J. exp. Biol. (1980), 84, 119–136
With 27 figures
Printed in Great Britain

ELECTRICAL INTERACTIONS BETWEEN THE GIANT AXONS OF A POLYCHAETE WORM (*SABELLA PENICILLUS* L.)

BY DEFOREST MELLON JR*, J. E. TREHERNE†, N. J. LANE, J. B. HARRISON AND C. K. LANGLEY
Department of Zoology, University of Cambridge, U.K.

(*Received* 23 *April* 1979)

SUMMARY

Intracellular recordings demonstrated a transfer of impulses between the paired giant axons of *Sabella*, apparently along narrow axonal processes contained within the paired commissures which link the nerve cords in each segment of the body. This transfer appears not to be achieved by chemical transmission, as has been previously supposed. This is indicated by the spread of depolarizing and hyperpolarizing voltage changes between the giant axons, the lack of effects of changes in the concentrations of external divalent cations on impulse transmission and by the effects of hyperpolarization in reducing the amplitude of the depolarizing potential which precedes the action potentials in the follower axon.

The ten-to-one attenuation of electronic potentials between the giant axons argues against the possibility of an exclusively passive spread of potential along the axonal processes which link the axons. Observation of impulse traffic within the nerve cord commissures indicates, on the other hand, that transmission is achieved by conduction of action potentials along the axonal processes which link the giant axons. At least four pairs of intact commissures are necessary for inter-axonal transmission, the overall density of current injected at multiple sites on the follower axon being, it is presumed, sufficient to overcome the reduction in safety factor imposed by the geometry of the system in the region where axonal processes join the giant axons.

The segmental transmission between the giant axons ensures effective synchronization of impulse traffic initiated in any region of the body and, thus, co-ordination of muscular contraction, during rapid withdrawal responses of the worm.

INTRODUCTION

Giant axons are conspicuous nervous structures which have evolved independently in a variety of invertebrate groups, notably in annelids, arthropods and cephalopod molluscs (Bullock & Horridge, 1965), for the rapid and reliable conduction of nervous impulses involved in certain critically important reflex responses.

* Present address: Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Va. 22903, U.S.A.
† To whom correspondence should be addressed.

In annelids, which exhibit considerable variation in the structural organization of giant axon systems, these axons appear to share a common involvement in the rapid withdrawal responses that apparently confer valuable selective advantages (Nicol, 1948*a* Dorset, 1978). These systems are most highly developed in sabellid polychaetes, in which the giant axons can be exceptionally large, generally extend for the entire length of the nerve cord and occur in all species in the family (Nicol, 1948*a*). This is seen most dramatically in *Myxicola infundibulum*, in which the extremely rapid escape response is mediated by a single very large axon, of up to 1·0 mm in diameter (Nicol & Young, 1946; Nicol, 1948*b*), in which action potentials are propagated with a velocity of 20 m s^{-1} (Nicol, 1948*a*). Other sabellids possess two giant axons, rapid and symmetrical body contractions being achieved by their synchronization via an anastomosis in the brain (Nicol, 1948*a*) that enables the action potentials from one giant axon to be transmitted to the other (Bullock, 1953). Synchronization of the activities of these paired giant axons was also observed in posterior segments of the bodies of *Protula* and *Spirographis*, in which 'cross-over' of impulses occurs in a synaptic manner (Bullock, 1953). The existence of such transmission between the giant axons was subsequently confirmed by Hagiwara, Morita & Naka (1964) in *Eudistylia polymorpha*, using intracellular recording techniques. These authors showed that an action potential induced a potential change in the other axon that resembled a synaptic potential and which could initiate an action potential. They concluded that electrotonic spread would be insufficient to explain this transmission and postulated that it is mediated by numerous chemical synapses, while recognizing that 'no histological evidence has been obtained of such connexions'.

In this paper we describe results which provide electrophysiological and structural evidence for electrical transmission of impulses between the paired giant axons in the mid-body region of *Sabella penicillus*.

METHODS AND MATERIALS

Specimens of *Sabella penicillus* L., trawled from the Tamar Estuary, were obtained from the Marine Biological Association, Plymouth, U.K. They were kept in marine aquaria at Cambridge in circulating sea water (at 8–10 °C) before use.

The electrophysiological experiments were carried out using isolated lengths of body wall, containing the paired nerve cords and giant axons, as previously described (Carlson, Pichon & Treherne, 1978; Treherne & Pichon, 1978).

One or both of the giant axons were stimulated, at a distance of at least 2·0 cm from the recording electrodes, by rectangular current pulses (50–300 μs in duration) from a Farnell pulse generator through an R.F. isolating unit and a pair of platinum wire electrodes. Resting and action potentials were recorded simultaneously with up to three intracellularly located KCl-filled glass microelectrodes, with resistances of from 5 to 20 MΩ, via separate high-impedance, negative capacitance, converters (W.P. Instruments Inc., Model M 701) a Tektronix 561 oscilloscope and a Medelec, four-channel, fibre optic oscilloscope. In some experiments, hyperpolarizing and depolarizing current pulses were delivered from the Farnell pulse generator, via one of the impedance converters, through an intracellularly located microelectrode.

The artificial sea water used in this investigation had the following composition

Na^+, 470 mM, K^+ 10 mM, Ca^{2+} 11 mM, Mg^{2+} 55 mM, Cl^- 609·7 mM and HCO_3^- 2·3 mM. Variations in calcium and magnesium concentrations were accommodated by appropriate alteration in sodium concentration. Low chloride saline was produced by substituting NaCl for sodium methyl sulphate.

For ultrastructural studies the ventral nerve cord and giant axons were flooded with fixative *in situ* for 15–20 m. The ventral nervous system, together with a strip of ventral body wall, was then removed and left in fixative at room temperature from 2 to 15 h.

The fixative solution was modified from Baskin (1971) and included 2% glutaraldehyde plus 0·5 M sucrose in 0·1 M phosphate buffer, pH 7·4, plus 1 drop of 1% $CaCl_2$ per 10 ml fixing solution, followed by washing in 0·1 M buffer, pH 7·4, plus 0·64 M sucrose. Subsequent treatment included 1 h in 1% OsO_4 in 0·1 M buffer, plus sucrose. After this, the tissues were stained for 30–60 min *en bloc*, in 2% aqueous uranyl acetate, dehydrated through an ascending series of ethanols, treated with propylene oxide and embedded in Araldite. Thick sections were cut both transversely and longitudinally through the ventral nerve cord and its associated giant axons and were stained with toluidine blue for examination under the light microscope. These sections were often cut serially through the region of the septum of the body wall in order to clarify the structural details of the relationship between the giant axons and the nerve cord. At intervals, thin sections were cut, mounted on grids, stained with uranyl acetate and lead citrate and examined in a Philips EM300.

Tissue was also fixed after 4% procion black was directly injected into the giant axons; this induced enhancement of the constriction of the giant axons at the septa. The fixing solution for this was similar to that outlined above. After embedding, both thick and thin sections were cut at the regions where the constriction had occurred.

RESULTS

Transmission between the giant axons

Intracellular recordings were made with microelectrodes inserted into each of the two giant axons (*ca.* 200 μm in diameter) in a segment from the mid-body region. As shown in Fig. 1, the giant axons lie at the inner margins of the paired nerve cords which are linked by a pair of commissures in each segment of the body (see also Fig. 13).

When one of the two giant axons was stimulated in action potential was first recorded in that axon and, after a delay of at least 0·7 ms, an action potential was recorded in the other axon in the recording segment (Fig. 2). With increasing stimulus frequency, the delay between the action potentials increased and a pre-potential, reminiscent of a synaptic potential, became more obvious before the rising phase of the action potential recorded in the distal giant axon. As shown in Fig. 2, transmission between the giant axons eventually failed during prolonged stimulation at frequencies of between 3 and 10 Hz.

In the experiment illustrated in Fig. 3, action potentials were recorded in the two giant axons in the same, mid-body, segment following stimulation of, first, the right (Fig. 3 B) and, subsequently, the left giant axon (Fig. 3 C). Essentially similar responses were recorded in the unstimulated axons when either the left or right ones were

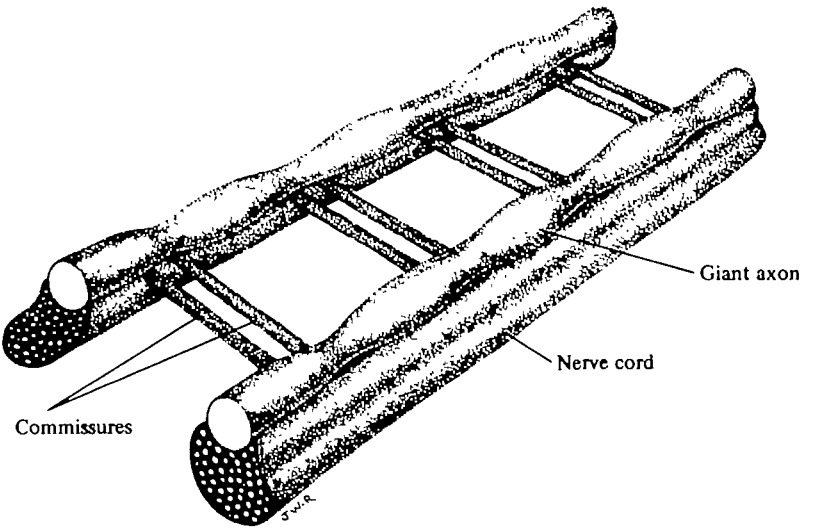


Fig. 1. Schematic representation of the giant axons and their relations to the paired nerve cords and commissures in *Sabella*.

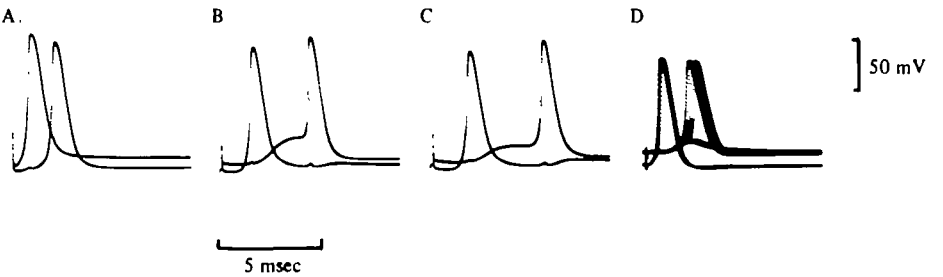


Fig. 2. Action potentials recorded, intracellularly, from both giant axons in the same, mid-body, segment. When one giant axon was stimulated directly an action potential was first recorded in this axon. After a variable delay, an action potential was subsequently recorded in the other giant axon. (A) The effects of a single stimulus applied to one giant axon; (B, C) successive effects of stimulation at 10 Hz; (D) in another preparation, the effects of stimulation at 10 Hz on action potentials recorded in the directly stimulated or in the follower axon.

stimulated. When both giant axons were stimulated simultaneously (by placing the stimulating electrodes across both giant axons) synchronous action potentials were recorded (Fig. 3 A). When the giant axon was cut (three segments before the recording segment) at a distance of approximately 20 mm from the stimulating electrodes, delayed action potentials with characteristic pre-potentials were recorded following stimulation of the other axon. This eliminates the possibility of any direct stimulation of this axon.

In a few preparations, deterioration occurred in transmission between the giant axons. This was seen in the progressive prolongation of the pre-potential and in the delay of the associated action potential in the indirectly stimulated axon (Fig. 4). This alteration cannot be attributed to effects on the giant axons themselves, for normal directly excited action potentials persisted even after impulse transmission between them had ceased. At a critical stage, when the delay between the directly and

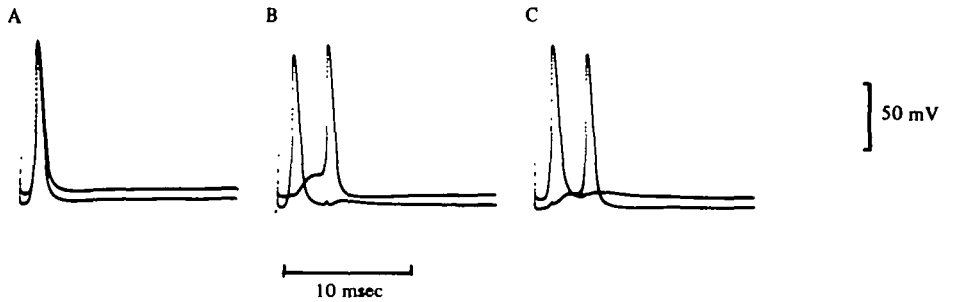


Fig. 3. (A) Synchronous action potentials, recorded with intracellular microelectrodes in left and right giant axons (in the same mid-body segment), with the stimulating electrodes placed between the giant axons so as to stimulate both directly. (B) Effects of direct stimulation of only the right giant axon. (C) Effect of direct stimulation of only the left giant axon.

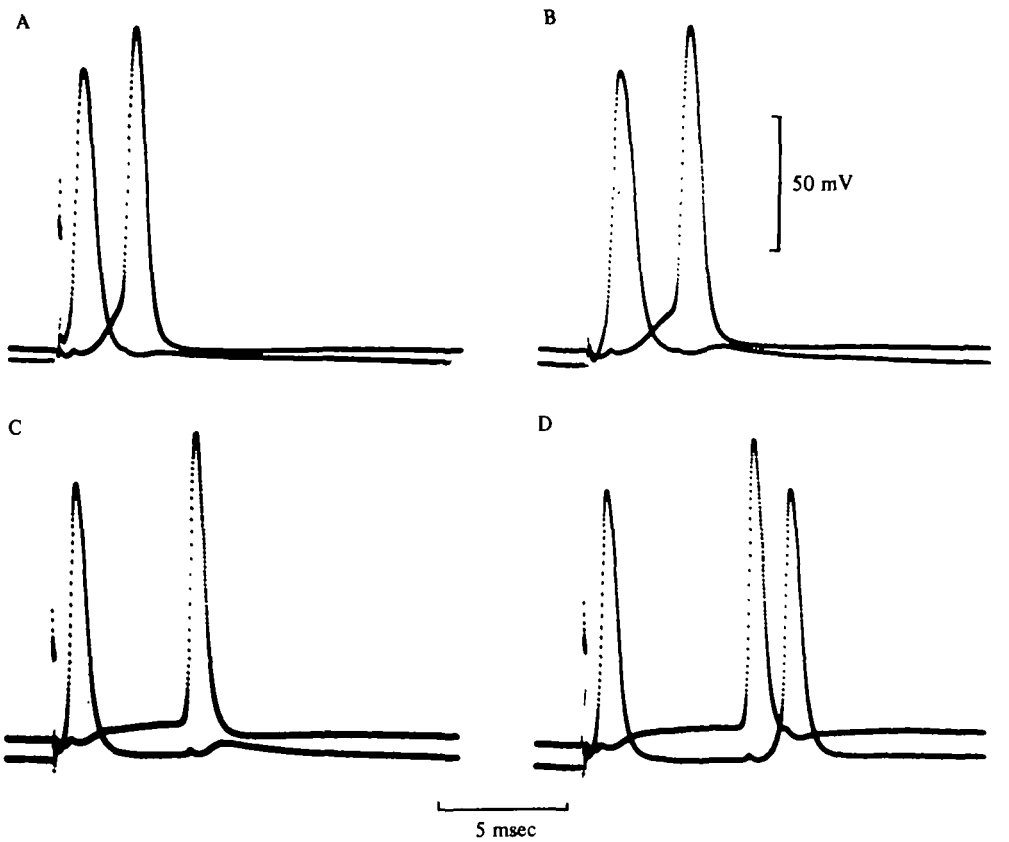


Fig. 4. Successive recordings of action potentials from each of the giant axons in the same segment of a deteriorating preparation. (A) Initial recording from the directly stimulated and follower axon. (B) After 2 min, (C) after 30 min, (D) after 40 min, when the delay between the directly stimulated and indirectly stimulated action potentials had increased sufficiently to enable the latter to evoke another action potential in the initially stimulated giant axon.

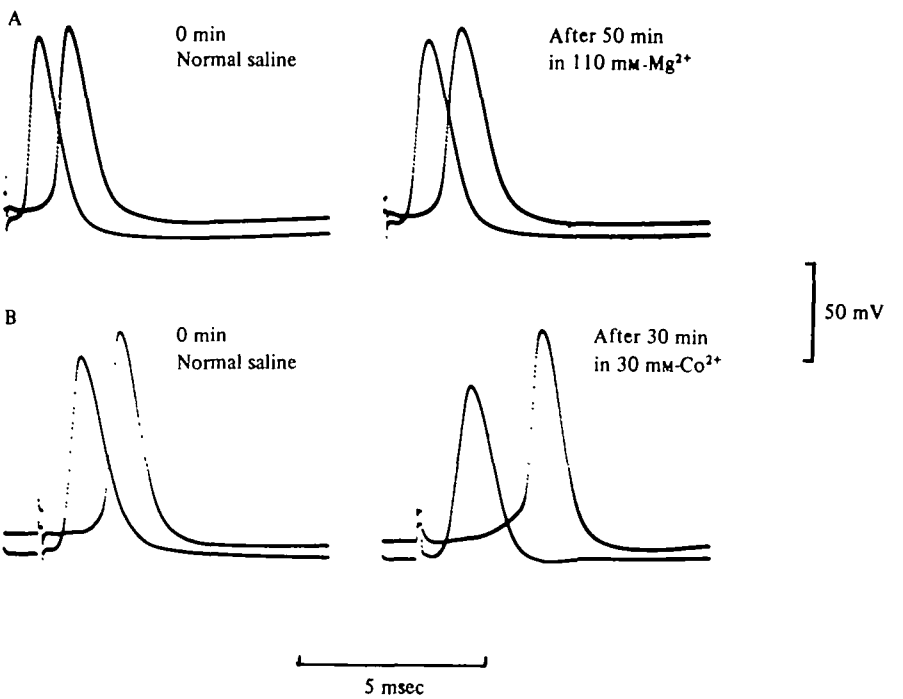


Fig. 5. (A) Effects of calcium-deficient saline (*ca.* 50 μM-Ca^{2+}) containing 110 mM-Mg^{2+} on the directly and indirectly stimulated giant axons in the same, mid-body, segment of *Sabella*. (B) Effects of addition of 30 mM-Co^{2+} to the calcium-deficient saline.

indirectly stimulated action potentials had increased to 5·8 ms, a second action potential was sometimes evoked in the first axon, shortly after the delayed action potential had occurred in the indirectly stimulated one (Fig. 4 D). Thus if transmission of an action potential between the axons is delayed sufficiently, so as to fall outside the refractory period of the initially stimulated axon, then it is possible for it to re-excite the axon. These observations also show that delayed transmission in one direction can occur in the presence of more rapid transmission in the other.

Effects of divalent cations on transmission between the giant axons

The above observations demonstrate that transmission can occur between the giant axons in *Sabella* and that an action potential in one can initiate a pre-potential, resembling a synaptic potential, which precedes the action potential in the other giant axon. As concluded by Hagiwara *et al.* (1964), in studying *Eudistylia*, this indicates the possibility of some chemical synaptic connexion between the giant axons. Experiments were, therefore, performed to test the effects of alterations in the concentrations of external divalent ions which are known to affect chemically mediated synaptic mechanisms.

Exposure of preparations to calcium deficient saline (50 μM-Ca^{2+}) had no significant effect on the transmission of action potentials between the giant axons. Elevated concentration of magnesium ions (110 mM) and addition of cobalt ions (30 mM) to the saline bathing the preparations also failed to block transmission between the giant axons (Fig. 5). The cobalt ions caused an increase in the delay between the action potential recorded in the two axons and a slight increase in the duration of the in-

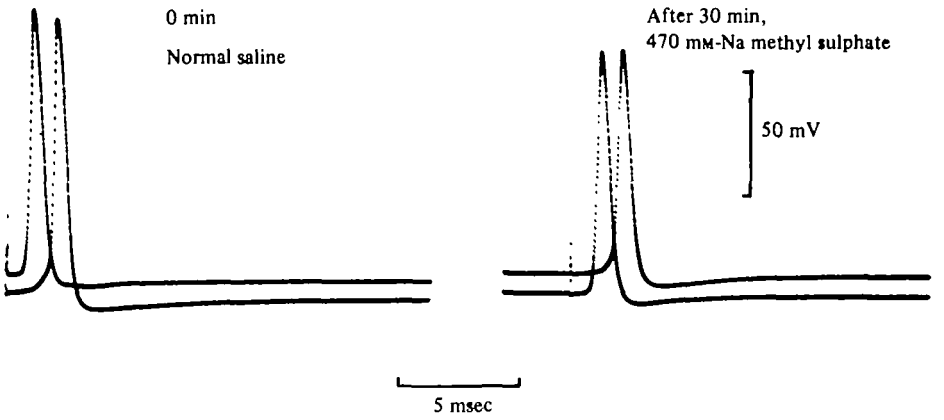


Fig. 6. The effects of substitution of sodium chloride in the saline (470 mM) for sodium methyl sulphate on the action potentials recorded in the directly stimulated and follower axons.

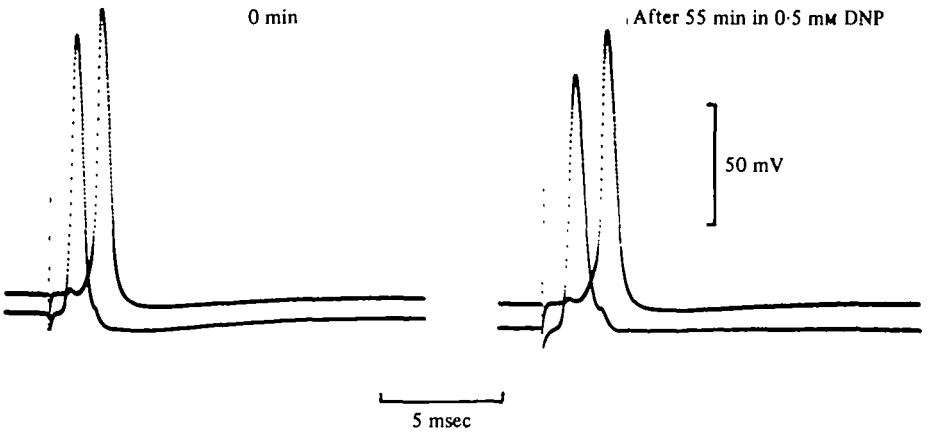


Fig. 7. Effects of dilute dinitrophenol (0·5 mM) on the action potentials recorded in the directly stimulated and follower giant axons.

directly stimulated action potential (Fig. 5B). However, this effect can be related to the effects on the directly stimulated axon in which there was a noticeable increase in the duration of the potential.

Effects of low-chloride saline on transmission between the giant axons

Reduction in the chloride concentration of physiological saline, by a variety of anion substitutes, is known to be effective in uncoupling the junctions which mediate electronic communication between nerve cells (cf. Asada & Bennett, 1971). In these experiments substitution of NaCl (by sodium methyl sulphate 470 mM) was found to have no significant effects on the transmission of action potentials between the giant axons (Fig. 6).

Effects of dinitrophenol on transmission between the giant axons

This metabolic inhibitor is known to produce rapid electrical uncoupling of the low resistance junctions, for example in the septate lateral giant axons of the crayfish (Peracchia & Dulhunty, 1976). The effects of dilute dinitrophenol (0·5 mM) was,

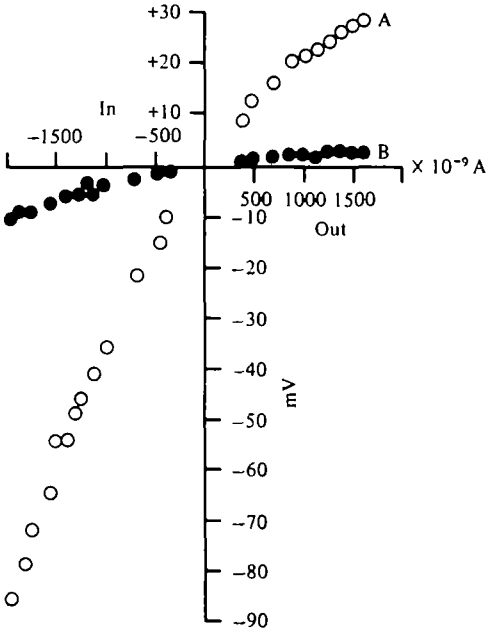


Fig. 8. The relationship between applied current, injected into a giant axon through a microelectrode, and the displacement of membrane potential measured by microelectrodes in the same axon (A) and in the other giant axon (B).

therefore, tested on the transmission of action potentials between the giant axons. As shown in Fig. 7, the metabolic inhibitor had no effects on the transmission of action potentials such as were observed in the crayfish lateral giant axons. Here the effects on transmission were presumed to be a consequence of the accumulation of free intracellular calcium ions (Peracchia & Dulhunty, 1976) or, possibly, as suggested for embryonic cells (Turin & Warner, 1977), changes in intracellular pH.

Voltage spread between the giant axons

In these experiments alternating hyperpolarizing and depolarizing current pulses (500 ms duration) were passed into one giant axon. The resultant displacement of membrane potential was simultaneously recorded in both giant axons in the same segment. The relationship between applied current and the resulting voltage changes is illustrated in Fig. 8. The slope of the I/V curve obtained from the same axon is similar to that obtained in other neurones. The attenuation factor for electrotonic voltage spread between the axons is approximately 10 to 1 (Fig. 8). There is thus appreciable attenuation in the passive spread of voltage between the axons, which is however not as great as that observed in *Eudistylia* by Hagiwara *et al.* (1964) in which the attenuation factor was greater than 30.

Effects of axonal hyperpolarization on inter-axon transmission

Experiments in which the follower axon was hyperpolarized (by current delivered through a second intracellular microelectrode) lend support to the idea that transmission of excitation between the giant axons is electrical in character. As shown in

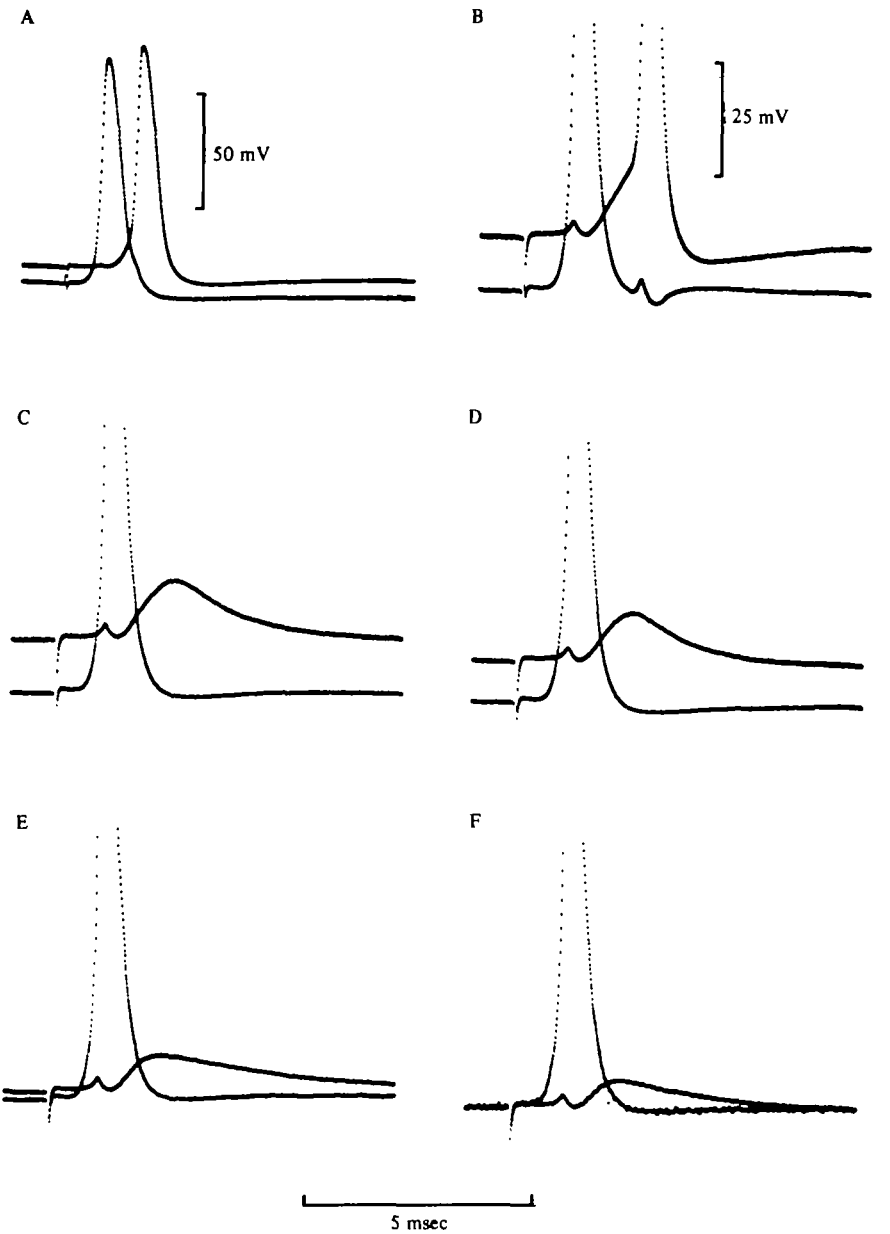


Fig. 9. Effects of hyperpolarization on the electrical response of a giant axon following direct stimulation of the other axon. In this experiment the follower axon was hyperpolarized by current delivered through a second intracellular microelectrode. (A) Initial response, showing, action potentials recorded from the directly stimulated and the follower giant axon. (B) Initial response displayed at higher gain. (C–F) The effects of progressive hyperpolarization of the follower axon in abolishing the action potential and reducing the amplitude of the pre-potential in this axon.

Fig. 9, increasing hyperpolarization was associated with action potential blockage and a reduction in size of the prepotential recorded in the indirectly excited axon following initiation of impulses in the directly stimulated axon. These data are inconsistent with those expected from a conventional chemically mediated synapse, in which the post-synaptic current density would be increased during post-synaptic hyperpolarization. The continuous decline in amplitude of the pre-potential, as observed, suggests that the strength of excitatory current responsible for the pre-potential was reduced in proportion to the degree of hyperpolarization. One explanation for this effect could be a progressively more distant blocking of regenerative activity in pathways between the giant axons.

Effects of cutting nerve cord commissures

In these experiments a length of worm containing eight successive pairs of commissures was isolated by severing all other commissures anteriorly and posteriorly (Fig. 10). The effects of successive cutting of the remaining commissures on transmission between the giant axons was then recorded as indicated in Fig. 10.

Impairment of transmission between the axons did not occur until only four pairs of commissures remained intact (i.e. at stage 5 in Fig. 10). At this stage transmission became intermittent. With only three pairs of commissives intact the secondary spike response was abolished (stage 5 in Fig. 10). Further cutting of commissures resulted in the slight reduction of the depolarizing response which had preceded the action potential in the indirectly stimulated axon. These observations suggest that the nerve cord commissures are involved in transmission of action potentials between the giant axons, that a minimum of four adjacent pairs are necessary for post-axon excitation to occur and that current density from the directly excited axon is reduced in a graded manner by successive elimination of commissures.

Electrical recordings from the nerve cord commissures

More direct support for the possibility of impulse passage along pathways between the giant axons was provided by intracellular microelectrode recordings from within the commissures which link the nerve cords associated with the giant axons (see Fig. 1). In these experiments three microelectrodes were used to record simultaneously in the two giant axons (in the same segment) as well as in one of the pair of commissures.

Various impalements in the commissures indicated the penetration of numerous nervous processes. In six cases excitable responses were recorded which were all associated with the activity of the giant axons. As shown in Fig. 11, the responses measured in the commissures often consisted of a fast rising prepotential (apparently due to activity in the directly stimulated giant axon) superimposed upon which was a more rapidly rising excitable component having an amplitude comparable to that of the impulses in the giant axons. These responses were obtained in both the anterior and the posterior of the commissure of the pairs, although not in the same preparation.

Although the commissures were impaled apparently midway between the giant axons the action potentials recorded there often occurred synchronously with those recorded in the indirectly stimulated giant axon (Fig. 11 B). This appears to be an

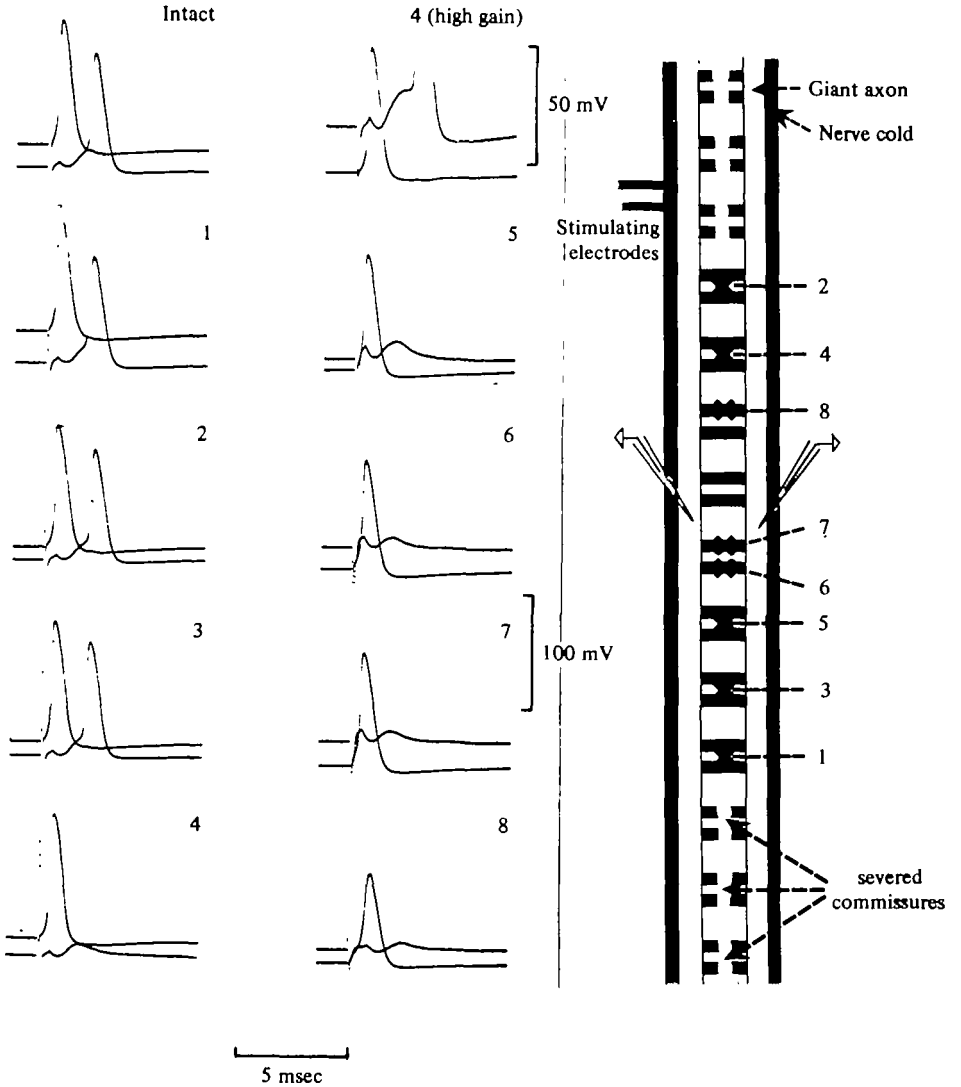


Fig. 10. Experiment in which a length of worm containing eight successive pairs of commissures between the nerve cords (see also Fig. 1) was isolated by cutting all the commissures anteriorly and posteriorly. The effects of successive cutting of the remaining commissures on the follower giant axon are illustrated. Impairment of inter-axonal transmission occurred when only four pairs of commissures were intact (stage 4). At this stage transmission became intermittent. A prepotential and follower action potential recorded at this stage is shown, readjusted, at higher gain (4 (high gain)). Further severance of the commissures caused progressive reduction in the amplitude of the depolarizing pre-potential recorded in the follower giant axon.

artifact of impalement, for extracellularly recorded action potentials or those measured during partial impalement (Fig. 12), occurred midway in time between the action potentials recorded in the directly stimulated and the follower axon.

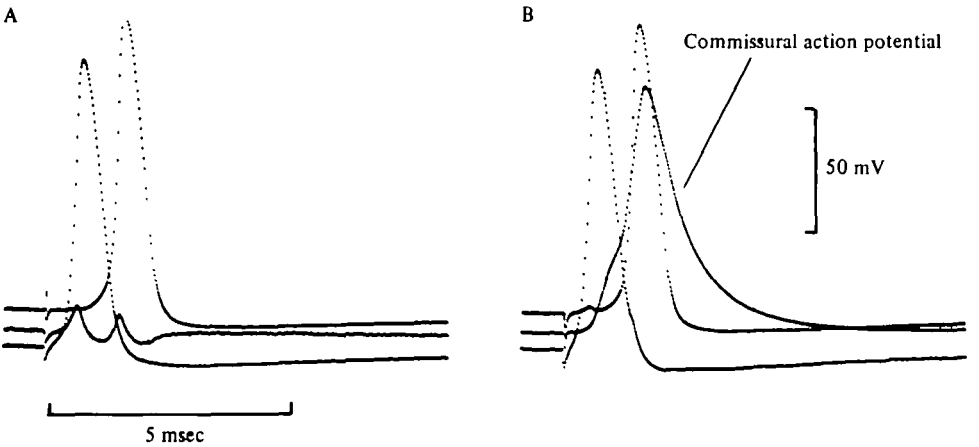


Fig. 11. Simultaneous recordings from the indirectly stimulated axon, the follower axon and from the posterior of one of the pair of commissures in the same, mid-body, segment. (A) The indirectly stimulated and follower action potentials and the electrical responses measured in an intracellular position within the commissure. (B) Shows the pre-potential and action potential, recorded during a subsequent impalement of the commissure, together with the action potentials recorded in the indirectly stimulated and follower giant axons. Note that the peak of the commissural action potential is approximately synchronous with that of the follower action potential.

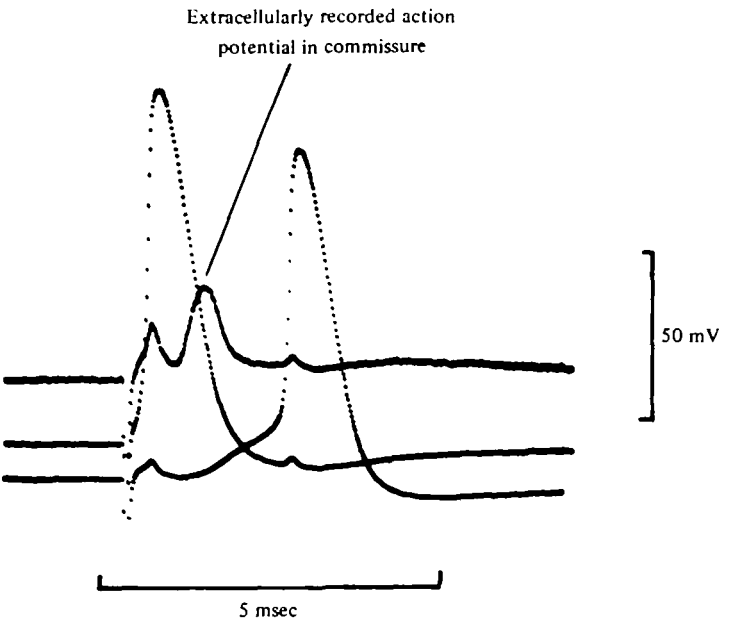


Fig. 12. Intracellular recordings from the directly stimulated and follower giant axons together with an extracellular recording from within an associated commissure. In this case the action potential recorded within the commissure occurred between the directly stimulated and follower action potentials.

Fine structure of the giant axons, nerve cords and commissures

As illustrated in Fig. 1, *Sabella* possesses two ventral nerve cords which are joined in each segment at the level of the septum by two commissures. Each nerve cord contains a single giant axon, of up to 250 μm in diameter (Fig. 13A), together with many small axons of variable diameter (Fig. 16).

The giant axons contain mitochondria, fibrils, and vesicles of smooth membrane but few neurotubules (Fig. 16, inset); many nerve terminals containing both small electron-lucent and larger electron-opaque granules are found directly apposed to their axolemma, especially in areas where branching occurs (Fig. 17).

At points on either side of the septum where two body segments adjoin, each giant axon can be seen to become flattened as it produces a branch (Figs. 13B, 13C and 18) which joins the commissures that connect the two nerve tracts (Figs. 13C, 14, 15 and 21). These branches are of variable diameter (from 5 μm or less to 10 μm) (Figs. 14 and 15) but their precise geometry, ascertainable only by serial sectioning through a number of commissures, has not yet been fully analysed. Such branches can be identified in thin sections as areas of increasingly attenuated axoplasm. As they run into and become part of the commissures, they contain irregular smooth vesicles, especially near their periphery (Fig. 18) and in some cases these become aligned across the axoplasm. In the areas near the body septa, where the commissures (Figs. 19 and 20) fuse with the ventral nerve tract (Fig. 21A, B and C) and where the giant axons contribute a process to the commissure (Figs. 13*c*, 14, and 15) there is a great reduction in the diameter of the giant axons from 200 μm or more (Fig. 13A) to about 70 μm (Fig. 13B, C). This appears to be a normal phenomenon, which is, however, considerably enhanced under conditions of stress, such as when foreign materials (Procion black, Fast green or cobalt and horseradish peroxidase) were injected into the axoplasm (Fig. 22). This axonal constriction could account for the difficulties in demonstrating transfer of injected dyes and tracer substances from one giant axon to the other via the commissure processes, as well as between adjacent segments of the same axon. In these experiments only faint staining or reaction product could be detected in the adjacent axon, or segments of the same axon, presumably as a result of the restricted access into the contricted region of the giant axon (cf. Fig. 22).

The other, smaller axons in the nerve cords contain mitochondria and neurotubules, but little smooth membrane (Fig. 16). There are glandular cells, probably containing mucopolysaccharide (Fig. 18) as well as muscle fibres (Fig. 18), characterized by very large paramyosin filaments lying around the ventral nerve tracts, in close association with the giant axons (Fig. 26). There is very little glial investment of the smaller axons although the giant axons may have a number of attenuated glial layers wrapped like a very loose mesaxon round them (Figs. 16, 17, 18 and 26). Such processes are often separated from one another by regions of extracellular matrix containing collagen-like fibres (Figs. 17 and 26); although less frequently two glial cell processes may lie very close to one another and be associated by gap junctions (Fig. 27). The glial cells send attenuated cytoplasmic processes out in several directions from their cell bodies which lie at random in the mass of axons (Fig. 16). The glial cytoplasm is characterized by clumps of fibrillar material (Fig. 27) of considerable

electron opacity and often contains extensive Golgi complexes (Fig. 25); in some cases, dense bodies which may be gliosomes (lysosomes) (Fig. 17) also occur.

The ultrastructure of the commissures (Figs. 19 and 20) appears to be similar to that of the ventral nerve tract in that they possess many small axons, and a few glial cells (Fig. 23). They have some larger axons in their periphery, as can be seen in cross-sections (Fig. 23), and one of these must represent the branch that arises from the giant axon. The commissures lack a very definite outer perineurial layer, which appears to be formed of loose attenuated cell processes separated by connective tissue (Fig. 23) as occurs in the ventral nerve tract. Many neurosecretory-bearing fibres are present as is also the case in the ventral nerve tract and these are particularly striking in longitudinal section (Fig. 24). Glia once more exhibit the characteristic dense fibrillar cytoplasmic inclusions (Fig. 24) and as in the nerve cord, some muscle fibres lie in the connective tissue sheath around the commissures.

DISCUSSION

The electrophysiological observations described above fully confirm those of Hagiwara *et al.* (1964) on *Eudistylia* in showing a transfer of impulses between the giant axons in each body segment in the mid-body region of *Sabella*. However, our

Figs. 13–15. These are light micrographs of the ventral nerve cords of *Sabella*, cutting through the nerve tract (VNC) and giant axons (GA) in transverse section and through the commissure (C) in longitudinal section. S, septum of the ventral body wall. In each case the intestinal tract and related organs have been dissected away to reveal the underlying nervous system.

Fig. 13 (A) Section through the nerve cord in an area where the giant axon is at its normal, unrestricted diameter, × 175. (B) Section through an area where the giant axons are becoming reduced in diameter prior to producing a branch into the commissure. × 175. (C) Elliptical giant axon giving rise to a branch (arrow) which runs into and forms part of the cross-commissure (C); × 175.

Fig. 14. Branch (large arrow) about 10 μm in diameter, from the giant axon, forming part of the commissure and running through it (small arrows) recognizable by virtue of its relatively greater diameter in comparison with the other axons present. × 357.

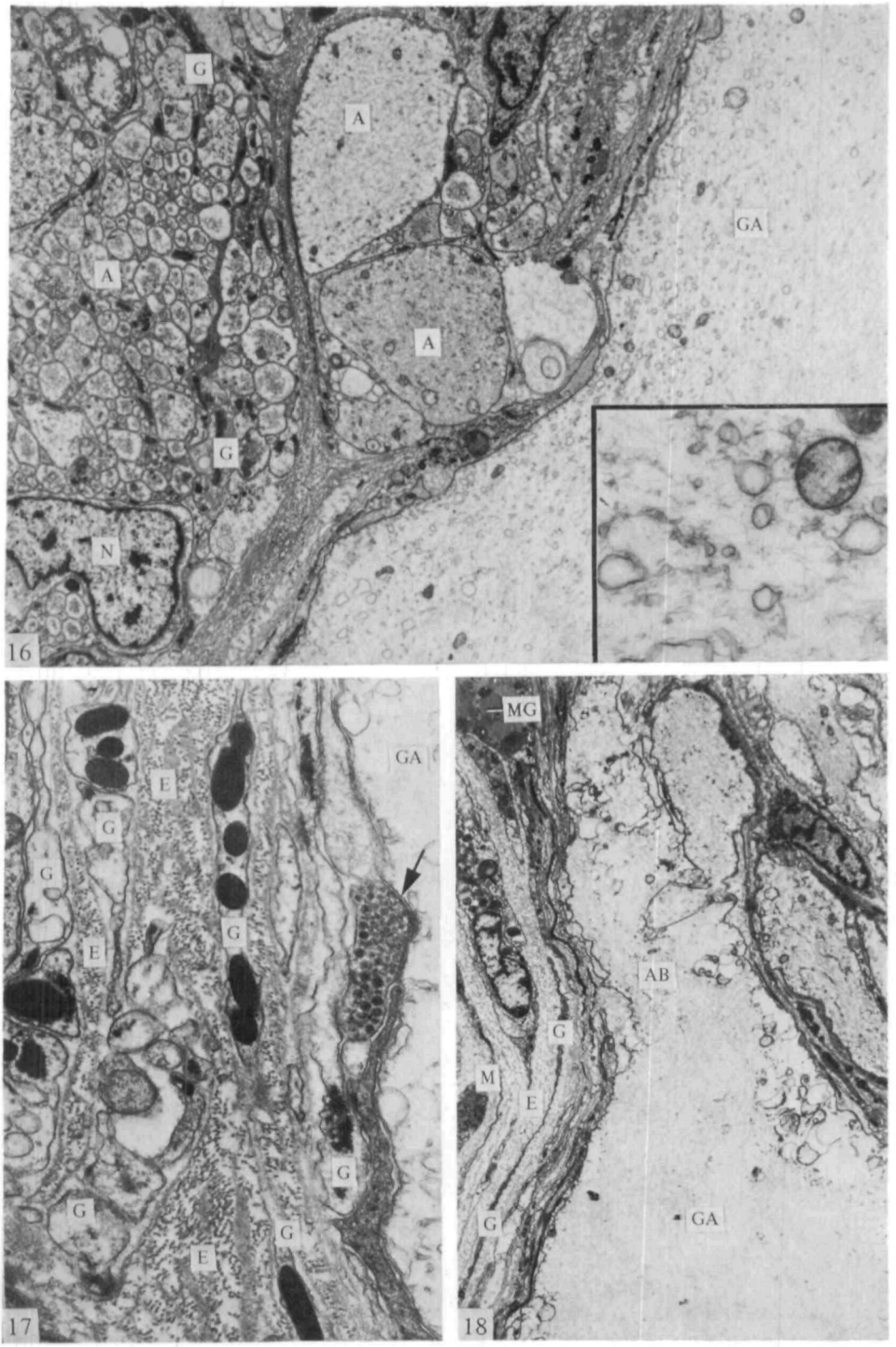
Fig. 15. Branch (arrow), of a much smaller diameter, from the giant axon, illustrating the variable nature of the contribution from the giant axon to the cross-commissure. × 233.

Figs. 16–18. These are electron micrographs from thin sections of the ventral nerve cord of *Sabella*.

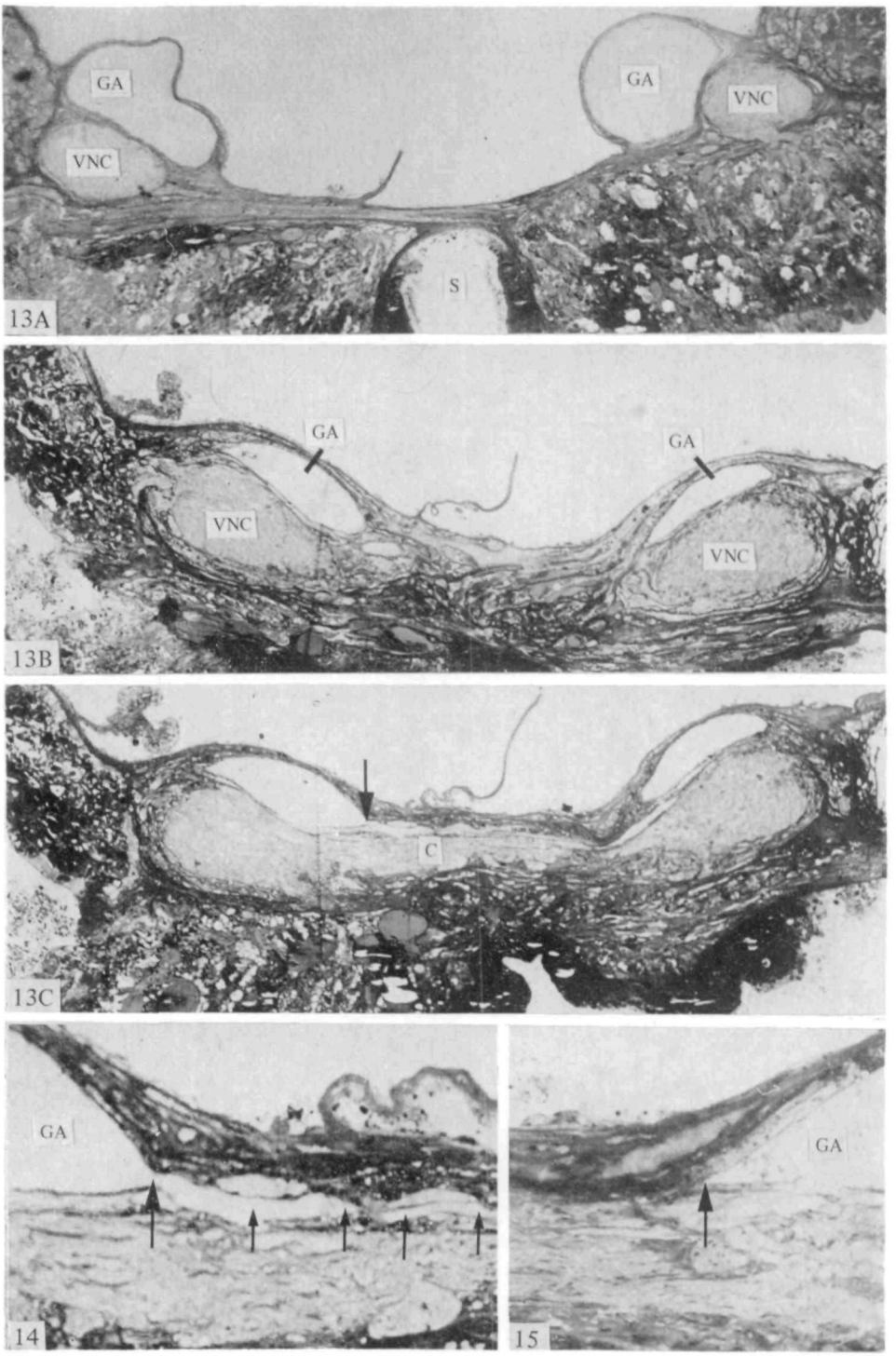
Fig. 16. Cross-section through nerve cord demonstrating its numerous small axons (A) and occasional glial cells (G). The glia, together with the extracellular matrix, form a loose investment around the giant axon (GA). N, glial cell nucleus. Insert shows enlargement of an area of giant axoplasm to illustrate its component mitochondria, smooth vesicles and microfibrils. × 5600; insert, × 25900.

Fig. 17. Glial cells (G) bordering the edge of the giant axon (GA) cut in transverse section. Note the loose packing of the attenuated glial cell processes which lie in the extracellular space (E) containing collagen-like fibres. As occurs fairly commonly, there is a nerve terminal abutting directly onto the giant axolemma (arrow); this contains both small electron-lucent and larger dense-cored vesicles, possibly representing a neurohormone of some kind. × 20600.

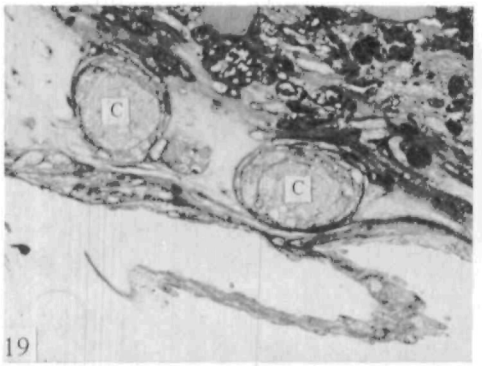
Fig. 18. Point where the giant axon (GA) narrows down to produce a small axonal (AB) contribution to the cross commissure. A number of vesicles and fragments of membrane of unknown significance frequently occur here. The axonal branch is surrounded by glial cells (G), gland cells, probably mucopolysaccharide-containing (MG), and by muscles (M), which lie in the extracellular space (E). × 4000.

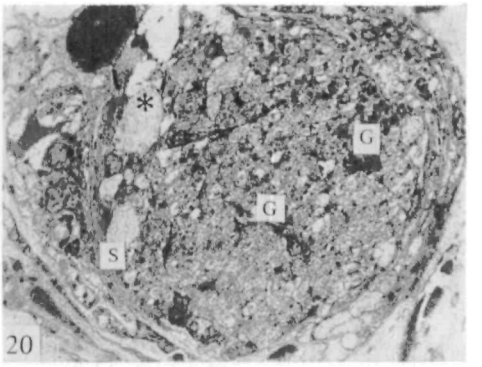


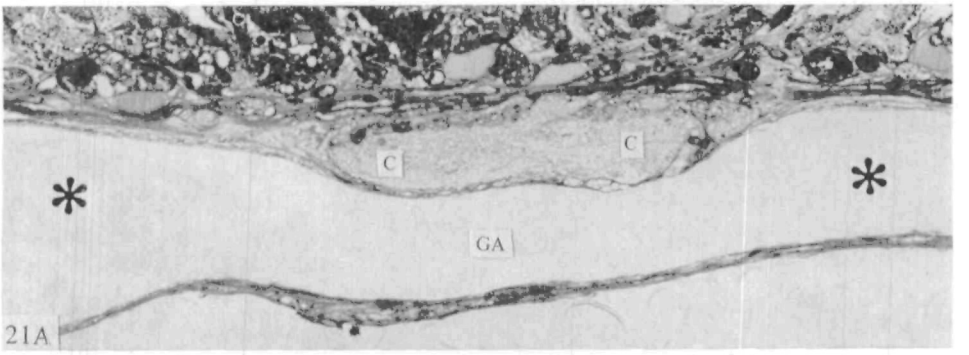
Figs. 16–18. For legends see p. 132.

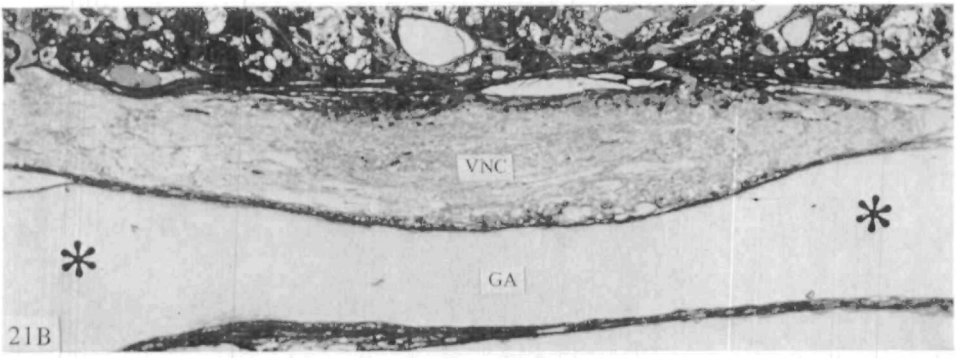


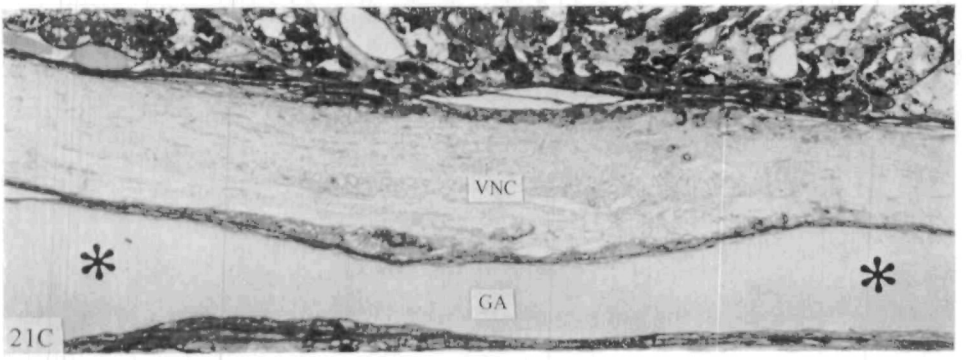
Figs. 13–15. For legends see opposite.



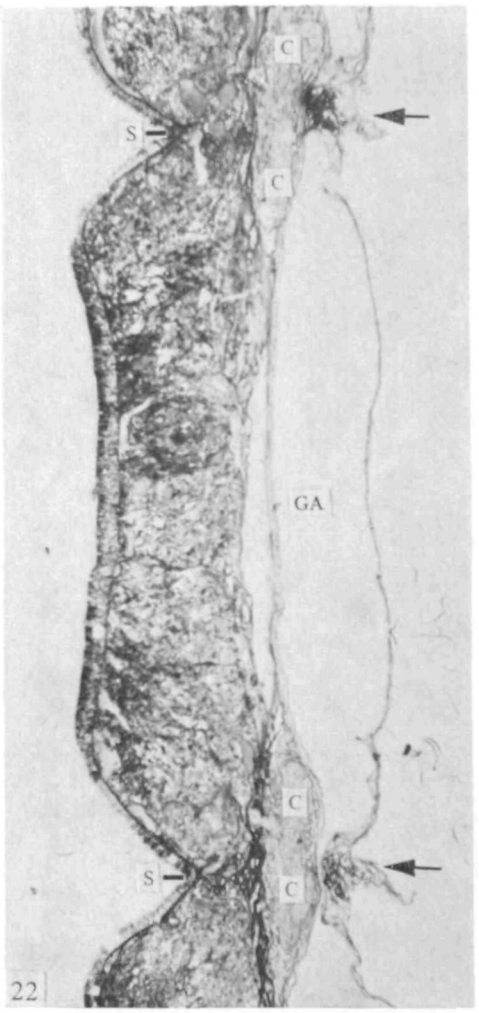


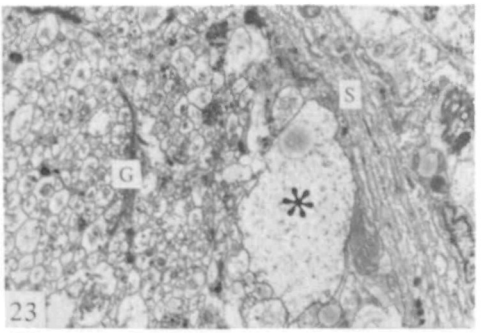


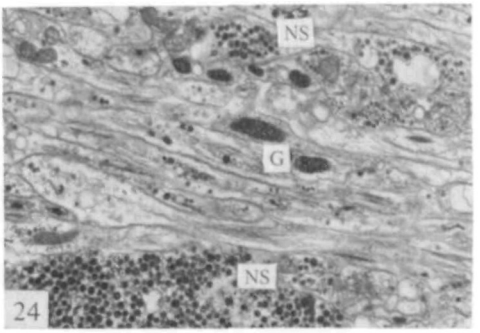


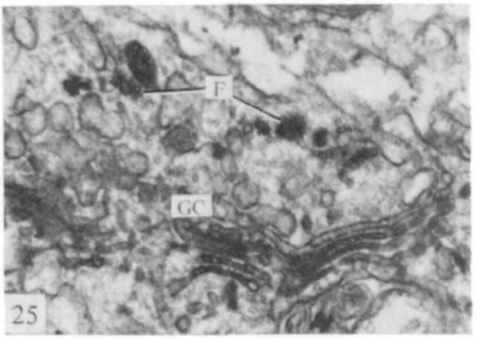


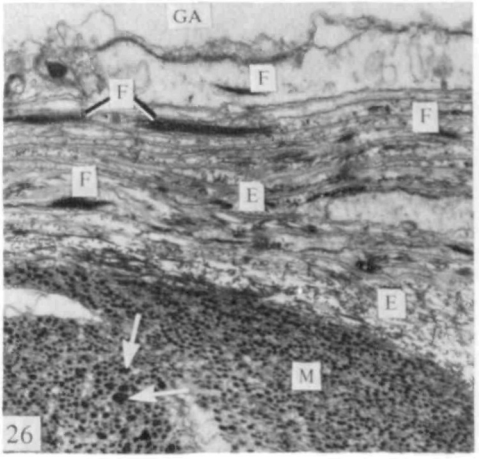
Figs. 19–21. For legends see p. 133.

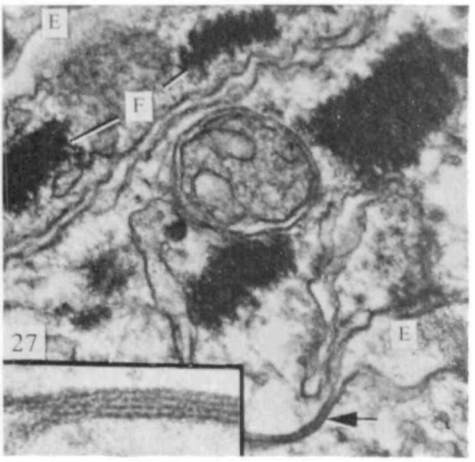












Figs. 22–27. For legends see opposite.

observations do not support the hypothesis that this transfer is mediated by chemical synapses. This is indicated by the spread of depolarizing and hyperpolarizing voltage changes between the giant axons, and the lack of effects of increased concentrations of magnesium and of cobalt ions in inter-axonal transmission. Furthermore, the effect of hyperpolarization of the indirectly stimulated axon in reducing the amplitude of the depolarizing prepotential is inconsistent with the expected behaviour of a conventional chemical synapse.

The histological evidence shows that the commissures which link the two halves of the central nervous system contain processes from both of the giant axons. These axonal processes are approximately 5–10 μm in diameter and are larger than the majority of the axons linking the nerve cords in the mid-body region. The presence of the axonal processes between the giant axons confirm the prediction of Hagiwara *et al.*

Fig. 19. Light micrograph of a cross-section through the commissures (C) that cross between and link the two ventral nerve cords, before they begin to become incorporated into the nervous tract. × 192.

Fig. 20. Electron micrograph of a commissure cut in cross-section, to show the numerous small axons as well as a peripherally located larger axon (*) which by its size could represent the contribution of the giant axon to the commissure. G, glial cells, S, sheath around axons and glia. × 1056.

Fig. 21. Light micrographs of longitudinal sections through the nerve cord and giant axon as the commissures (as seen in Fig. 19) fuse with one another (A) and become incorporated into the ventral nerve cord (VNC) (B) until their point of entry is no longer discernible (C) along the length of the nerve tract underlying the giant axon (GA). Note that the non-commissure regions (*) of the giant axons are larger than the point where the commissures enter. This flattening or constriction of the giant axon at the point of exit of its branch into the commissure is clearly seen in transverse sections in Fig. 13. (A), × 175; (B), × 175; (C), × 175.

Fig. 22. Light micrograph of a segment of *Sabella* showing the giant axon (GA) exhibiting constrictions (arrows) at points next to both the septa (S) in the body wall and the places of entry of the commissures (C) into the underlying ventral nerve cord (not yet sectioned into). This 'sausage-like' appearance has apparently been induced by injection with procion black, fixation taking place about 1 h after treatment. × 175.

The following figures are all electron micrographs from the nervous system of *Sabella*.

Fig. 23. Cross-section through part of one of the commissures that links the two lengths of ventral nerve cord. Note the large number of small axons, and one larger one (*) about 6 μm or more in diameter, which may be the branch that arises from the giant axon. Glial cells (G) are scattered amid the axon bundles, and a loose glial or perineurial investment interspersed with connective tissue ensheathes (S) the whole structure. × 4500.

Fig. 24. Longitudinal section through one of the cross-commissures between ventral nerve tracts. Axons laden with neurosecretory granules (NS) abound and glial cells (G) are characterized by intracellular bundles of filaments. × 8100.

Fig. 25. Glial cell body from the ventral nerve tract showing an active Golgi complex (GC) as is typical of these cells. F, small clumps of fibrillar material, characteristic of the glia throughout the nervous system. × 27600.

Fig. 26. Attenuated glial processes which contain bundles of fibrillae (F), lying around the giant axon (GA). Note the collagen-containing extracellular space (E) in which they lie and the muscle bundle (M) lying nearby (as in Fig. 18); arrows indicate its paramyosin filaments. × 13300.

Fig. 27. Glial cell processes at higher magnification to illustrate the fibrous intracellular bundles (F). The extracellular space (E) between the cells is usually extensive but occasionally it becomes reduced where the adjacent glial cells are associated by gap junctions (arrow). The insert shows one of these junctions at higher magnification to demonstrate the reduced 2–3 nm intercellular gap. × 54400; insert, × 199500.

(1964), that 'there must be some kind of fibre-like connexion between' the giant axons, and they provide a structural path for the action potentials recorded in the commissures during transmission between the giant axons in our study.

We lack detailed structural evidence as to the precise nature of the connexion represented by the processes between the giant axons. There are two possibilities. There could be direct cytoplasmic continuity, as occurs in the first order giant axons of the squid (Young, 1939), or there could be electrotonic communication across gap junctions such as occurs, for example, at septate gap junctions between the segmented giant axons of the crayfish (e.g. Perrachia, 1973*a*, *b*). The electrophysiological evidence does not support the latter possibility, since the agents which are known to uncouple electronic junctions in other nerve cells (Ca-free saline, dilute dinitrophenol and reduced external chloride concentration) were without effect on impulse transmission between the giant axons of *Sabbella*. It is difficult to attribute the lack of effect of these uncoupling agents to restricted intercellular access to the axonal surfaces, for previous electrophysiological observations indicate that there is no appreciable restriction to the intercellular diffusion of water-soluble ions and molecules to the axon surfaces in *Sabella* in isosmotic conditions (Treherne & Pichon, 1978). The present ultrastructural observations reveal no morphological evidence for restriction since the axons show only loose glial coverage, intercalated with connective tissue, with only occasional gap, not tight, junctions occurring between adjacent glial processes. The available evidence thus tends to favour the possibility that the processes within the commissures are in cytoplasmic communication with both of the giant axons.

The observation of impulse traffic within the commissures indicates that transmissions between the giant axons are not achieved solely, if at all, by passive spread of potential. Furthermore, the ten-to-one attenuation of electronic potentials between the axons also argues against the possibility of an exclusively passive spread of potential along the axonal processes linking the giant axons across the commissures. Thus depolarizing currents passed across an axonal membrane were ineffective in producing action potentials even when they generated voltage drops of 20 mV. The measured action potentials of 110 mV would therefore be insufficient to excite action potentials in the follower axon with the ten-to-one alteration of a passive potential spread between the axons.

As recognized by Hagiwara *et al.* (1964) (as a less favoured alternative to the hypothesis of chemically mediated transfer of impulses) the transmission delay could be due to conduction time of action potentials along the connecting processes between the giant axons. In the *Sabella* preparation the conduction time along the axonal processes (*ca.* 10 μm in diameter and 0·32 mm in length) can be roughly estimated by comparison with the measured conduction velocity (2·7 ms^{-1}) and diameter *ca.* 200 μm) of the giant axons. This yields an estimated conduction time for transmission along the axonal processes within the commissures of 0·53 m s^{-1}. This falls short of a minimal measured transmission delay of approximately 0·7 m s^{-1}. This discrepancy can be accounted for by the marginal nature of the transfer of excitation to the follower axon due to the very large size discrepancy between the very much smaller axonal processes and the giant axons themselves. Action potentials occurring along the axonal process within the commissures will be severely loaded as they approach the

expanded membrane area of the follower giant axon. Given that the space constant of the giant axons is around 7 mm and that the conduction velocity is 2·7 m s^{-1}, then it follows that at least five pairs of commissural axonal processes will be excited simultaneously within the transmission time of the action potential. We assume, therefore, that the overall density of injected current at the multiple sites on the follower axon are sufficient to overcome the reduction in safety factor imposed by the geometry of the system. Our findings that at least four pairs of intact commissures are necessary for inter-axonal transmission supports this interpretation. However, the marginal nature of the transmission between the commissural processes and the giant axons may also account for additional conduction delay between the measured action potentials in the directly stimulated and follower axons. Thus, the depolarizing pre-potential which precedes the follower action potential could be an electrotonic reflexion of the action potential within the processes which increases appreciably the transmission delay between the peaks of the action potentials in the directly stimulated and in the follower axon. This interpretation is supported by our observation that hyperpolarizing potential changes in the follower axon reduce the amplitude of the depolarizing pre-potentials while decreasing the latency of the maximal change of pre-potential (see Fig. 9). The above results would be expected if the imposed hyperpolarization blocks inter-axonal impulses at progressively greater distances from the recording site along the follower giant axon.

Bullock (1953) describes the transmission between the giant axons of sabellid worms as occurring 'in a synaptic manner, delaying and labile but unpolarized'. However, Hagiwara *et al.* (1964) showed, in *Eudistylia*, that repetitive stimulation of one giant axon caused fatigue in transmission but did not affect conduction in the opposite direction, an effect which was attributed to the properties of chemically mediated synapses. Our observations in *Sabella*, which indicate electrical transmission, also show that apparent polarization of impulse transmission can occur in certain conditions (see Fig. 4). These observations could be explained in terms of geometrical asymmetries in the system which are known to operate along equivalent electrically conducting pathways in other nervous preparations (cf. Tauc & Hughes, 1963; Mellon & Kaars, 1974; Spira, Yarom & Parnas, 1976).

An important aspect of inter-axonal transmission in *Sabella* is the appreciable constriction of the giant axons, in the septate regions, where the axonal processes originate (Figs. 13C and 22). The extreme narrowing of the diameter of the giant axons would be expected to increase current density from action potentials within the processes and, consequently, the efficacy of post-synaptic currents generated by other central neurones in this region. It is probably significant that numerous nerve terminals are present, in the constricted region of the giant axon, which contain dense and hollow core vesicles (Fig. 17). It is conceivable, therefore, that transmission between the giant axons could be influenced by synaptic inputs and could, thus, provide an explanation for the otherwise inexplicable observations of Bullock (1953) who described the interactions between the giant axons as being mediated by 'quasi-artificial synapses', in which transmission occurs 'at a localized spot, but this spot may shift smoothly along, millimetres in seconds'.

The available evidence shows that the co-ordinated activities of the two giant axons in sabellids is achieved in the brain by a single anastomosis (Nicol, 1948*a*;

Bullock, 1953) and by electrical transmission, apparently, in each segment of the body. As in the earthworm (Wilson, 1961), the presence of the segmental transmission between the giant axons ensures effective synchronization of impulse traffic initiated in any region of the body and, thus, co-ordination of muscular contractions during rapid withdrawal responses of the worm. Furthermore, this segmental transmission preserves the co-ordinated body contractions even in portions of truncated worms, a mechanism which is likely to be of selective advantage in animals capable of body regeneration.

This research was supported in part by a grant from the U.S. European Research Office.

REFERENCES

ASADA, Y. & BENNETT, M. V. L. (1971). Experimental alteration of coupling resistance at an electrotonic synapse. *J. Cell Biol.* **49**, 159–172.

BASKIN, D. (1971). The fine structure of neuroglia in the central nervous system of vereid polychaetes. *Zeit. Zellforsch.* **119**, 295–308.

BULLOCK, T. H. (1953). Properties of some natural and quasi-artificial synapse in polychaetes. *J. comp. Neurol.* **98**, 37–68.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates.* San Francisco and London: Freeman.

CARLSON, A. D., PICHON, Y. & TREHERNE, J. E. (1978). Effects of osmotic stress on the electrical activities of the giant axon of a marine osmoconformer, *Sabella penicillus*. *J. exp. Biol.* **75**, 237–251.

DORSETT, D. A. (1978). Organization of the nerve cord. In *Physiology of Annelids* (ed. P. J. Mill), pp. 115–160. London and New York: Academic Press.

HAGIWARA, S., MORITA, H. & NAKA, K. (1964). Transmission through distributed synapses between two giant axons in a sabellid worm. *Comp. Biochem. Physiol.* **13**, 453–460.

MELLON, D. & KAARS, C. (1974). Role of regional cellular geometry in conduction of excitation along a sensory neuron. *J. Neurophysiol.* **37**, 1228–1238.

NICOL, J. A. C. (1948*a*). The giant axons of annelids. *Q. Rev. Biol.* **23**, 291–323.

NICOL, J. A. C. (1948*b*). The giant nerve fibres in the central nervous system of *Myxicola* (Polychaeta, Sabellidae). *Q. Jl. microsc. Sci.* **89**, 1–45.

NICOL, J. A. C. & YOUNG, J. Z. (1946). Giant nerve fibre of *Myxicola infundibulum* (Grube) *Nature Lond.* **158**, 167–168.

PERRACHIA, C. (1973*a*). Low resistance junctions in crayfish. I. Two arrays of globules in junctional membranes. *J. Cell Biol.* **57**, 54–65.

PERRACHIA, C. (1973*b*). Low resistance junctions in crayfish. II. Structural details and further evidence for intercellular channels by freeze-fracture and negative staining. *J. Cell Biol.* **57**, 66–76.

PERACCHIA, C. & DULHUNTY, A. F. (1976). Low resistance junctions in crayfish. Structural changes with functional uncoupling. *J. Cell Biol.* **70**, 419–439.

SPIRA, M. E., YAROM, Y. & PARNAS, I. (1976). Modulation of spike frequency by regions of special axonal geometry and by synaptic inputs. *J. Neurophysiol.* **39**, 882–899.

TAUC, L. & HUGHES, G. M. (1963). Modes of initiation and propagation of spikes in the branching axons of molluscan central neurons. *J. gen. Physiol.* **46**, 533–549.

TREHERNE, J. E. & PICHON, Y. (1978). Long-term adaptations of *Sabella* giant axons to hyposmotic stress *J. exp. Biol.* **75**, 253–263.

TURIN, L. & WARNER, A. (1977). Carbon dioxide reversibly abolishes ionic communication between cells. of early amphibian embryo. *Nature, Lond.* **270**, 56–57.

WILSON, D. M. (1961). The connections between the lateral giant fibers of earthworms. *Comp. Biochem. Physiol.* **3**, 274–284.

YOUNG, J. Z. (1939). Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. *Phil. Trans.* **229**B, 645-503.