

Conduction pathways in the nervous system of *Saccoglossus* sp. (Enteropneusta)

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Abstract: A species of *Saccoglossus* from Barkley Sound, British Columbia, was observed in the field and found to exhibit a startle withdrawal response. Optical and electron microscopy of the nerve cords failed to reveal giant axons. The dorsal collar cord and ventral trunk cord consist of small axons with a mean diameter of $0.4 \mu\text{m}$. The majority of the axons run longitudinally and there is no indication of a specialized integrative centre. Electrical recordings from the nerve cords show events interpreted as compound action potentials. The potentials are through-conducted from proboscis to trunk. Such propagated events probably mediate startle withdrawal. Conduction velocities did not exceed $40 \text{ cm} \cdot \text{s}^{-1}$ in any part of the nervous system.

Résumé : Une espèce de *Saccoglossus* de Barkley Sound, Colombie-Britannique, a été observée en nature et cet organisme manifeste une réaction de fuite en cas d'alerte. L'examen des cordons nerveux au microscope optique et au microscope électronique n'a pas révélé la présence d'axones géants. Le cordon dorsal du cou et le cordon ventral du tronc se composent de petits axones de $0,4 \mu\text{m}$ de diamètre moyen. Les majorité des axones sont disposés longitudinalement et il ne semble pas y avoir de système central d'intégration. La mesure des courants électriques le long des cordons nerveux a démontré l'existence de potentiels d'action composés. Les potentiels se propagent directement du proboscis au tronc et sont probablement responsables de la réaction de fuite. La vitesse de conduction n'excède jamais $40 \text{ cm} \cdot \text{s}^{-1}$ dans le système nerveux.

[Traduit par la Rédaction]

Introduction

The enteropneusts, with the pterobranchs, constitute the small deuterostome phylum Hemichordata, generally regarded as an early offshoot from the chordate line of evolution (Ruppert and Barnes 1994; Wada and Satoh 1994). Such a group might be expected to hold clues concerning early chordate neural evolution, but the nervous system and behaviour have been little studied since the work of Bullock (1940, 1944, 1945). The nervous system appears to be very primitive, consisting of an intraepithelial nerve plexus thickened locally into longitudinal fibre bundles or "cords." In the collar region the dorsal cord sinks below the surface in a manner reminiscent of the formation of the dorsal neural tube in vertebrate embryos. Despite its internal location, the collar cord does not resemble an integrative centre histologically (Bullock 1945; Bullock and Horridge 1965; Silén 1950; Knight-Jones 1952; Dilly et al. 1970). It appears to be a transmission pathway much like the other nerve cords, but with the interesting addition of giant axons.

Giant axons have been found in several species of enteropneusts (Spengel 1893; Bullock 1944). Their cell bodies are located in the collar cord and their axons decussate and run back into the general epithelial plexus of the trunk or into the ventral cord, presumably terminating in the longitudinal muscles. Their numbers are variable: Bullock (1944) counted between 15 and 20 in *Glossobalanus minutus*, fewer than a

dozen in another species of *Glossobalanus*, and 161 in a *Balanoglossus* species. Counts for species of *Saccoglossus* range from 15 to 30. One electron microscope study (Dilly et al. 1970) has confirmed their presence in a member of this genus.

Most of what is known of enteropneust behavioural physiology is summarized by Bullock and Horridge (1965). Conduction is diffuse and decremental in many regions but through-conduction pathways are also thought to exist. The clearest example of a through-conducted response is the startle withdrawal response (Bullock 1940), where a gentle poke on the proboscis results in a rapid contraction of the longitudinal muscles of the trunk. Observed in the dark, startle withdrawal is accompanied by luminescence (Baxter and Pickens 1964).

The only neurophysiological investigation of an enteropneust reported to date is that of Pickens (1970) on *Ptychodera* sp. In this study, through-conducted signals were recorded from the dorsal and ventral trunk nerve cords and from the collar cord. Pulse amplitude was found to vary with shock strength. This, together with other evidence, suggested that the signals were compound action potentials. No fast pathways suggestive of giant axons were discovered and an electron microscope examination showed that most fibres in *Ptychodera* sp. were less than $0.33 \mu\text{m}$ in diameter (Pickens and Ferris 1969).

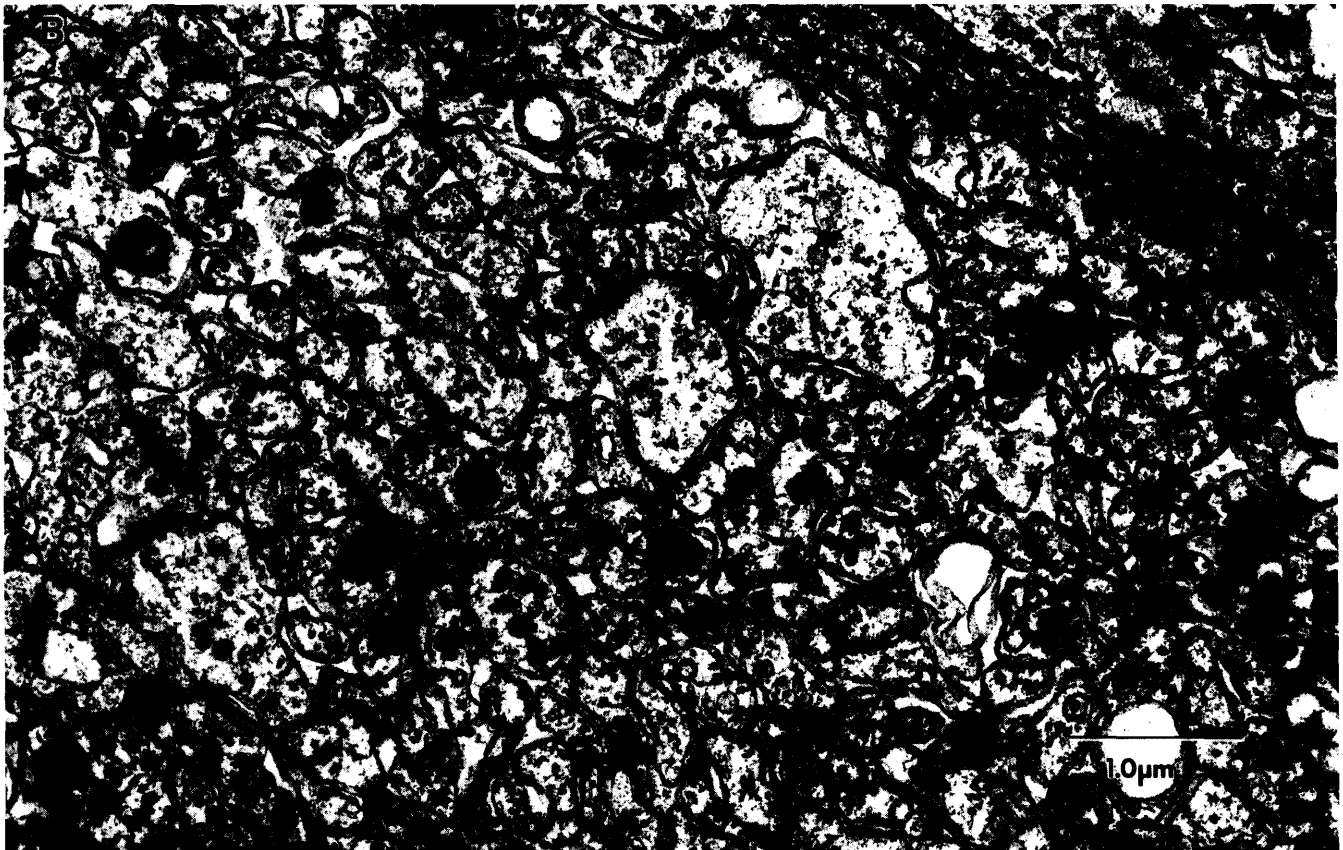
Given the paucity of information on enteropneust neurophysiology, neural fine structure, and behaviour, we decided to investigate a species of *Saccoglossus* from Barkley Sound, British Columbia. Our goals were (i) to determine if this enteropneust showed a startle response, (ii) to carry out a microscopical examination of the nerve cords with a view to describing the numbers and distribution of giant axons, which we assumed would be present, as in other members of

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Fig. 1. Transverse sections through the collar cord. (A) Paraffin section through the collar cord, showing the nerve fibre layer (*nf*) beneath the epithelial cell body layer (*ep*). (B) Electron micrograph of a section through the fibre layer in a region corresponding to that indicated by the arrowhead in A. Other parts of the fibre layer showed a similar range of axon diameters, and data from numerous sections such as this were used to prepare the histogram shown in Fig. 2.



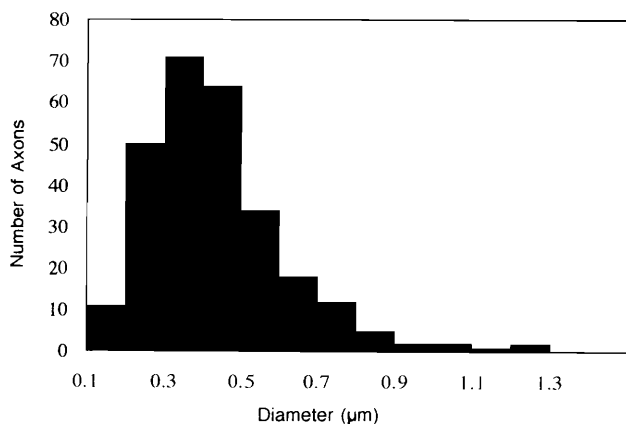
the genus, and (iii) to record electrical events from its nerve cords to determine if through-conduction pathways existed as reported by Pickens (1970) for *Prychodera* sp., but with the additional expectation of finding fast pathways corresponding to the giant axons.

Material and methods

The species of *Saccoglossus* used for this study has not yet received a formal taxonomic description, and will be provisionally design-

nated *Saccoglossus* species A, pending determination by a specialist. This species is quite distinct from the only other enteropneust known to occur locally, *Saccoglossus bromophenolosus* (King et al. 1994). The two species differ in size, coloration, and habitat. *Saccoglossus bromophenolosus*, from Padilla Bay and Willapa Bay, Washington (Woodwick 1951), is an intertidal mud dweller, while species A has most frequently been found subtidally in coarse-grained calcareous debris of biogenic origin. Specimens were obtained at approximately 10 m depth in the Ross Islets, Barkley Sound, during the summer and fall, 1994. Field observations were made by SCUBA on the enteropneusts in their natural habitat and

Fig. 2. Size-frequency histogram of axon diameters measured in the collar cord and ventral trunk cord; $n = 272$, $\bar{x} = 0.438$, $SD = 0.188$.



in aquaria at the Bamfield Marine Station. Specimens were transported to the University of Washington Laboratories at Friday Harbor, where the electrophysiological recordings were made, and to the University of Victoria, where the optical and electron microscope work was carried out.

Specimens were fixed in Bouin's fixative for paraffin embedding. Four specimens were serially sectioned from proboscis to trunk. A single animal was fixed in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer at pH 7.4 followed by postfixation in 1% osmium tetroxide in 0.2 M phosphate buffer. Pieces of tissue were dehydrated and embedded in Epon 812. Thick epon sections (ca. 1.0 μm) were cut at about 180 points along the length of body from posterior proboscis to anterior trunk and stained in Toluidine Blue. Thin sections were cut from the same blocks and were stained with uranyl acetate and lead citrate for electron microscopy.

Twenty specimens were used for recordings of nervous activity. They were pinned out on Sylgard platforms. Long thin polyethylene suction electrodes with internal tip diameters of 50–100 μm were used. Flexibility was important because the worms showed a great deal of peristaltic movement, tending to dislodge the electrodes. Recordings from the collar cord were made by removing the overlying tissues, exposing the cord. Signals were amplified, digitized, and displayed on an oscilloscope using conventional procedures. Stimuli were delivered through bipolar metal electrodes held in a micromanipulator. Shocks of 2 ms duration in the 5.0- to 10.0-V range were usually effective.

Results

Histology and ultrastructure

Sections were cut through the nerve cords in the proboscis, collar, and trunk. Particular attention was paid to the collar and anterior trunk region, where giant axons have been described in other species of *Saccoglossus* (Bullock 1945). Sections were cut through the collar cord (Fig. 1A) and selected regions were examined by electron microscopy (Fig. 1B). Most of the cord tissue consists of longitudinal bundles of small axons seen as subcircular profiles in sections cut transversely to the body axis. There is no suggestion of a cortical region with cell bodies surrounding a central neuropil. The axons are unmyelinated and contain microtubules, mitochondria, and dense-cored and clear vesicles, as described by previous workers (Pickens and Ferris 1969; Dilly et al. 1970). In the collar cord and ventral trunk cord, axon diameters were found to vary within the range 0.1–

1.3 μm, showing a normal distribution, with a mean of around 0.4 μm (Fig. 2). No giant axons were found in any part of the nervous system examined.

Behavioural observations

Specimens observed in the natural habitat showed a "startle" response, pulling themselves rapidly down into their burrows, as described in *S. pusillus* by Bullock (1940). In aquaria, the response was evoked by tactile stimulation and by gently tapping the wall of the tank. In the field, the approach of a SCUBA diver would initially evoke slow withdrawal, but any sudden movements in the water near the animal or disturbance of the sediment layer within approximately 1 m of its burrow evoked the startle response. The animals are evidently sensitive to vibrations transmitted through both the water and through the substrate. Withdrawal can occur when the proboscis and collar are extended above the substrate and seems to be dependent on the corkscrew-like orientation of the animal in the substrate and the contraction of the longitudinal muscles of the trunk (C.B. Cameron and A.R. Fontaine, unpublished data). These muscles are most strongly developed medioventrally.

Electrophysiology

Single electric shocks applied to the longitudinal cords in several regions evoked trains of propagated electrical events (Figs. 3a, 3b). These events rarely exceeded 50 μV in amplitude and were often so small as to be indistinguishable from base-line noise. The best recordings were obtained from the neck of the proboscis, where numerous nerve bundles converge and enter the collar, and from the ventral cord of the trunk. Signals were also recorded from the collar cord, but we were unable to record signals consistently from the dorsal trunk cord or the lateral body wall. Increasing the strength of shocks above the threshold for production of propagated events typically increased their amplitude and complexity, indicating that they are compound action potentials. Bursts of potentials evoked by single shocks often lasted for more than 50 ms, typically showing declining amplitudes. The earlier (more rapidly propagated) events in such series sometimes showed fairly consistent wave forms following shocks of similar strength given a few seconds apart (as in Fig. 3b), but we never saw potentials of the sort generated by giant axons in other animals, i.e., large, sharp, spikey events conducted at conspicuously high velocities, whose amplitudes are not affected by variations in shock strength.

It was possible to demonstrate through-conduction following single shocks within the dorsal proboscis cord, the collar cord, and the ventral trunk cord. The highest velocities were seen in the anterior region of the ventral trunk cord, regarded as the major nerve pathway in the trunk (Bullock 1945). Conduction velocity declined posteriorly. Shocks on the neck of the proboscis and on the collar cord evoked potentials that propagated through to the ventral cord, showing that there are continuous conduction pathways linking the proboscis with the trunk via the collar. These findings are summarized in Fig. 4 and Table 1.

These experiments were carried out on pinned specimens, so it was not possible to be certain that the trunk contractions exhibited following shocks in anterior regions represent the startle withdrawals seen in the natural environment, but it is

Fig. 3. Shock-evoked compound action potentials recorded extracellularly between two points on the dorsal midline of the proboscis (A) and ventral midline of the trunk (B, two sweeps, 1 s apart). Asterisks show shock artefacts. Conduction times were measured between the beginning of the shock artefact and the peak of the first, negative-going (downward) potential.

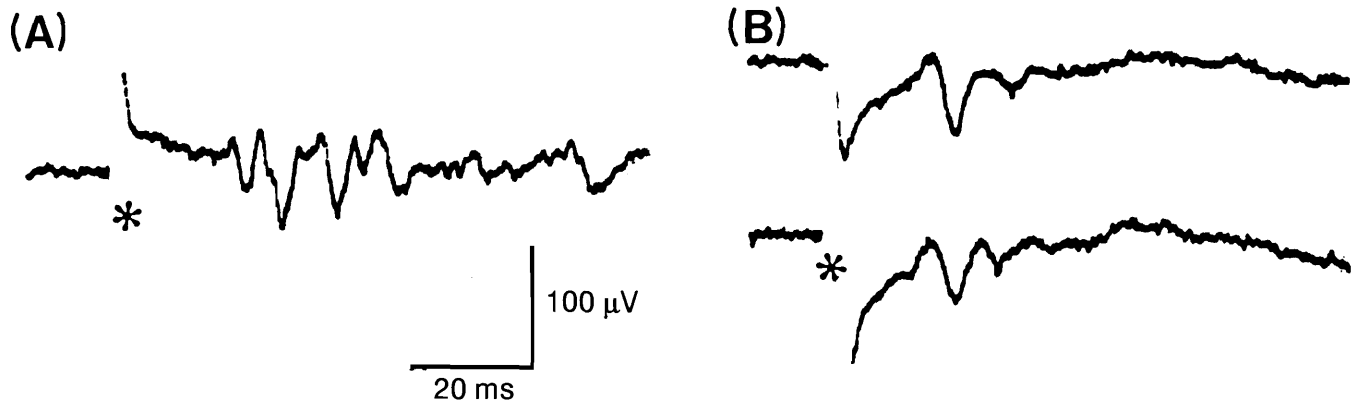
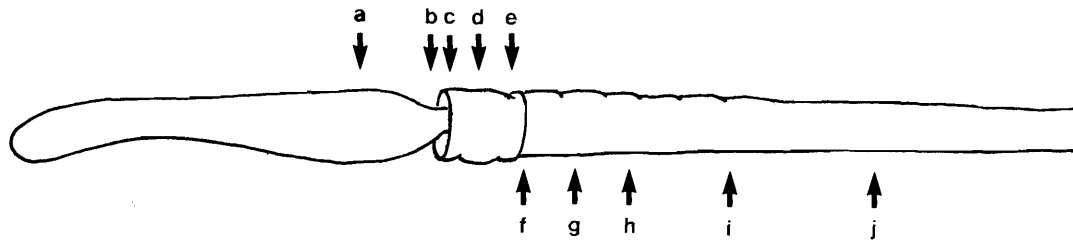


Fig. 4. Conduction velocities measured between various points along the dorsal and ventral midlines of *Saccoglossus* sp. Points *a* and *b* are on the proboscis, *c–e* are on the collar, and *f–j* are on the trunk (for details of paths see Table 1).



reasonable to assume that they do. The contractions are powerful and are through-conducted, occur with short latency rather than spreading by peristalsis, and primarily involve the longitudinal muscles of the anterior trunk region.

Discussion

The results reported here show that *Saccoglossus* sp. A has a startle response similar to that described in other enteropneusts, and demonstrate the existence of through-conduction pathways within the proboscis, collar, and trunk. These pathways are located in the dorsal nerve cords of the proboscis and collar and in the ventral cord of the trunk. They probably mediate the startle response, but a fuller electrophysiological analysis on unrestrained animals would be necessary to demonstrate this conclusively.

Knight-Jones (1952) showed that propagation of contraction waves in the trunk during fast withdrawals was blocked by lesions through the ventral nerve cord, indicating that the fast conduction pathways were located in this part of the nervous system. The fastest pathways identified in the present study were also in the ventral cord. The ventral cord lies directly adjacent to the trunk muscles whose contraction brings about startle withdrawal.

The electrical potentials recorded in this study closely resemble those published by Pickens (1970) for *Ptychodera* sp. In neither case was there any indication of fast pathways of the kind elsewhere associated with giant axons. The structural evidence reported here likewise indicates that giant axons are completely absent from the nerve cords. Instead,

Table 1. Lengths and velocities of conduction pathways.

Path	Path length (mm)	Conduction velocity ($\text{cm} \cdot \text{s}^{-1}$)
<i>a–b</i>	4.0	21.1
<i>c–e</i>	4.4	16.0
<i>f–h</i>	6.0	39.6
<i>f–i</i>	11.8	25.6
<i>f–j</i>	20.0	17.9
<i>d–g</i>	6.7	24.0
<i>b–g</i>	10.0	13.7

we seem to be dealing with conduction in bundles of small axons. A normal distribution of axon diameters is seen and none exceed $1.3 \mu\text{m}$ in diameter. Such units are evidently sufficient for through-conduction and for mediation of startle behaviour. The question of the function of giant axons in those species having them thus remains to be addressed.

Our findings agree with those of previous workers who found no hint of “central” neural specialization in the dorsal cord. This structure appears to be a simple longitudinal transmission pathway linking the proboscis with the trunk rather than being specialized as an integrative centre.

Recent insights into the patterns of expression of key developmental genes in insects and vertebrates have led to a revival of the old idea that somewhere in the line of chordate evolution, the dorsal and ventral sides became inverted

(Arendt and Nübler-Jung 1994). According to this view, the ventral nerve cord of annelids and arthropods is homologous to the dorsal nerve cord of chordates. How do enteropneusts fit into this picture? Commenting on the inversion theory, Peterson (1995) states that “many authors have accepted the homology between the dorsal nerve cords of enteropneusts and chordates, with both structures dorsal, hollow, possessing giant nerve cells, and formed by invagination or delamination of the neuroectoderm. The ventral nervous system of enteropneusts is clearly of invertebrate design with circumenteric connectives and a main ventral nerve cord.” As Peterson points out, if structures homologous to both ventral and dorsal nerve cords exist in the same animal, it becomes impossible to argue that one represents the other.

To the present writers it is not obvious that the nerve cords of enteropneusts are homologous to either invertebrate ventral nerve cords or chordate dorsal nerve cords. The nerve cords of enteropneusts are local thickenings of the ectodermal nerve plexus; they show no special concentrations of nerve cell bodies and no ganglionic organization, and give off no nerves laterally. They remain intraepithelial even where, as in the collar, the epithelium containing the nerves becomes internalized. Though “hollow” in some species, this structure is quite unlike the dorsal tubular nerve cord of chordates and, as we have shown here, giant axons may be absent. It is true that “circumenteric connectives” exist in the form of tracts running from the ventral cord up and around the body wall on each side, converging toward the dorsal midline. There is also a nerve ring around the proboscis base. Such connectives would presumably be necessary in any worm-like animal possessing concentrations of nervous tissue on both dorsal and ventral sides, and it is by no means certain that they are the homologues of the circumenteric connectives of annelids and arthropods. Regarding the ventral nerve cord, this structure may be the “main” nerve cord in terms of the number of axons in it and their conduction velocity, but it is built on exactly the same principle as the other nerve cords, differing from them only in degree.

Rather than looking for homologies between the nerve cords of enteropneusts and those of arthropods and chordates, it seems to us more appropriate to regard the cords as ad hoc specializations of a diffuse ectodermal nerve plexus inherited from a common ancestor with the echinoderms. The equivalent system in echinoderms would be the ectoneural nervous system.

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