Cell Tissue Res (1985) 242:685–688

Cell and Tissue Research

The motor innervation of the oral plate ligament in the brittlestar *Ophiura ophiura* (L.)

James L.S. Cobb
Gatty Marine Laboratory, St. Andrews, Fife, Scotland

Summary. The innervation of the connective tissue ligaments between the oral plates on the arm of a brittlestar by small branches of the hyponeural motor system is described. These branches arise from the hyponeural part of each segmental ganglion and pass laterally round the nerve cord and then orally across the epineural sinus to penetrate a small intersegmental node of juxtaligamental tissue located centrally. The endings of the nerve branches contain numerous small agranular vesicles and make chemical synapses onto the juxtaligamental cells. Processes from the juxtaligamental cells containing large granular vesicles ramify amongst the connective tissue of the oral ligaments. This innervation is associated with rapid changes in the stiffness of the connective tissue.

Key words: Connective tissue – Innervation – Synapse – Echinoderm – Ophiuroidea

There is growing evidence that collagenous connective tissue in echinoderms can rapidly change its viscoelastic properties in response to nervous stimulation, and that this phenomenon is widespread. Takahashi (1967) first proposed connective tissue changes in the catch apparatus in sea urchin spines, and Hidaka and Takahashi (1983) and Hidaka (1983) have recently reviewed progress on this preparation and compared it to similar phenomena described in other echinoderm tissues. Wilkie (1978a, b, 1979) provided evidence that these connective-tissue viscoelastic changes are nervously mediated in the intervertebral ligaments of brittlestars via a group of cells he termed 'juxtaligamental'. The present paper describes the motor innervation of the connective tissue of the oral plate ligaments in the brittlestar *Ophiura ophiura* by small branches from the segmental ganglia of the hyponeural nervous system.

Materials and methods

Tissue for initial light- and transmission electron microscopy was fixed in 1% phosphate-buffered osmium tetroxide solution for 1 h. The tissue was rinsed in distilled water and subsequently decalcified in a 1:1 mixture of 2% ascorbic acid and 0.36 M sodium chloride for 24 h (Dietrich and Fontaine 1975). The decalcified tissue was subsequently dehydrated in acetone and finally embedded in Araldite resin. Light-microscope sections were stained with toluidine blue prior to examination. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 E.M. This decalcification produces poor fixation but was sufficient to identify juxtaligamental tissue by serial section. Subsequent to this work the identified juxtaligamental node of tissue was fixed in situ for 15 min in a fixative produced by mixing immediately prior to use 2% glutaraldehyde in phosphate buffer at a pH of 7.2 with 2% aqueous osmium tetroxide in equal volumes. The tissue was dissected free, avoiding contamination with fragments of calcite ossicles using watchmakers forceps, and processed for ultrathin examination. This technique used without decalcification provided a much enhanced quality of cytoplasmic detail.

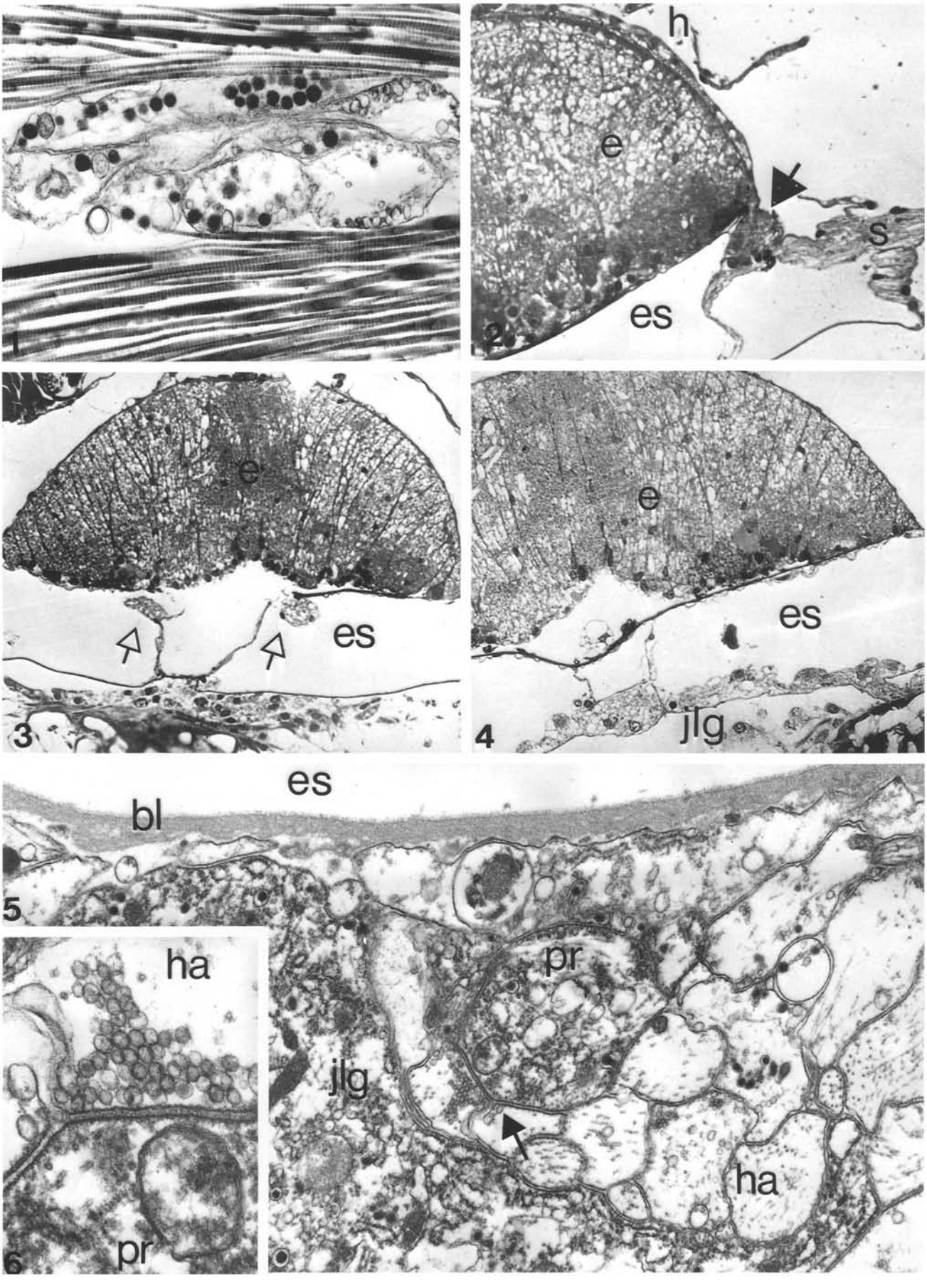
Send offprint requests to: Dr. J.L.S. Cobb, Gatty Marine Laboratory, St. Andrews, Fife, Scotland

Results

1. Oral plate ligaments

The oral plates are triangular calcite structures which are attached by ligaments to the lateral arm plates. Tissue can be excised both for morphological and physiological examination by removing most of the lateral and aboral plates, as well as the vertebral ossicles, ligaments and muscles, thus leaving the radial nerve cord attached to the oral plates and part of the lateral plates. The connective tissue between the oral plates is composed of massive bundles of collagen fibrils with small numbers of cellular processes between them. This cellular material consists of interstitial cells presumably associated with the secretion of the collagen, and juxtaligamental cell processes (Fig. 1) (see Wilkie 1984) which contain large granular vesicles. These granular vesicles containing profiles can be traced back to a plaque of cell bodies in a central position on the oral floor of the epineural sinus (Figs. 2–4). This plaque is innervated by two small bundles of hyponeural motor axons. These axons can be shown to arise from the hyponeural tissue which lies in ganglionic nodes on the aboral side of the nerve cord in each arm segment. The branches pass laterally and orally around both sides of the nerve cord and through the epineural sinus to penetrate the central juxtaligamental plaques. Other small branches from the ganglia also pass to ligaments between the lateral plates but these were not studied in detail.

The endings of the hyponeural nerves within the juxtali-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gamental tissue contain large numbers of small agranular vesicles 35–50 nm in diameter. In places they form a chemical synapse (Figs. 5, 6) against juxtaligamental cells or processes from them. The chemical synapse is characterized by a regular cleft containing densely staining amorphous material between the pre- and postsynaptic elements. There is some cytoplasmic material present as a fuzz associated with both cell membranes at the synapse but not substantially more than is found in some non-synaptic regions.

Discussion

Interest in echinoderm connective tissue was generated by Takahashi (1967) who drew attention to the interesting properties of the catch apparatus in sea urchin spines. Wilkie (1984) has recently reviewed the work done by others showing connective tissue structural changes to be involved in crinoid ligaments, holothurian and asteroid body wall and tubefeet in echinoids. Wilkie (1978a, b, 1979) in a series of papers described the connective tissue between the vertebral ossicles in the arm of a brittlestar and showed that it could rapidly change its properties. He made it clear that this change is so rapid that it must be nervously mediated. The evidence of Wilkie and, more recently, Hidaka and Takahashi (1983) indicates that the changes are brought about by the collagen fibrils sliding past each other rather than any change in the collagen fibrils themselves. All these authors stress the importance of Ca^{2+} ions in altering the links between the collagen fibres formed by the extracellular protein/carbohydrate matrix in which they are embedded.

Motokawa (1981, 1982a, b) in a series of papers on holothurian body wall has identified two different factors in the coelomic fluid which either stiffen or relax echinoderm connective tissue. It is not certain, however, whether these factors affect the connective tissue directly or whether the nervous system is involved. The relationship between these substances and the putative transmitter substance in the juxtaligamental cells is also unresolved. The present study shows a plaque of juxtaligamental tissue to be innervated by small branches from the segmental ganglionic hyponeural nervous system. Recently Stubbs and Cobb (1981) showed that hyponeural nerves apparently also provided motor innervation to connective tissue in the juxtaligamental nodes associated with the intervertebral ligaments. The fixation of this tissue was of poor quality, mainly because decalcification was necessary, and the situation is confused because the hyponeural nerves in this situation mainly innervate the large intervertebral muscles. The previous anatomical evidence for innervated connected tissue was therefore not entirely satisfactory. The improved fixation of the present study has allowed description of a typical chemical synapse showing specialization of the cleft in particular but also of the pre- and postsynaptic membranes. There are no muscles present in the vicinity of the oral plate ligaments and the hyponeural nerve branches end in, and are only associated with, juxtaligamental cells. The hyponeural ganglion cells from which the nerve branches arise were originally described using the electron microscope (Stubbs and Cobb 1981). Recent work (Cobb 1985) has confirmed the anatomy of these neurones using iontophoretic dyefills of individual cells and demonstrated physiologically that they are indeed motor. Propagated action potentials can be recorded intracellularly from the motor axons and no other type of nerve cell is present. A most fascinating question remains to be answered about these changes in connective tissue properties. This is whether the uniqueness lies in the substances released, or (and more likely) in the protein carbohydrate matrix, or perhaps in both. A pharmacological study is required of this unique system to examine both the types of substance released by the juxtaligamental cells and the biochemical changes that occur in the proteins and carbohydrates of the connective tissue associated with rapid changes in viscosity. Wilkie (1979) and Byrne (1982) have provided some anatomical evidence for two types of juxtaligamentous cells and it is possible these findings may be related to the two coelomic factors of Motokawa (1981). There are now a number of suitable preparations for investigation but the oral plate system described here may be particularly useful since large amounts of it can be quickly prepared free of epithelium, muscle and radial nerve for extraction procedures and testing of putative chemicals. A further valuable feature of this preparation is that conventional intra- and extracellular electrophysiological experimentation can be carried out on both the ectoneural and hyponeural nervous system that are presynaptic to the juxtaligamental cells (Cobb 1985).

Fig. 1. Section through the oral plate ligament showing extensive collagen fibrils. Small cellular profiles containing granular vesicles of various sizes are also present, and these can be shown to be processes from juxtaligamental cells. × 30000

Fig. 2. Transverse section of part of the radial nerve cord of *Ophiura ophiura*. The bulk of the tissue is ectoneural (*e*) but a small crescent of hyponeural (*h*) is also present, separated by a basement membrane. Part of this hyponeural tissue (*large arrow*) lies at the edge of the epineural sinus (*es*) and a side branch nerve (*s*) passes to the periphery. × 650

Fig. 3. Section further along nerve cord showing 2 hyponeural nerve branches (*arrows*) derived from the tissue at the edge of the epineural sinus (*es*) in Fig. 1. × 250

Fig. 4. The serial section analysis shows the nerve branches illustrated in Fig. 2 enter a plaque of juxtaligamental tissue (*jlg*). × 400

Fig. 5. Part of the juxtaligamental tissue in an identical position to that shown in Fig. 3. Juxtaligamental cells (*jlg*) are present which contain typical nuclei and endoplasmic reticulum, and are characterized by granular vesicles of various sizes. Processes from these cells (*pr*) intermingle with hyponeural motor axons (*ha*) characterized by small agranular vesicles and numerous microtubules. Some of these hyponeural motor neurones make chemical synapses onto juxtaligamental cell processes (*arrow*). × 20000

Fig. 6. Enlargement of synapse at *arrow* in Fig. 5 between a hyponeural motor axon (*ha*) and a process from a juxtaligamental cell (*pr*). The synapse is relatively unspecialized but does show an even cleft which contains amorphous material, and a slight pre- and post-synaptic fuzz of cytoplasmic material. × 65000

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Accepted June 24, 1985