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Sensory Innervation of the Tentacles of the Polychaete, *Sabella pavonina*

R. M. Santer and M. S. Laverack
Gatty Marine Laboratory and Department of Natural History,
University of St. Andrews, Fife, Scotland

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Summary. Following observation of conical groups of stiff, but motile cilia on the tentacles of the branchial crown of *Sabella pavonina*, these were examined with the electron microscope. The bundles consist of about 40 unenclosed "standard" cilia supported by one or two primary sense cells with centrally directed axons of 0.1–0.2 μ diameter. Axons in the distal portions of the branchial crown occur in small bundles surrounded by a basement membrane. More centrally, glial elements appear and the nerves are surrounded by a collagenous sheath. The branchial nerve trunk shows similarities in organisation to other previously investigated annelid central nervous tissue in that the whole nerve is surrounded by a fibrous sheath central to which there is a layer of glial cells with processes penetrating a central neuropile. The 0.1–0.2 μ axons commonly occur in glial-enveloped groups of < 40 whilst other axons of larger and mixed diameter are found together.

Each tentacle has two branchial nerves on the oral side, and each nerve gives rise to two small 75-axon branches running to each pinnule. The branchial nerves fuse to form the branchial nerve trunk running to the supra-oesophageal ganglia.

Sections of the branchial nerves of the branchial crown at progressively more central levels show that the branchial nerve trunk contains enough axons of 0.1–0.2 μ diameter to account for all the sensory cells on the tentacles. This is taken as evidence for the sensory cells having axons terminating within the central nervous system and that there is no peripheral confluence or fusion of these afferent axons.

Key-Words: Tentacles — Polychaetes — Sensory cells — Sensory innervation — Cilia — Fine structure.

Introduction

The crown of tentacles of *Sabella pavonina* is well known because of the part it plays in respiration (Fox, 1938) and feeding (Nicol, 1930). The gross morphology and some aspects of histology were outlined by Nicol in 1930 and this paper is the main source of information about the system. It is a matter of common observation that withdrawal of the crown occurs when an appropriate stimulus is applied. This may be rapid or, in some cases, rather slower (Krasne, 1965). Although the rapid withdrawal response, mediated via the giant fibre system, has been described on a number of occasions, little or nothing is known of the pathways by which this in stimulated; that is to say the nature of the sensory receptors that are brought into play. In some cases the appropriate input may be from proprioceptors located at the base of the tentacles, but it seems equally possible that mechanoreceptors located on the tentacles themselves may be active. Equally possible is the likelihood that chemoreceptors, involved in feeding responses, are present on the tentacles. None have so far been described, in tubicolous polychaetes, although there is some information for errant worms (e.g. Dorsett and Hyde, 1969).

Material and Methods

Specimens of *Sabella pavonina* were obtained from the Scottish Marine Biological Association Laboratory at Millport, Isle of Cumbrae. They were maintained within the laboratory aquarium in constantly circulating aerated sea water and were not fed. The animals maintained themselves in good condition feeding upon the content of the circulating sea water. The smaller specimens supplied were those used for histological purposes.

Four methods of fixation were utilised in an attempt to obtain satisfactory fixation. The required pieces of the branchial crown were removed from the live animals and immediately placed in the fixative. The alternatives were as follows:

1. 1% osmium tetroxide in filtered sea water for 1 hour at 4° C.
2. 1% O_8O_4 in veronal acetate buffer at pH 7.3 (Palade, 1952) for 1 hour at 4° C.
3. Prefixation in phosphate buffered 6% glutaraldehyde for 3 hours followed by fixation in 1% O_8O_4 in phosphate buffer for 1 hour at 4° C.
4. Triple fixatives (Imaizumi and Hama, 1969) 1% O_8O_4 in phosphate buffer for 1 hour at 4° C. 6% glutaraldehyde in aqua dist. for 1 hour. 1% O_8O_4 in aqua dist. for 30 mins. 2% uranyl acetate in aqua dist. for 30 min.

All material was dehydrated in a graded acetone series. The material was then embedded in Epon using a vacuum embedding technique. Thin sections were cut on an LKB ultramicrotome, mounted on unfilmed copper grids and examined on an AEI EM6B electron microscope at 60 kV.

Material fixed by the first three methods was stained with 2% uranyl acetate followed by lead citrate (Reynolds, 1963) whilst triple fixed material was block stained prior to dehydrating (Imaizumi and Hama, 1969).

Observations

The Branchial Crown

Each half of the crown consists of about 20 branchial filaments which arise from a common base but soon separate throughout their length. Each filament is approximately 500 microns in width and carries about 400 pinnules. These pinnules are arranged alternately along either side of the oral surface of the filament and are about 1.5 mm in length. They are found along the whole length of the filament except at the tip, where they are shorter. The anterior 1 mm of the filament has no projections.

At the tip of each filament and on each pinnule there are cells bearing stiff cilia in cone-shaped bundles. Direct observation using Nomarski interference optics showed them to be quite distinct from the cilia whose beat provides the feeding currents. Whilst the feeding cilia on the filaments and pinnules beat continuously the stiff cilia remain immobile for long periods. They were, however, occasionally observed to flick, all the cilia of a bundle moving in unison. Careful observation showed there to be two such bundles of stiff cilia at the tip of each pinnule and about 20 on the tip of each filament. Of these 20 about 10 are clustered around the actual tip whilst the other 10 bundles are situated slightly further back from the tip, more often on the oral surface. Stiff cilia were not seen on any other parts of the branchial crown.

Electron microscopical examination of sections taken through the filament and pinnule tips showed that the cells bearing the stiff cilia were distinct from the other cells in the same tissue and appeared to be primary sensory cells. These cells are about 1.5–3 microns in diameter at the distal end and taper into an axon-like process with an average diameter of 0.1–0.2 microns at the proximal end (Fig. 1). In some cases a bundle of stiff cilia arose from two adjacent cells but all cells,

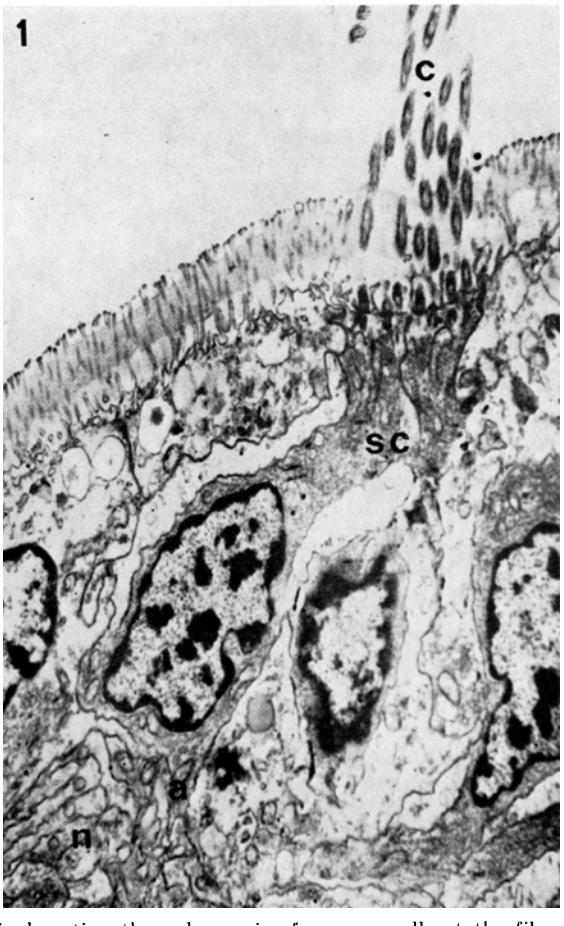

Fig. 1. A longitudinal section through a pair of sensory cells at the filament tip. A single bundle of cilia (*c*) arises from the two cells (*sc*) distally whilst, proximally, an axon process (*a*) of 0.2 μ diameter runs towards a collection of similar nerve fibres (*n*). It is in this region that synapses were occasionally observed. Triple fixation. × 10000

whether supporting cilia or not, have desmosomal regions at the distal surface. Interposed supporting cells are unspecialised.

The cytoplasm of the sensory cells is more electron dense than that of surrounding epithelial cells. These cells also possess more intracellular organelles. Many hollow vesicles 1000 Å in diameter, small round mitochondria and small granules occur in the sensory cells, but epithelial cells contain more vesicles. Multivesicular bodies about 0.3 microns in diameter and containing many loosely packed pale cored vesicles are found in the sensory cells (Fig. 2). The cuticle covering the sensory cells does not differ in thickness from that overlying the rest of the epithelium but fewer microvilli penetrate to the exterior.

Each sensory cell carries about 40 cilia interspersed among the microvilli of the distal surface. At the level of the cuticle the cilia are normal to the cell surface

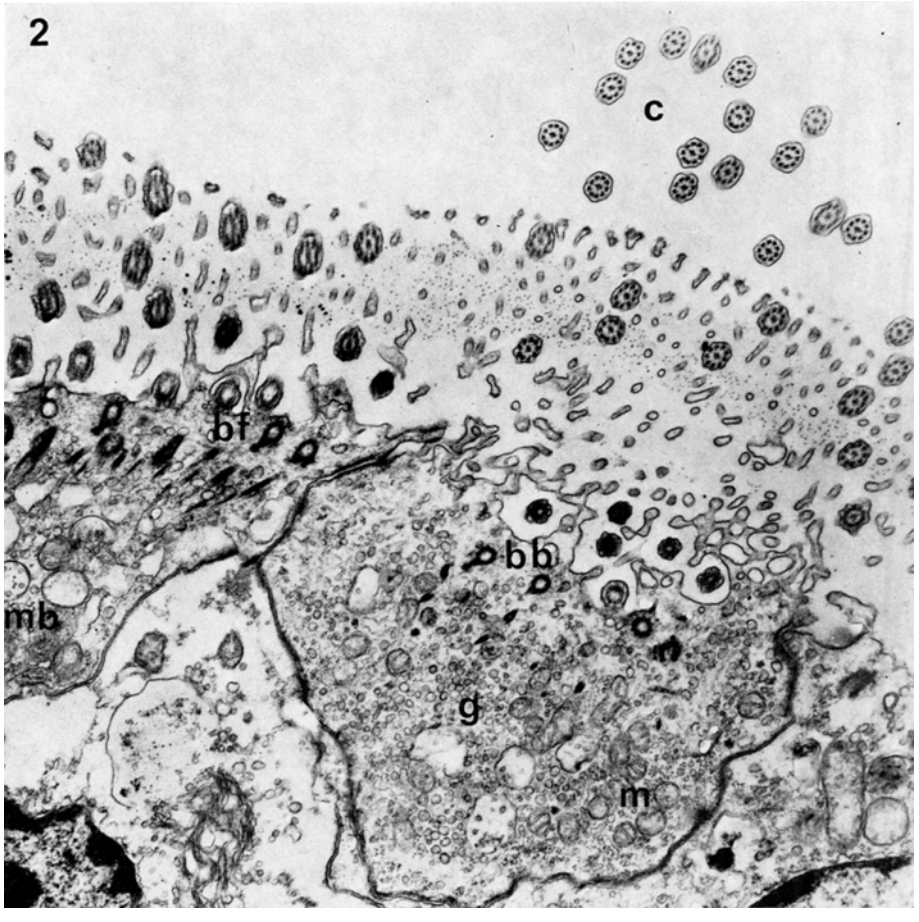

Fig. 2. Oblique section through two adjacent sensory cells. Their cytoplasm is darker than the surrounding epithelial cells and is filled with multivesiculate bodies (*mb*), small round mitochondria (*m*) and small granules (*g*). Cilia (*c*), basal bodies (*bb*), and basal feet (*bf*) are also evident. Triple fixation. × 15000

but they tend to become more closely apposed distally to form the characteristic cone-shaped bundle. At no point is the ciliary bundle surrounded by a limiting membrane. Each cilium is about 0.2 microns in diameter and approximately 30 microns in length. The arrangement of ciliary fibrils is typically $9+2$ and within a bundle all the central pairs of fibrils are orientated in the same plane. This might be expected from a consideration that all cilia within a bundle beat in unison in the same direction. The basal bodies are simple and have a wedge-shaped end. From each basal body arises a ciliary root that is at an angle to the cilium itself and also a small basal foot. The root shows a stripe repeat of 500 Å similar to those described in other ciliary systems.

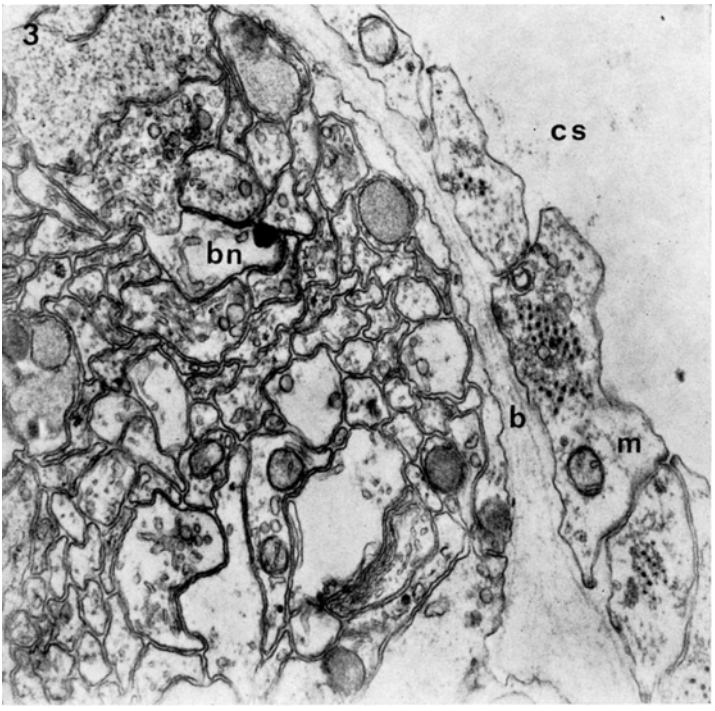

Fig. 3. Section through a branchial nerve (*bn*) which is separated by a basement membrane (*b*) from the single layer of muscle cells (*m*) which lines the coelomic space (*cs*). Triple fixation. × 30000

The proximal end of the sensory cells tapers into a thin axon-like process which runs towards a bundle of nerve fibres with which it becomes associated but does not form any synaptic contact.

The question of synaptic contact is important in this respect, since vesicles occur within the processes of sensory cells though they are never numerous. There are no other physical features of synaptic contact such as membrane specialisations or presynaptic bars to be found. Structures interpreted as synapses have, however, been seen amongst the nerves of the filament and also at the filament tips. In these cases groups of vesicles of 440 Å diameter are closely packed together and the membrane with which they arre associated is usually straight and shows slight thickening. The synaptic cleft between adjacent units is a constant 75 Å in width as opposed to the more variable width of other interaxonal spaces. Occasional large hollow vesicles may occur in the postsynaptic axon but these are few in number. All tentatively identified synapses that have been observed were axo-axonic. No synapses were seen in the pinnules or at the junction of the pinnule axons with the branchial nerves.

The Nervous Anatomy of the Branchial Crown

The branchial nerve trunk gives off two branches (branchial nerves) to each filament along which they run on the oral side (Nicol, 1930; Thomas, 1940). Earlier

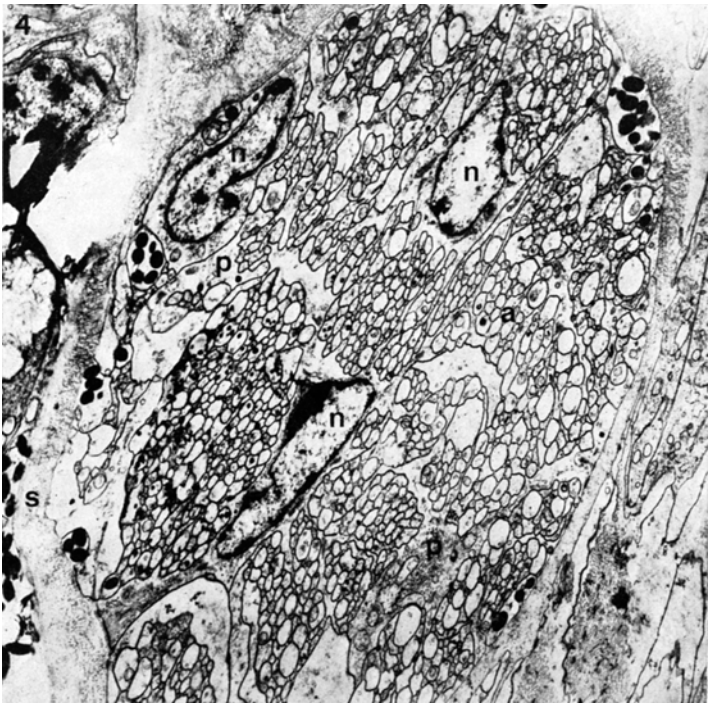

Fig. 4. Transverse section through a branchial nerve mid-way down the filament. A collagenous sheath (*s*) surrounds the nerve, which at this level contains about 850 axons. Glial nuclei (*n*) and glial processes (*p*) are present, delimiting the axons (*a*) into bundles. Veronal buffered O_sO_4. $\times 10000$

authors note the presence of a single layer of muscle cells in both pinnule and filament (Fig. 3) but describe no innervation.

At the tip of each branchial filament the axons of the 20 sensory cells converge into 4 or 5 bundles each containing 12 to 14 axons. At a level just proximal to the end of the pinnule region each branchial nerve is composed of about 150 axons. Midway along the branchial nerve there are approximately 850 axons (Fig. 4). At the base of the filament where there are still two distinct branchial nerves each branchial nerve contains about 1000 axons. The branchial nerve trunk itself contains 40000 axons and there are no branches occurring before the nerve enters the supraoesophageal ganglia.

Two nerves run the length of each pinnule, on the oral side. At the base each pinnule nerve contains about 75 axons. The axons of the two pinnule sensory cells run into small 15 axon bundles at the tip, which are joined by two other bundles to form the pinnule nerves (see Fig. 5).

At two sites in the branchial crown, namely at the filament and pinnule tips, there are structures formed by coalescence of small nerves into the larger branchial and pinnule nerve which resemble primitive neuropiles. At these points section reveals axons cut in transverse section and others in oblique and longitudinal

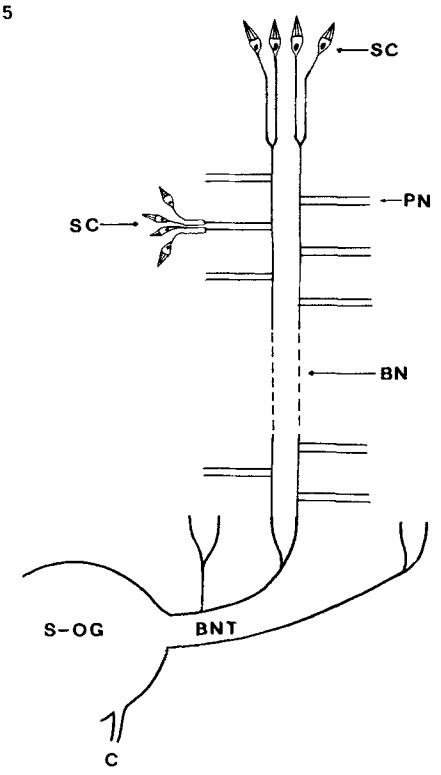

Fig. 5. Diagram of the branchial nervous system of *Sabella* showing the innervation of one filament in detail. Sensory cells (*sc*) exist peripherally and their axons form the pinnule nerve (*pn*). The branchial nerve (*bn*) and branchial nerve trunk (*bnt*) are collections of pinnule nerves together with a number of motor axons. Supra-oesophageal ganglion (*s–og*)

section. These latter elements presumably originate at the periphery and the site represents one of two places where synaptic structures may be discovered. No neuron cell bodies are evident in these areas nor is there any trace of glia which is present in other parts of the branchial nervous system.

Distribution of Axon Diameters

Sections of the branchial nerve were taken at three levels to determine the number and distribution of axon diameters (Fig. 6). Of the axons of the branchial nerve at the filament tip 85% are between 0.1 and 0.2 microns in diameter and 15% between 0.3 and 0.8 microns in diameter. Midway down the filament 71% of the axons were between 0.1 and 0.2 μ, 29% between 0.3 and 0.8 μ. At the base of the filament 74% are of 0.1 to 0.2 μ and 26% between 0.3 and 0.7 μ. The proportion of 0.1 to 0.2 μ diameter axons remains almost constant throughout the length of the branchial nerve whilst the absolute number increases progressively along the nerve.

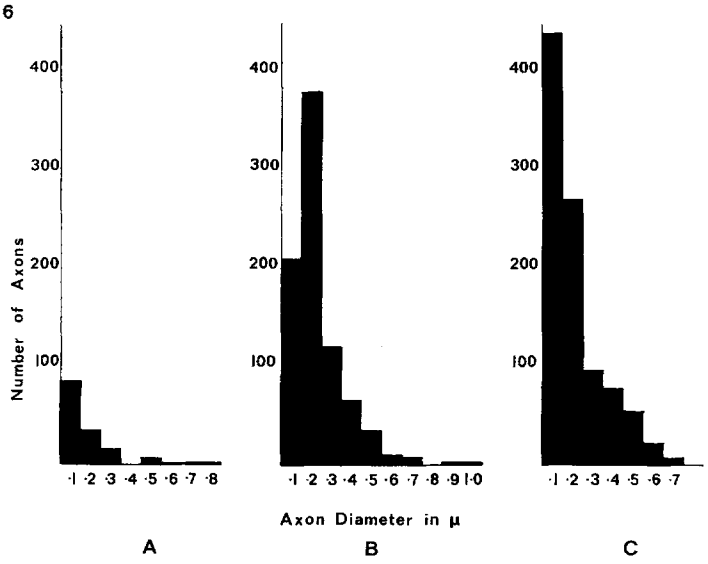

Fig. 6 A–C. Histogram to show the distribution of axon diameters at three different levels in the branchial nerve. The proportion of 0.1–0.2 μ diameter axons remains constant at all levels but the actual numbers increase progressively at more central regions. A) distal end, B) mid-way down filament, C) base of filament

Fine Structure of Peripheral Nerves

The small bundles of axons at the distal end of the branchial nerves are bounded not by a sheath but by a limiting unit membrane closely apposed to the membranes of the unsheathed axons and contains mitochondria and hollow vesicles. The axoplasm is granular, due possibly to deformed nerve filaments and neurotubules 300–1 000 Å diameter. This arrangement is identical to the distal ends of the pinnule nerves. At this level neither shows any glial elements. At the pinnule base there are a few glial elements within the nerve bundle but otherwise there is no difference apart from the number of axons from the distal end.

Midway down the filament the branchial nerves show considerable advances in complexity (see Fig. 4). The nerve is bounded by a collagenous sheath 500 μ thick, but most noticeable is the appearance of glia. Glial nuclei are common and projections of glial cells demarcate the nerve into bundles of axons each bundle consisting of approximately 100 axons of varying diameter. The axons within the bundle are not individually sheathed and lie in close approximation to one another. At the base of the filament the collagen sheath increases in thickness but the only other obvious changes are in the increase in axon number.

Fine Structure of the Branchial Nerve Trunk

The branchial nerve trunk is about 0.2 mm in diameter and is formed as a result of the coalescence of the branchial nerve and runs from that point to the base to the dorsal lateral aspect of the supraoesophageal ganglia.

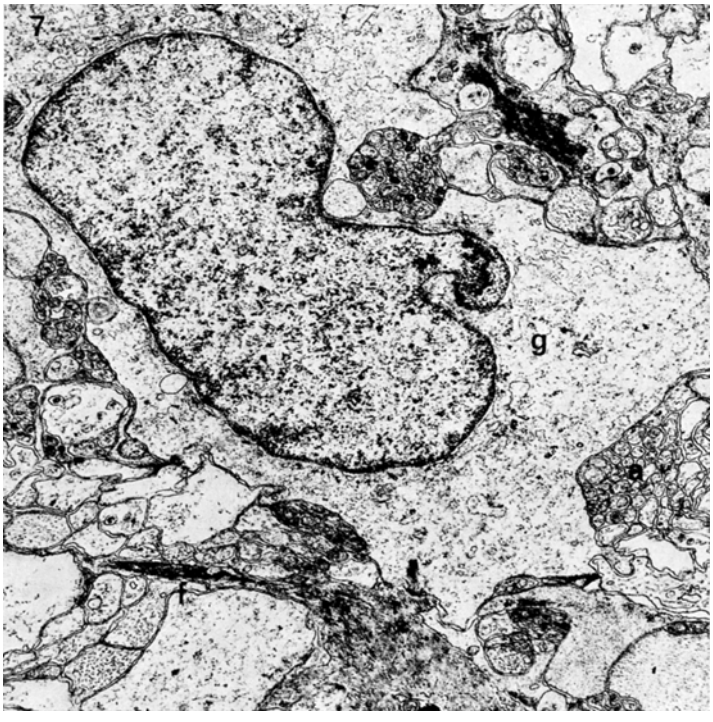

Fig. 7. Peripheral region of the branchial nerve trunk showing a large glial soma (*g*). Note bundles of small axons (*a*) of 0.1–0.2 μ diameter and intracellular fibrillar material (*f*). Veronal buffered O_8O_4. × 20000

The nerve trunk is surrounded by a collagenous sheath of variable thickness and smooth paramyosin-like muscle is attached at certain points on the sheath. Glial elements are numerous with cell bodies situated at the circumference of the nerve but not at all in the centre. Within the branchial nerve trunk approximately 60% of the axons have a diameter between 0.1 and 0.2 μ. They are grouped in aggregations of 10–40 and occasionally together with axons of other diameters (Fig. 7). The large axons between 0.3 and 0.8 μ diameter contain hollow or pale cored vesicles of varying diameters and also neurofibrillar material. Yet other large axons are somewhat irregular in profile outlines and contain 700 Å vesicles and dense cored vesicles between 600 and 800 Å in diameter. Such dense cored vesicles were occasionally seen in small axons but most often in the larger ones.

The peripherally situated glial cell bodies are somewhat irregular in outline and internally the cytoplasm is dark in electron micrographs due to the presence of many fine granules and hollow vesicles (see Fig. 7). They do not appear to contain extensive endoplasmic reticulum and golgi complexes. Mitochondria were not evident in glial cell bodies but were numerous in glial processes (Fig. 8).

Throughout the branchial nerve trunk but mostly at the circumference there are many electron-dense elongated structures situated within cellular elements. The dense nature of these is similar to that described in the ventral nerve cord of the

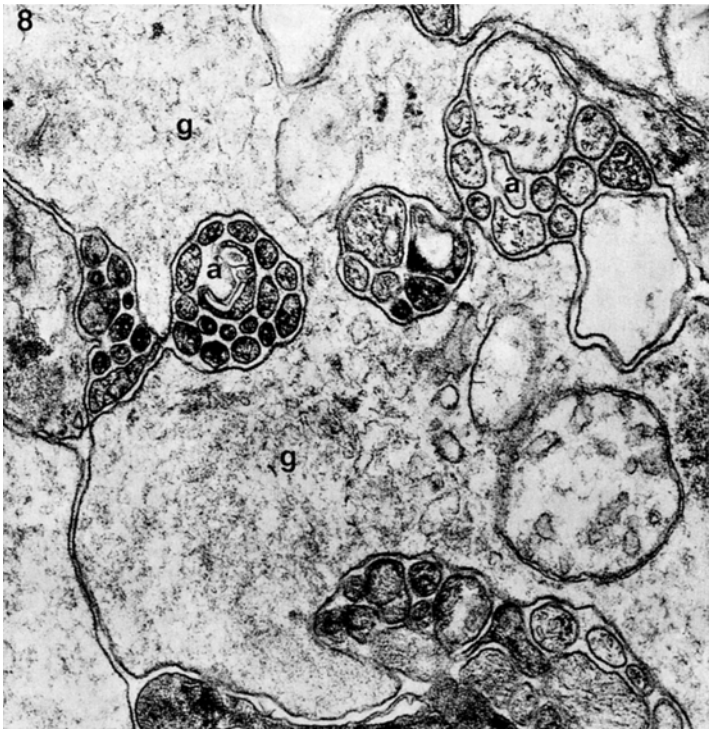

Fig. 8. Central region of the branchial nerve trunk showing large glial processes (*g*) surrounding bundles of 0.1–0.2 μ diameter axons (*a*). Veronal buffered O_sO_4. × 40000

leech (Gray and Guillery, 1963). They are believed to be the processes of the fibrous neurilemma found limiting the branchial nerve trunk. This type of organisation is essentially the same as that described in the earthworm ventral cord (Coggeshall, 1965) although some features of that system seem to be absent.

Discussion

The cilia of the sense cells on the branchial crown are stiff but not immotile; they can be seen to flick, all the cilia of a bundle moving in unison. It is interesting to note that they often flick spontaneously as well as a result of being struck by water-borne particles. Preliminary physiological investigations suggest that these sense cells are mechanoreceptive, monitoring stimuli of a vibrational and tactile nature. This is the case in stiff cilia found in other invertebrates such as Chaetognaths (Horridge, 1966), Ctenophores (Horridge, 1966) and Echinoderms (Cobb, 1968). There is no reliable information available regarding a chemoreceptive role for these invertebrate stiff cilia.

One of the major problems that has faced physiologists working on invertebrates within the last few years has been the question of resolving the number of axons involved in any particular system. Nowhere has this been more evident than in the annelids where there has been some disagreement and reassessment

regarding the total number of fibres contained within any particular nerve. Earlier workers who relied upon classical histological methods, such as Smallwood (1926) and Smith (1957), were misled in that such methods did not enable the resolution of the finest axons. The use of the electron microscope by such authors as Horridge (1963), Mill and Knapp (1970), Lawry (1967), indicates that the total number of fibres is much greater than was previously suspected. Horridge believed that in *Harmothoë* the number of axons contained within the segmental nerve approximated quite closely to the number of peripheral sensory cells. He therefore concluded that these primary sense cells have their terminations within the central nervous system. However Lawry (1967) found evidence to suggest a certain degree of peripheral confluence of sensory axons in the ventral parapodial cirrus of *Harmothoë*. He suggests that some fibres of less than 1 μ in the centre of the neuronal core of the cirrus might well be continuous with the fibres greater than 1 μ at the base of the cirrus and that these fibres might well have afferent endings upon them. Lawry also found synapses at the periphery. Parapodial ganglia (e.g. in *Aphrodite*) suggest peripheral integration in some polychaetes.

In the branchial crown of *Sabella* there are enough sensory axons of 0.1–0.2 μ diameter to account for the estimated total number of peripheral sense cells. The constancy of proportion of axons of that diameter throughout the length of the branchial nerve is interpreted as evidence that the peripheral sensory cells have axons that terminate within the central nervous system. As expected, the actual numbers of 0.1–0.2 μ axons increase due to recruitment towards the base of the filament where it reaches its maximum for the branchial nerve. The length (over 2 cm) over which some of these axons have to run and their very small diameters makes them impossible to trace individually.

The existence of peripheral synapses in the branchial crown of *Sabella* would suggest a certain degree of peripheral interaction of axons but since it is not possible to identify the origins of the pre- and post-synaptic elements, no definite conclusions can be drawn as to the significance of these synapses. The synapses could correspond to the axons of bipolar sensory cells very occasionally seen in *Harmothoë* to run together as if in direct anastomosis and described as a "rare anomaly" by Horridge (1963). Few records of synaptic structures have been obtained in *Sabella*. Peripheral synapses imply peripheral integration to a certain degree and it is possible that if these synapses are of the "en passant" axo-axonal type between afferent sensory axons, excitation of one sensory cell could be manifested in two or more (depending on the number of synapses) axons at a more central level. Hence there could take place a multiplication of the input to the CNS. However it is by no means certain that the observed synapses are between sensory axons. The other axons within the branchial nerves are probably motor to the muscle cells of the pinnule and filaments or could possibly be from free nerve endings in epidermis. Neither these nor neuromuscular junctions were observed. Functionally, however, the small size of these axons may be a question of massed synchronous firing of whole populations.

The fine structural organisation of the branchial nervous system shows similarities with other annelid nervous systems that have been investigated with the electron microscope. The organisation of the branchial nerve trunk follows the same pattern as the ventral cords of the earthworm (Coggeshall, 1965) and the

leech (Gray and Guillery, 1963) in that the whole nerve is surrounded by a fibrous sheath underneath which there is a layer of glial cells with processes penetrating a central neuropile.

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R. M. Santer
Gatty Marine Laboratory and
Department of Natural History
University of St. Andrews
Fife, Scotland