960; Rathmayer, 1965), (2) the motor nerve *B*, and (3) the large sensory nerve *C*, which contains all the receptor axons. Since each leg segment carries further sense organs, more axons are added to the nerve *C* as it proceeds towards the central nervous system. Figure 11 indicates this gradual increase in the number of sensory fibres within the first leg.

Since it was clear that leg I must contain more than 7,000 sensory fibres (Table 5), it seemed interesting to determine the total number of sensory

Tarsus	Tactile hairs	Chemosensitive hairs
I	87	48
II	83	38
III	75	28
IV	58	16

Table 3: A typical count of tarsal hair sensilla obtained from the different legs of one female *Zygiella x-notata*.

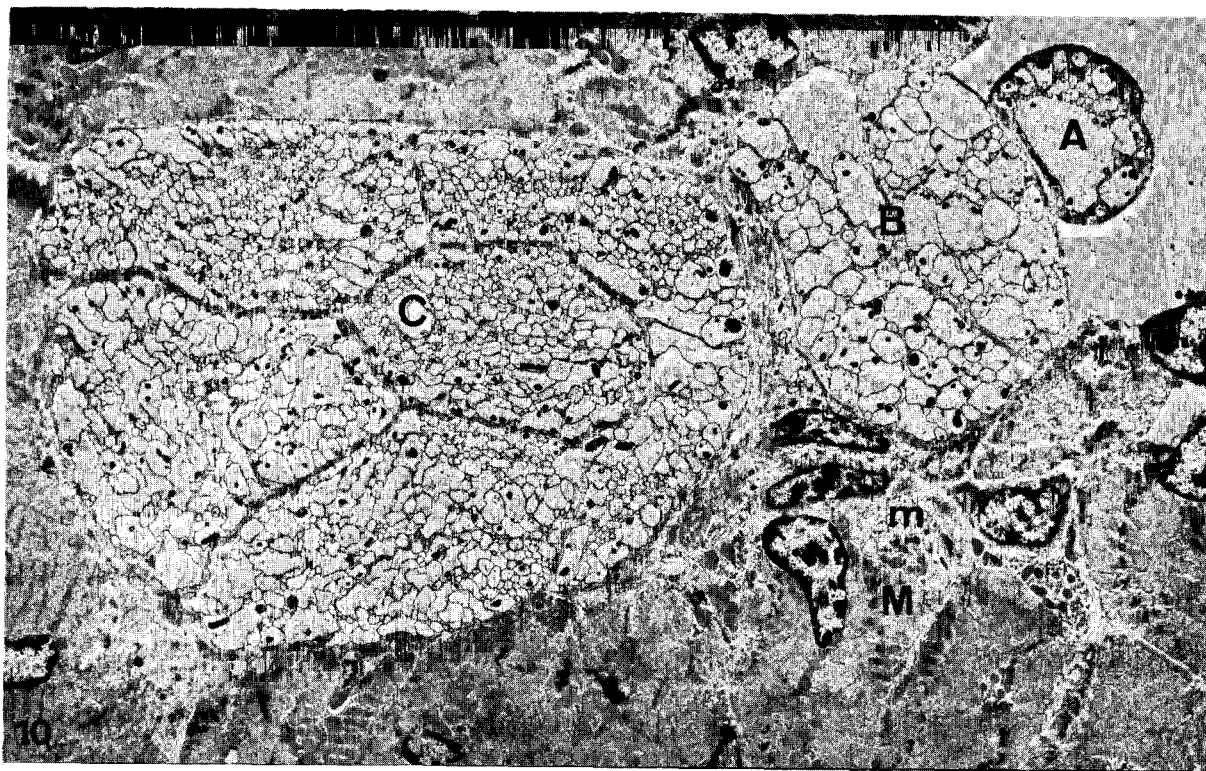


Fig. 10: The three leg nerves in the femur of *Zygiella x-notata* seen in a cross-section. The small nerve *A* contains about 50, mainly sensory, fibres; nerve *B* has about 100 motor fibres of relatively large diameter, and nerve *C* possesses several thousand small sensory axons. Motor endplates (*m*) on muscle cells (*M*) are indicated on the right. x 3,300.

axons for the other appendages. Axon counts taken at the coxal level yielded the figures shown in Table 5.

Surprisingly, the short third leg contains more sensory fibres than the rather long fourth leg. However, this can be explained by the fact that the density of hair sensilla is higher on the third than on the fourth leg (cf. Table 3). From Table 5 it can also be deduced that the total sensory input from the appendages to the CNS must be over 42,000 units.

Discussion

The main result of the present investigation is that the actual number of tarsal sensory axons is larger than that expected from the number of tarsal hair sensilla. This certainly rules out any possibility of axon fusion as was formerly discussed for certain insect antennal nerves (Wigglesworth, 1959). On the

contrary, several hundred "extra" fibres were counted in our cross-sections, which calls for some explanation. Some contribution to the number of sensory fibres is certainly made by other tarsal receptors such as slit sensilla, the tarsal organ and the claw innervation (Foelix, 1970). However, these few receptors could not account for several hundred axons (e.g. it is known from the work of Barth (1969-71) that there are about 10 single slit sensilla/tarsus in *Cupiennius* (i.e. 20 axons), and *Zygiella*, judging from SEM, hardly has more). Possible sources of inaccuracy lie in misidentification of chemosensitive hairs and in variation in their numbers of axons, but these errors are also likely to be relatively small. A further explanation would assume that some of the tarsal nerve fibres are in fact efferent and probably of central origin. This would apply to those fibres which contain synaptic vesicles and/or neurosecretory granules. Such an interpretation was also

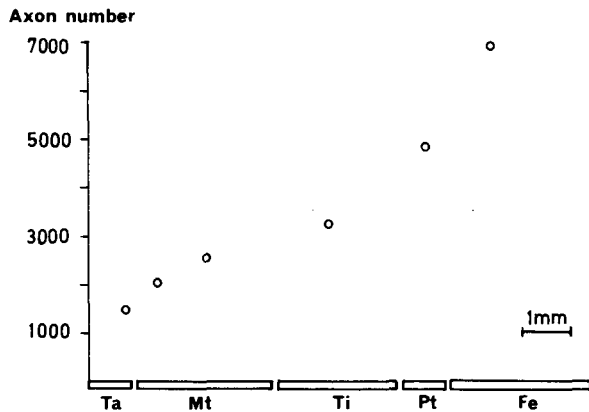


Fig. 11: Increase in the number of sensory fibres within the different segments of leg I in *Zygiella x-notata*.

given for nerve fibres within the joint receptors of spider legs (Foelix & Choms, 1979). These fibres may exert some central control on the receptor organs.

Using the high resolution of the electron microscope, the number of nerve fibres can be determined very accurately. Hence, quantitative data are provided for the sensory system of a spider for the first time: the total sensory input from the appendages is provided by more than 42,000 peripheral nerve cells. This figure applies only to the rather small orb-weaver *Zygiella x-notata* and higher numbers may be expected for larger spiders. Numbers of more than 100,000 have been cited for the combined sensory input from the antennal nerves in large cockroaches (Schafer, 1971; Schaller, 1978); for the horse-shoe crab *Limulus polyphemus* L. several million sensory nerve fibres were estimated (Wyse, 1971). In spiders the main contribution of sensory fibres comes certainly from the front legs, which carry most of the sense organs. This is also reflected in the spider's behaviour, since the front legs play the main role in

probing the environment (Reed & Jones, 1965), a possible prey, a conspecific (mate) or an enemy.

Although one would tend to consider a spider leg as mainly to serve locomotion, the number of sensory fibres compared with motor fibres emphasises its perceptual function: whereas the motor nerve *B* contains only a few hundred motor fibres, the large sensory nerve *C* is formed by several thousand sensory axons (Fig. 10).

The average diameter of the sensory axons is only about $0.4 \mu\text{m}$, so these fibres can hardly provide a high conduction velocity. This seems unnecessary, since most sense organs lie relatively close to the central nervous system. A giant fibre system as found for instance in the long antenniform legs of whip spiders (Amblypygi; Foelix, 1975) is not present in any araneid legs that have been examined. However, synaptic interactions take place in the peripheral nervous system, as testified by the presence of synaptic contacts within the tarsal nerves. Whether these synapses are mainly of an afferent/afferent or of an efferent/afferent nature, cannot be determined at present.

Acknowledgements

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		Expected number of axons	Counted number of axons
Tarsus	I	1269	1554
	II	1065	1344
	III	813	825
	IV	510	628

			Number of sensory axons
Palp			2200
	Leg	I	>7000*
II		4407	
III		4173	
IV		3861	

Table 5: Total number of sensory fibres counted at the coxal level of the different appendages of one female *Zygiella x-notata*.

*Leg I was counted at the femur level.

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favourable cross-sections of a few coxae were considered. One first leg was severed at each segment (about mid-region) and corresponding cross-sections were used for determining the increase of sensory fibres from distal to proximal levels.

Results

Tarsal nerves

a) Distribution and diameter of fibres

The tarsal sensory hairs are of two types, either

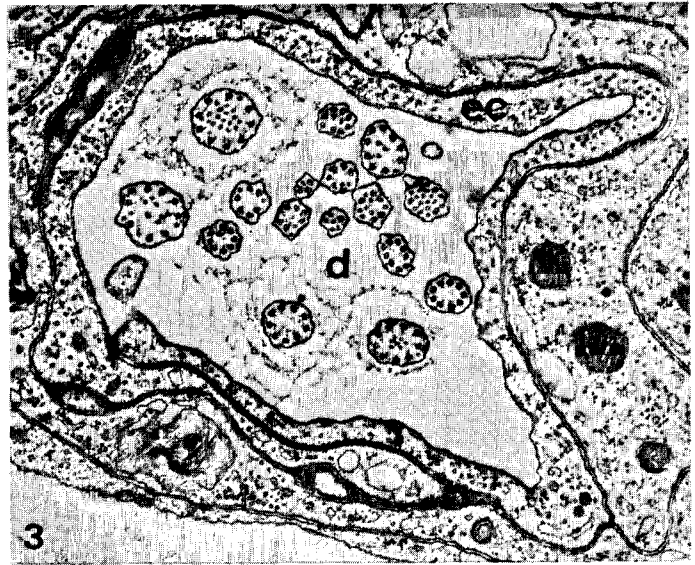
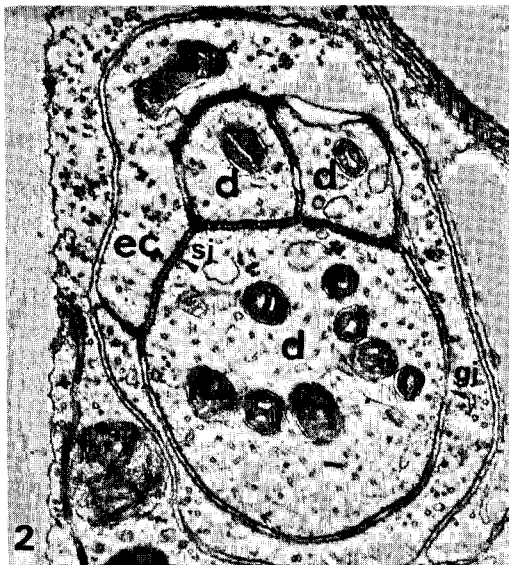
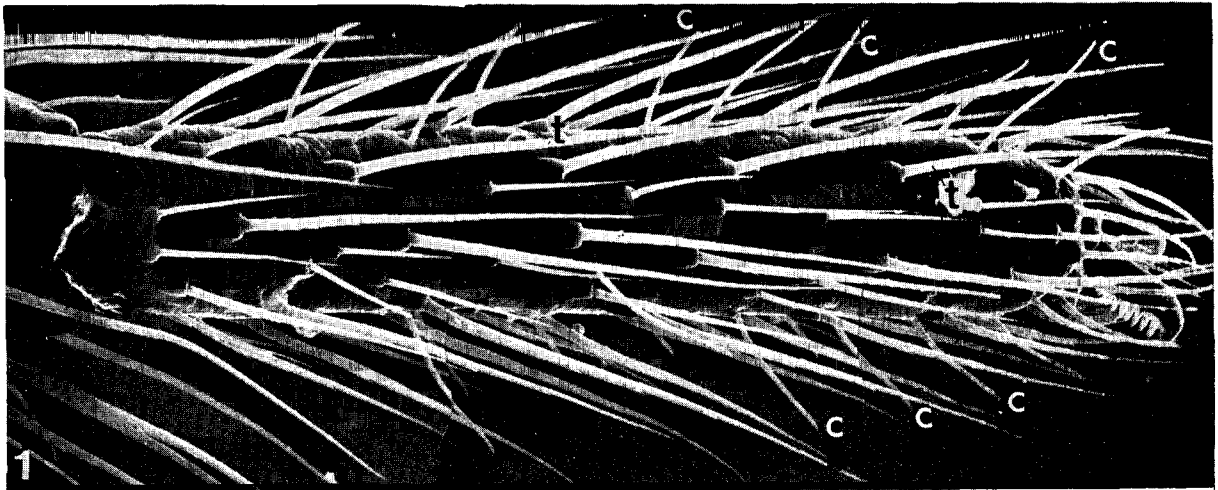


Fig. 1: Dorso-lateral view of tarsus I of *Zygiella x-notata*. The long tactile hairs (*t*) are clearly distinct from the shorter, blunt-tipped chemosensitive hairs (*c*). x 400.

Fig. 2: Cross-section of three dendrites (*d*) belonging to one tactile hair. Dendrites and surrounding enveloping cell (*ec*) exhibit membrane specialisations such as septate (*sj*) and gap junctions (*gj*). x 39,000.

Fig. 3: Cross-section of 16 dendrites (*d*) innervating one chemosensitive hair. The section passes through the narrow ciliary region of the dendrites. *ec* = enveloping cell. x 67,500.