

RADIAL SYMMETRY AND THE ORGANIZATION OF CENTRAL NEURONES IN A HYDROZOAN JELLYFISH

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Accepted 11 November 1983

SUMMARY

1. Two discrete networks of neurones in the outer nerve-ring of *Polyorchis penicillatus* can be identified by their physiological and morphological characteristics.

2. The 'B' system is characterized by the regular, spontaneous firing pattern that can be recorded intracellularly. Bursts of up to six spikes are produced in response to a rapid reduction in the light intensity.

3. Neurones of the 'B' system are electrically coupled to one another.

4. Action potentials in the 'B' system produce unitary EPSPs in swimming motor neurones and in epithelial cells overlying the outer nerve-ring.

5. Lucifer Yellow injected into a 'B' neurone diffuses rapidly through neighbouring neurones to reveal a condensed network of neurones in the centre of the nerve-ring and a more diffuse network passing up and around each tentacle.

6. The 'O' system is characterized by very regular (approx. 1 Hz), spontaneous membrane potential oscillations. Action potentials are never recorded.

7. Neurones of the 'O' system are electrically coupled to one another.

8. There is evidence of interaction between the 'O' system and swimming motor neurones.

9. Lucifer Yellow injected into an 'O' neurone diffuses through member neurones to show an anastomosing network of neurones extending across the width of the outer nerve-ring and tracts of neurones extending up the sides of each tentacle towards the ocelli.

10. The restriction of injected Lucifer Yellow to each of the networks and the blockade of interaction between systems by Mg^{2+} anaesthesia are evidence that signalling between different central networks is by chemical means.

11. The adaptive advantages of this type of functional organization of central neurones in radially symmetrical animals are discussed. Such an organization is compared with that found in bilateral animals.

INTRODUCTION

There is a wealth of information concerning the organization of neurones in the central nervous systems of bilaterally symmetrical animals, particularly the

protostomatous invertebrates. Present concepts of cellular organization in the central ganglia of the major protostome phyla (i.e. Annelida, Arthropoda and Mollusca) are derived from recent studies involving physiological characterization of individual neurones and subsequent morphological identification by intracellular iontophoresis of dyes. Such studies have generated the paradigm that invertebrate central nervous systems are constructed from identifiable cells or populations of such cells having similar functions. In most cases, these cells are bilaterally paired and homologous in related species (Beltz & Kravitz, 1983; Kramer & Goldman, 1981). Also, it is often possible to recognize homologous sets of neurones repeated in each segmental ganglion (Zipser, 1982). This applies particularly to motor neurones (Ort, Kristan & Stent, 1974) but it is also true of interneurones (Robertson & Pearson, 1983) and sensory neurones (Nicholls & Baylor, 1968; Yau, 1976). Local, unpaired, medial neurones are fairly common (Hoyle, Dagan, Moberly & Colquhoun, 1974; Evans & O'Shea, 1978), whereas asymmetrically paired or unilateral neurones are very rare and normally associated with a gross morphological asymmetry (Chapple, 1977; Mellon, Wilson & Phillips, 1981). The overall organization of central neurones in protostomes is a consequence of bilaterality and cephalization in animals that display a predominantly, anteriorly-directed locomotion.

In contrast, very little is known about the arrangement or functional connectivity of central neurones in radially symmetrical animals (e.g. Cnidaria, Ctenophora and adult echinoderms). *A priori* reasoning might predict a rather different arrangement of central neurones for animals whose locomotion is less polarized. While there may be a difference in the modality or degree of sensory stimulation for oral and aboral surfaces in most active, radially symmetrical animals the probability of receiving sensory stimulation is almost equally distributed throughout 360° in a horizontal plane. In addition the absence of bilaterally arranged locomotory structures can be expected to influence strongly the organization of central neurones in the Radiata.

Recently, with the introduction of the dye Lucifer Yellow, three neuronal populations have been identified in the Cnidaria: (1) swimming motor neurones in *Polyorchis* and other hydromedusae (Anderson & Mackie, 1977; Spencer, 1981; Satterlie & Spencer, 1983), (2) motor giant neurones in the trachymedusan *Aglantha* (Roberts & Mackie, 1980) and (3) the giant motor nerve-net in the scyphozoans *Carybdea* and *Cyanea* (Satterlie & Spencer, 1979; Schwab & Anderson, 1980; Anderson & Schwab, 1981). The motor networks of the hydrozoans *Polyorchis* and *Aglantha* are electrically and dye coupled. This is in contrast to the scyphozoans, *Carybdea* and *Cyanea*, where neurones forming the motor-net are connected by reciprocal chemical synapses (Satterlie, 1979; Anderson & Schwab, 1983). As yet, neurones whose major function is to transmit and integrate sensory information have not been identified both physiologically and morphologically in the Cnidaria. Furthermore, neurones which are presynaptic to motor neurones have not been morphologically characterized despite their presumed existence (Anderson & Mackie, 1977; Spencer, 1981).

A considerable amount of evidence has accumulated to suggest that the central nervous system of hydromedusae (Fig. 1B) is functionally partitioned into a motor centre, the inner nerve-ring and its radial projections, and a centre chiefly concerned with integrating sensory information, the outer nerve-ring. Much of this evidence comes from histological studies (Hertwig & Hertwig, 1878; Hyde, 1902; Little, 1911).

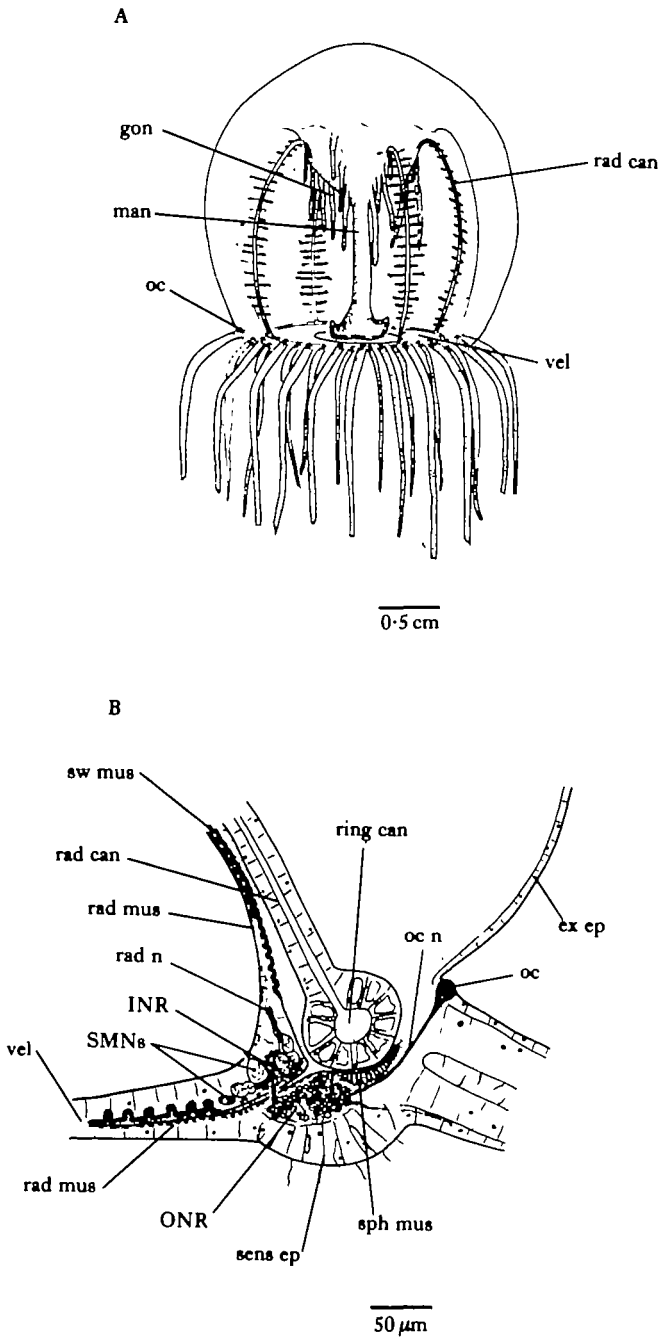


Fig. 1. Illustrations to show the gross morphology of *Polyorchis penicillatus* and the organization of nervous tissue in the margin. (A) A drawing of a medium-sized specimen of *Polyorchis*; the tentacles are truncated. (B) Semi-diagrammatic radial section through the margin in the region of a tentacle and radial canal. ex ep, exumbrellar epithelium; gon, gonad; INR, inner nerve-ring; man, manubrium; oc, ocellus; oc n, ocellar nerve; ONR, outer nerve-ring; rad can, radial canal; rad n, radial nerve; rad mus, radial muscle; ring can, ring canal; sens ep, sensory epithelium; SMNs, swimming motor neurons; sph mus, sphincter muscle; sw mus, swimming muscle; vel, velum.

Mackie, 1971; Spencer, 1979; Singla & Weber, 1982) which demonstrated a morphological division. Neurites have been seen crossing the mesogloal lamella which separates the inner and outer nerve-rings (Mackie, 1971; Singla, 1978; Spencer, 1979). The motor role of the inner nerve-ring is based on direct physiological evidence, whereas evidence for the integratory function of the outer nerve-ring is circumstantial (Spencer & Schwab, 1982).

In this study, we report two identifiable groups of central neurones in the outer nerve-ring of a hydromedusa, *Polyorchis penicillatus*. These two neuronal systems are identifiable using both morphological and physiological criteria. One of these neuronal systems is presynaptic to the swimming motor neurones while retaining a separate motor function, the other population does not have any obvious motor role and appears to be composed of first or second order photosensory neurones.

MATERIALS AND METHODS

Medusae of the hydrozoan *Polyorchis penicillatus* (Fig. 1A) were collected from Bamfield Inlet on the west coast of Vancouver Island, Canada. They were held in running sea water at 11–15°C until used, which was normally within 3 days, except for some dye-filling experiments when jellyfish were air-freighted to Edmonton. Experiments were conducted at room temperature, 18–21°C, on medium-sized medusae with bell diameters of from 8 to 25 mm.

Jellyfish were bisected and the apex of the bell removed down to the level of the attachment of the manubrium. If unanaesthetized animals were to be used, the radial muscles associated with each of the two remaining radial canals were transected close to their junction with the sphincter muscle at the margin. This reduced movements of the margin during crumpling. The radial muscles were left intact in anaesthetized jellyfish. Additionally, in most experiments all the tentacles were removed by cutting them just distal to the ocelli without damaging the ocelli or their nerves. In a few experiments the ocelli were excised, which necessitated removing a strip of exumbrellar mesogloea.

Anaesthetic media were prepared by mixing natural sea water with isotonic (0.33 M) MgCl₂. Normally, equal volumes of each were used, but occasionally a greater proportion of MgCl₂ was added: when used, such mixtures are specified in the text. Natural sea water was used when anaesthesia was not required.

Jellyfish were pinned, subumbrella-side down, to a Sylgard (Dow Corning) base formed in the bottom of a glass Petri dish by stainless steel pins and spines from the fruit of *Opuntia* sp. so that the outer nerve-ring could be seen through a dissecting microscope. Preparations were obliquely illuminated from below with a fibre-optic system and quartz-halogen light source. Photic stimulation was given by turning this light source on and off (1100 to <0.1 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$). All light experiments were done in a light-tight enclosure.

Conventional intracellular recording techniques were used as previously described (Spencer, 1981). The intracellular dye Lucifer Yellow CH was iontophoresed by continuous hyperpolarizing current of between 1 and 10 nA for variable periods up to 10 min. Micropipettes used for dye injection were tip-filled with a 3–5% solution of Lucifer Yellow and then back-filled with 1 M-LiCl₂. Useful electrodes filled in the

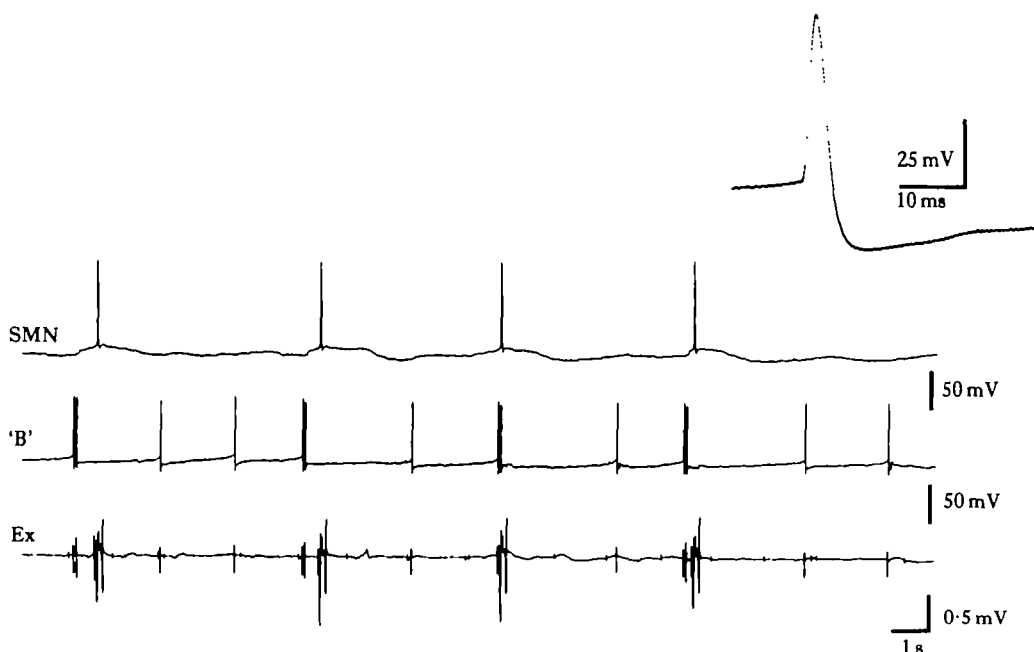


Fig. 2. Relationship between extracellularly and intracellularly recorded 'B' spikes. The middle trace shows spontaneous electrical activity recorded intracellularly in a 'B' neurone. An intracellularly recorded spontaneous 'B' spike is shown inset at high sweep speed. Pairs of 'B' spikes excite the swimming motor neurones sufficiently to cause firing (upper trace). The lower trace (Ex) is a suction electrode recording from the outer nerve-ring which records 'B' spikes that are time-locked with the intracellularly recorded spikes. In addition, the suction electrode records SMN spikes, swimming muscle action potentials and an unidentified impulse type.

way had resistances of 60–150 M Ω . Lucifer Yellow filled preparations were viewed live, with isotonic MgCl₂ being added to reduce movements when necessary. A mercury-vapour lamp with a BG 12 excitation filter was used as the u.v. source and a 500 nm cut-off barrier filter inserted in the microscope tube for viewing and photographing on 400 ASA black and white film. Drawings of neuronal networks were made by tracing the outlines of projected images of the negatives.

Extracellular recording was *via* a.c.-coupled, differential, plastic suction electrodes which used silver wire as the conductor.

RESULTS

Physiology of the 'B' system

While recording with extracellular suction electrodes attached to the outer nerve-ring (Fig. 1B), it is usually possible to detect short duration, biphasic potentials that produce unitary EPSPs in the swimming motor neurones (Anderson & Mackie, 1977; Spencer, 1981). These spikes occur spontaneously and continuously at fairly regular intervals, as single spikes, pairs, or bursts of up to six spikes (Fig. 2). In a previous study (Spencer, 1978), they were correlated with contractions of the longitudinal muscle of the tentacles. It is apparent that the spikes produced by this system are synonymous with marginal pulses – MPs (Passano, 1965), 'nerve-like' marginal pulses

– nMPs (Ohtsu & Yoshida, 1973), tentacle contraction pulses – TPs (Mackie, 1975) and pre-tentacle contraction pulses – PTPs (Spencer, 1975) recorded from other hydromedusae. The degree of synchronous tentacle contraction is dependent on the frequency and number of spikes in each burst. Single, intermittent spikes produce either a contraction localized in the tips of tentacles or no observable contraction (Spencer, 1978).

These extracellularly recorded spikes can be correlated with action potentials recorded simultaneously by microelectrodes penetrating member neurones of an identifiable network in the outer nerve-ring. We have called these neurones the 'B' system (Fig. 2). Neurones of the 'B' system have a mean resting potential of -40 mV and give action potentials with amplitudes of 75 – 80 mV and durations of approximately 5 ms (Fig. 2). A 12 – 20 mV hyperpolarizing phase lasting as much as 70 ms follows each spike. These after-hyperpolarizations can sum during a spontaneous burst so as to

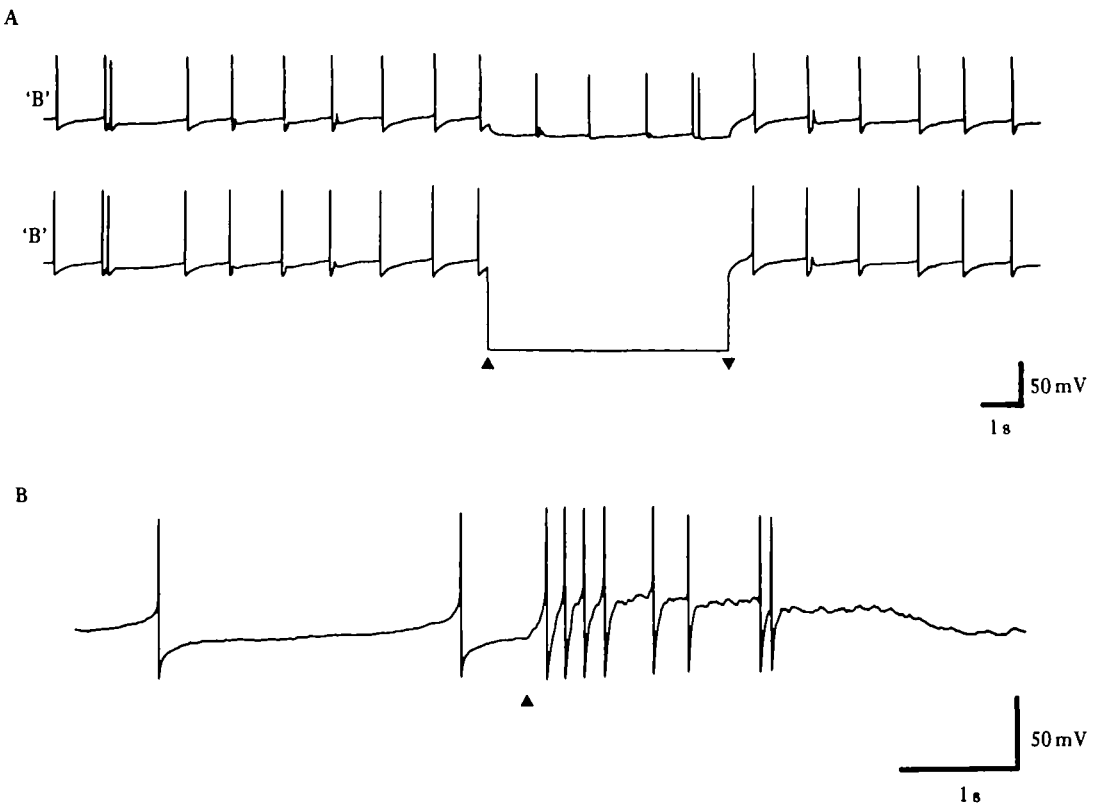


Fig. 3. Electrical coupling between member neurones of the 'B' system and a shadow-induced burst of 'B' spikes. (A) Recordings of spontaneous activity in two 'B' neurones $380 \mu\text{m}$ apart. A 3 nA hyperpolarizing current pulse was injected into one neurone at the arrow and a corresponding hyperpolarization was recorded in the second neurone (upper trace). The bridge circuit could not be balanced in the injected cell. Note the synchronous firing of the two 'B' neurones. (B) A shadow-induced burst of 'B' spikes recorded intracellularly. The stimulus was provided by turning the illuminating light off at the arrow. The change in light intensity was not quantified. Note the prolonged depolarization underlying the burst of spikes which appears to be composed of summed EPSPs.



Fig. 4. 'B' spikes produce EPSPs in both swimming motor neurones and epithelial cells. Simultaneous intracellular recording from an epithelial cell overlying the outer nerve-ring (upper trace, Ep) and an SMN (middle trace); the electrodes are separated by about $200\ \mu\text{m}$. A suction electrode monitors the outer nerve-ring (Ex) and records 'B' spikes (starred). This electrode also records one SMN spike and muscle action potential, and three epithelial action potentials. Note that the summing EPSPs in the SMN result in an action potential but that the EPSPs in the epithelial cell do not sum sufficiently to cause spiking. Epithelial spiking occurs after a swimming contraction and appears to be excited by some other mechanism. Epithelial spikes are associated with a long duration hyperpolarization in the SMNs.

delay generation of the next action potential. Thus the time of appearance of the next spike will depend on the number and frequency of spikes in a burst. Neurones of the 'B' system are electrically coupled to one another, as can be demonstrated by passing current into one neurone and recording the potential change in a neighbouring neurone. Only three such experiments were performed because of the difficulty of penetrating pairs of neurones. In one experiment, the distance between the recording electrode and the current-injecting electrode was $380\ \mu\text{m}$ and in others it was $430\ \mu\text{m}$ and $520\ \mu\text{m}$. The coupling is quite strong since a current pulse of $3\ \text{nA}$ produced a deflection of $17.5\ \text{mV}$ in the distant cell at an interelectrode separation of $380\ \mu\text{m}$. Thus, it is not surprising that spiking in member neurones is synchronous (Fig. 3A). Similarly, suction electrode recordings show that all 'B' neurones throughout the outer nerve-ring are normally spiking at the same time and that only occasionally can different firing patterns be recorded at different sites around the margin. On those occasions when there was no evidence of synchronous firing at different sites, physical damage to the outer nerve-ring was suspected and there was no evidence of electrical coupling. The observation that intracellularly-injected Lucifer Yellow diffuses rapidly

through the 'B' system corroborates the evidence for electrical coupling (see next section).

Although pairs of spikes can arise spontaneously, bursts of three or more spikes are more usually associated with photic stimulation. The most effective stimulus is to shadow rapidly the margin and ocelli by turning off the light-source. A burst of 'B' system spikes follows a shadow with a latency of approximately 300 ms (Fig. 3B). Preceding and throughout a shadow-induced burst there is a barrage of EPSPs which sum to produce a depolarization that lasts for at least the duration of the burst. After such a burst of spikes, the 'B' system is silent for a short period, but there is not a strong hyperpolarization as is seen after spontaneous bursts. If the light remains 'off' after a shadow, it may be as much as 30 s before the system returns to its former firing frequency. The 'bursting' response to a shadow is completely obliterated if all the tentacles are cut off at their bases so as to remove the ocelli.

Spikes in the 'B' system produce EPSPs in both the swimming motor neurones and the epithelial cells overlying the outer nerve-ring (Fig. 4). Although most intracellular recordings from SMNs show these EPSPs, they are not seen in some cells. In these cases, it must be assumed that the recording electrode is not close enough to 'B' neurone/SMN synapses for the EPSPs to be of sufficient amplitude to be resolved. In other cases, it may not be possible to resolve unitary EPSPs in a burst. The latency of EPSPs from the peak of the 'B' spike to the first appearance of the resulting EPSPs varies from 5 to 8 ms. Some of this variation is probably due to differences in the separation of the recording electrodes. Nevertheless, this distance was never more than 150 μm in experiments used to calculate synaptic latency. Thus, assuming a

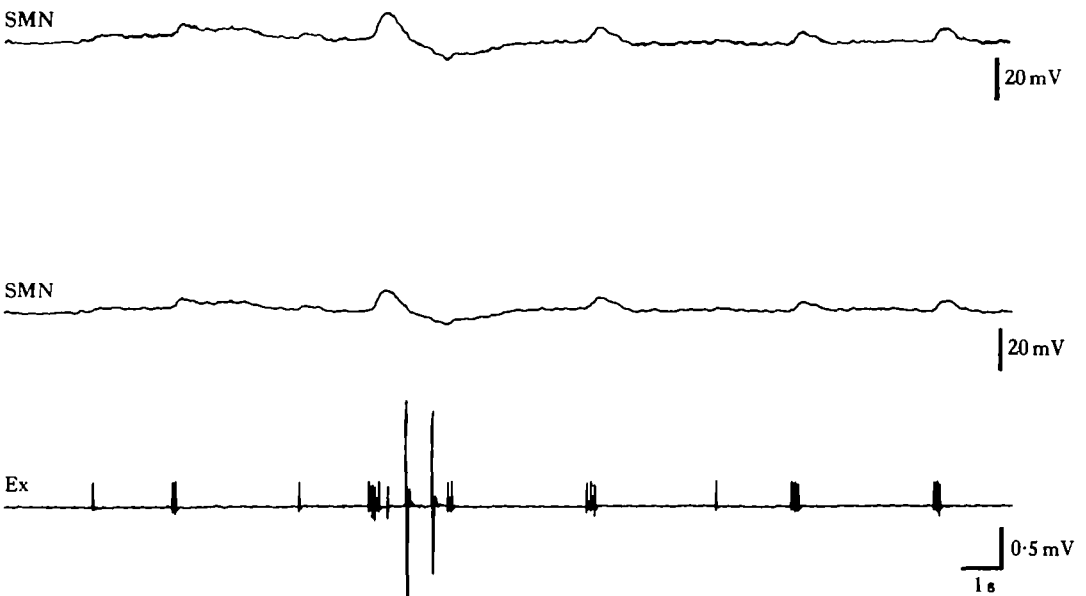


Fig. 5. Swimming motor neurones receive common excitatory input from the 'B' system even at fairly widely separated sites. The top two traces are records of spontaneous subthreshold activity from two SMNs separated by approximately 1 mm. The lower trace (Ex) is from a suction electrode simultaneously monitoring activity in the outer nerve-ring; the 'B' spikes are the lower amplitude spikes and the two large amplitude spikes are epithelial spikes which cause hyperpolarizations in the SMNs.

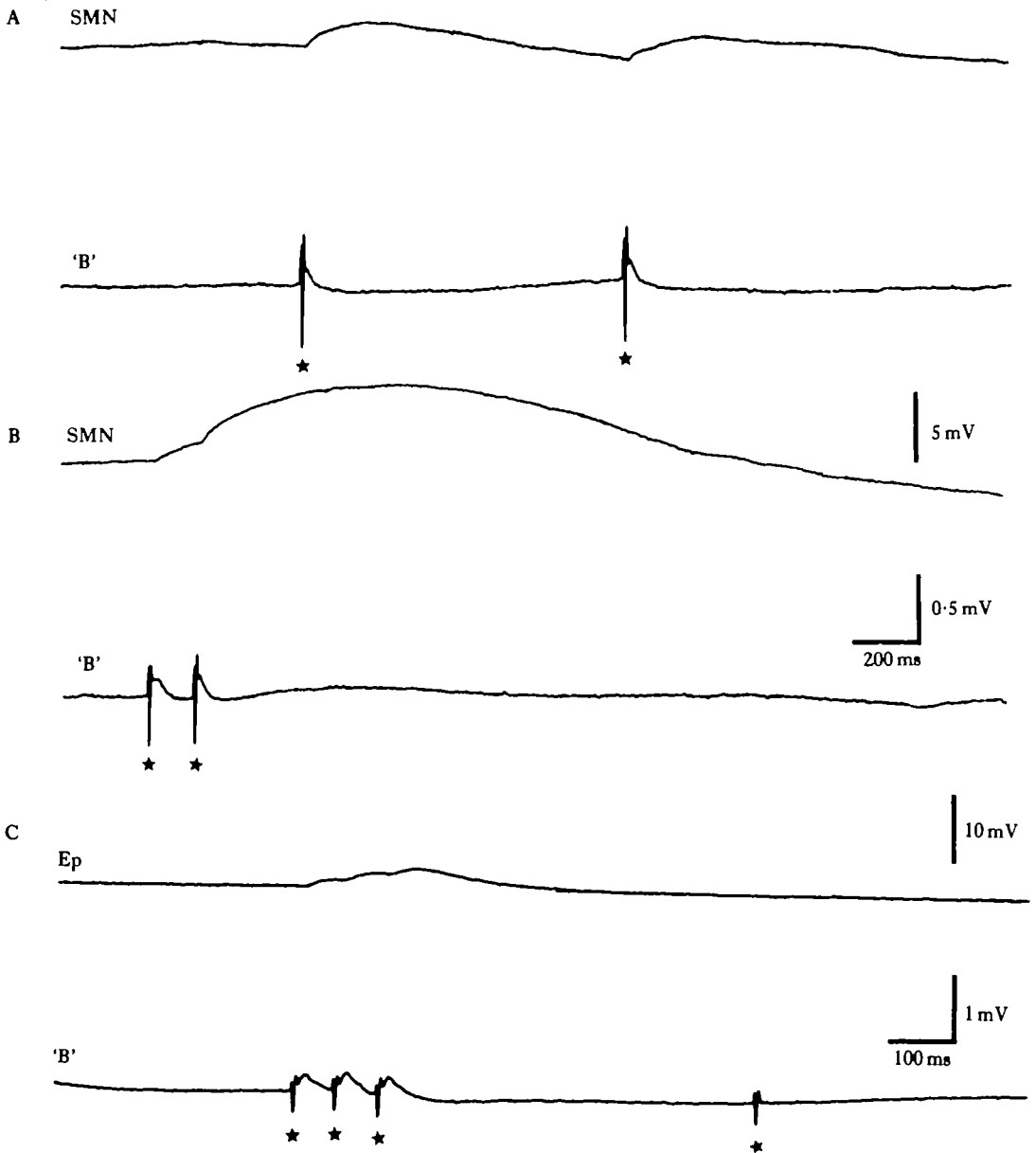


Fig. 6. Time course of 'B' spike-induced EPSPs in SMNs and epithelial cells. (A) There is no summation or facilitation of EPSPs if the interval between 'B' spikes (starred) is greater than about 1 s. (B) When 'B' spikes occur in pairs (or bursts) where the interval is short, there is noticeable summation and facilitation. SMN recordings are intracellular, upper traces in (A) and (B), while the recordings of 'B' spikes are with suction electrodes. The inter-electrode distance was less than 2 mm in both cases. (C) 'B' spikes (starred) produce shorter duration EPSPs in epithelial cells (Ep). EPSPs are only seen when the 'B' spikes have a following depolarization which is probably a smooth muscle potential (see text). 'B' spikes alone are not associated with EPSPs.

conduction velocity of approximately 50 cm s^{-1} for the 'B' spike (PTP conduction velocity: Spencer, 1978), the error due to conduction time should not be greater than 0.3 ms. Some of the variation may also be due to differences in the distance of the electrode recording EPSPs from the closest active synapse (Spencer, 1981). The maximum amplitude of unitary EPSPs is approximately 10 mV. Even in fairly widely separated SMNs, it is possible to record common EPSP activity resulting from 'B' system spikes (Fig. 5). Facilitation of the postsynaptic potential is the rule (Fig. 6A, B), so that while single 'B' spikes may not produce an EPSP, the second of a pair of spikes will give a large EPSP. Because these EPSPs decay quite slowly (mean duration of 1.3 s), a burst of presynaptic spikes will give a summing staircase of depolarization that is often sufficient to cause the SMN network to fire (Figs 2, 4).

Bathing a preparation with an anaesthetic solution containing 50 % isotonic MgCl_2 for more than 15 min does not disrupt the spontaneous rhythm of 'B' spikes yet abolishes the EPSPs in SMNs that are associated with spikes in the 'B' system.

'B' system spikes can also be correlated with EPSPs in ectodermal epithelial cells. Such EPSPs can be recorded locally from cells overlying the nerve-ring (Fig. 4). They have a mean amplitude of 1.5 mV and duration of 145 ms. The decay of EPSPs in epithelial cells is more rapid than in SMNs (Fig. 6C). The latency of EPSPs from 'B' spikes could not be measured accurately since it was not possible to position the intracellular microelectrode sufficiently close to the suction electrode recording 'B' spikes to avoid errors due to conduction time of the 'B' spike. In addition, the conduction direction of the spike was unknown. The functional significance of this connection is not obvious since epithelial action potentials do not normally result from these EPSPs, even though these epithelial cells are capable of producing overshooting potentials (Fig. 4). However, it is probably significant that EPSPs are only seen in epithelial cells when 'B' spikes (that are recorded extracellularly) are associated with an after-depolarization (Fig. 6C). This after-depolarization has been suggested to be the resulting muscle potential from either sphincter muscle or velar radial muscle (Spencer, 1978). Spiking in epithelial cells appears to result from some other source of excitatory input and is usually superimposed on an underlying slow depolarization (Fig. 4). A general contraction of all the radial muscles in the jellyfish accompanies such a burst of epithelial action potentials. This has been described previously in *Polyorchis* as 'crumpling' (Spencer, 1978; King & Spencer, 1981). A long duration, large amplitude IPSP in swimming motor neurones is usually associated with these epithelial action potentials (Spencer, 1981 and Figs 4 and 5).

Morphology of the 'B' system

After physiological characterization of the 'B' system, attempts were made to fill the neurones of the system with Lucifer Yellow. Five such preparations were successfully filled, one of which is shown in Fig. 7A. A generalized drawing of a portion of the 'B' system neurones (Fig. 8) has been synthesized from all the negatives obtained. Lucifer Yellow iontophoresed into a neurone of the 'B' system diffuses rapidly through all the member neurones of the system for several millimetres either side of the injection point. 'B' neurones form a condensed anastomosing network of mostly bipolar cells that is located in the centre of the outer nerve-ring. A diffuse network of bi- and multipolar neurones can be seen to pass from more central, condensed part

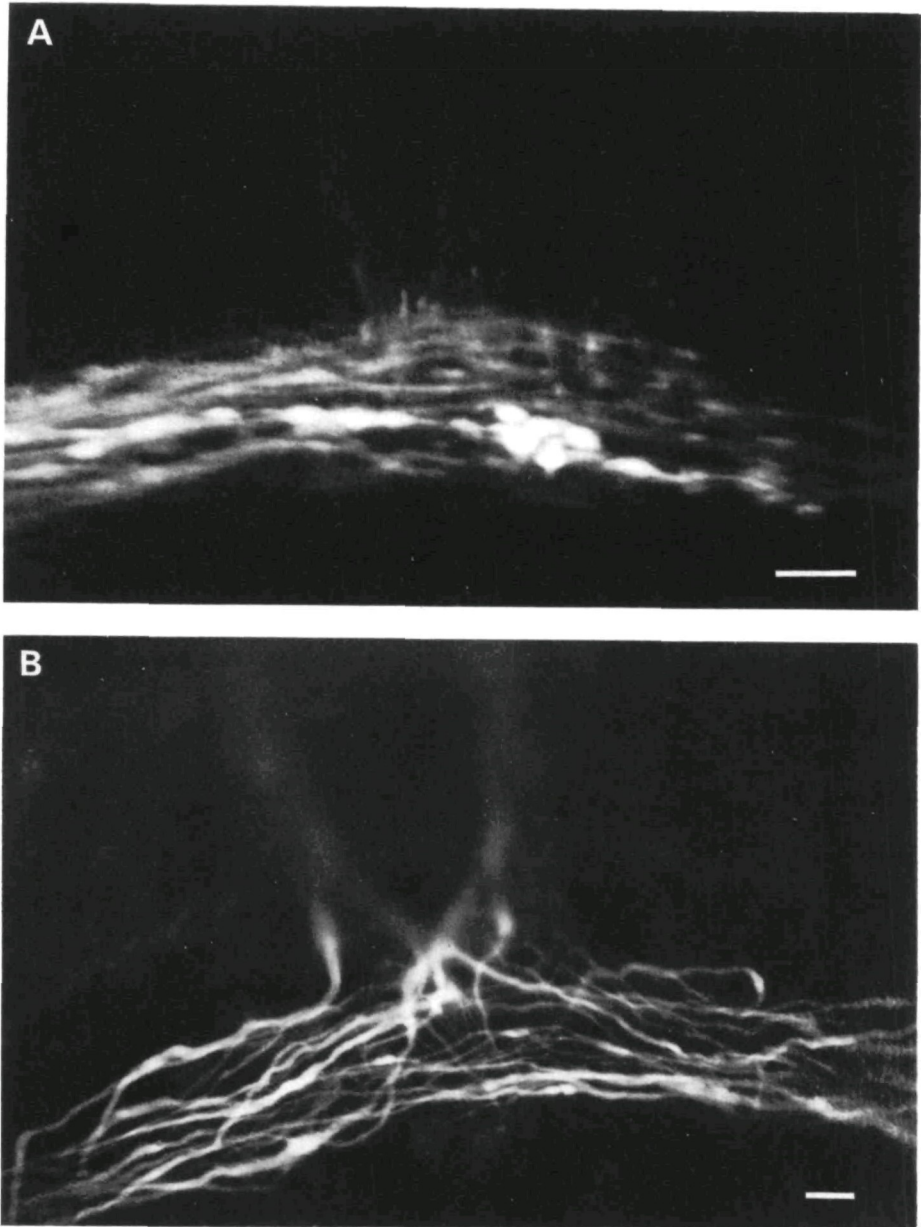


Fig. 7. Lucifer Yellow filled 'B' and 'O' networks in live, anaesthetized preparations. (A) Shows a portion of the 'B' network in the region of a tentacle. A diffuse network of neurones can be seen passing up a tentacle from the more centralized portions of the network in the outer nerve-ring. Scale bar = 10 μm . (B) A section of the 'O' network is shown in the region of a tentacle. Note the neurones passing up the sides of the tentacle in discrete tracts and the processes passing out onto the velum. Scale bar = 10 μm .

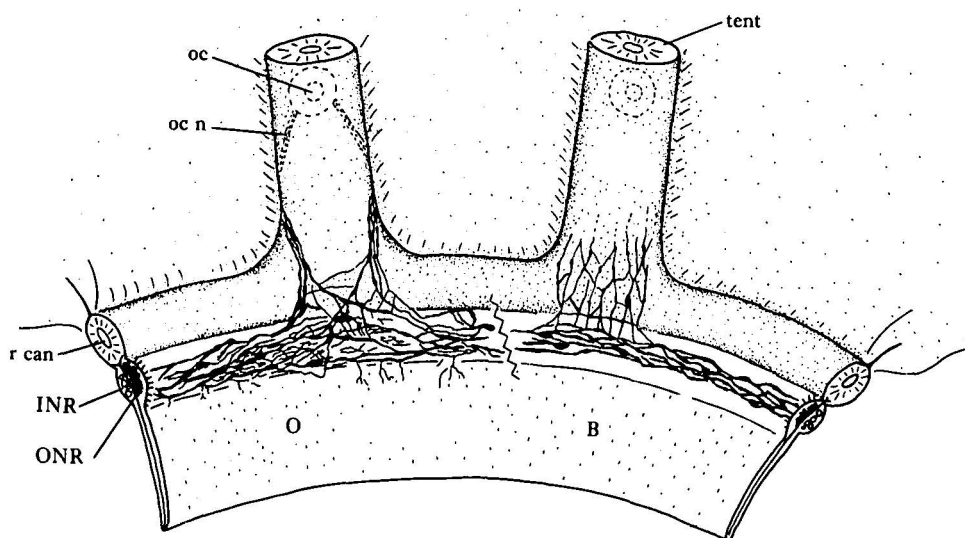


Fig. 8. Arrangement of the 'B' and 'O' networks in the outer nerve-ring to show their distribution with respect to each other and the tentacles. To the left of the zig-zag line, only the 'O' system has been illustrated, while to the right only the 'B' system is shown. The dotted line represents the presumed projection of the tracts of 'O' neurones in the tentacles. B, 'B' network; INR, inner nerve-ring; O, 'O' network; oc, ocellus; oc n, ocellar nerve; ONR, outer nerve-ring; r can, ring canal; tent, tentacle.

of the network within the nerve-ring to the tentacles. This distal portion of the 'B' system is localized within the ectoderm. It has been traced no further than the bases of tentacles. Neurones of the 'B' system are situated relatively superficially in the outer nerve-ring since they are penetrated just beneath the epithelial-cell layer.

Physiology of the 'O' system

Also within the outer nerve-ring is a population of neurones having some unusual but characteristic physiological properties. The spontaneous electrical activity that can be recorded from these cells is quite unlike that recorded from any other cnidarian neurone (Figs 9–12). Typically, it consists of periods when the membrane potential shows very regular depolarizing oscillations interspersed with periods when the resting potential remains fairly constant. The mean amplitude of these oscillations is 20 mV with a range of 2–38 mV (calculated from 115 examples in twelve animals). Resting potentials of between -40 and -75 mV have been measured. The level of the resting membrane potential influences the shape of the oscillations so that in cells with resting potentials more positive than about -55 mV, the depolarizations have rounded bases, while in cells with potentials greater than -55 mV, the oscillations are triangular, of lower amplitude and arise from a flat baseline. It does not appear that low resting potentials are the result of penetration damage since resting potentials have not been seen to change noticeably with time after penetration. The mean frequency of oscillations is 0.9 Hz (range of 0.5–1.1 Hz), and their duration averages 770 ms (range 150–1400 ms). These figures were calculated by only considering those portions of a recording in which the oscillations were regular. Often the frequency of oscillations decreases during a rhythmic period and then increases considerably at the

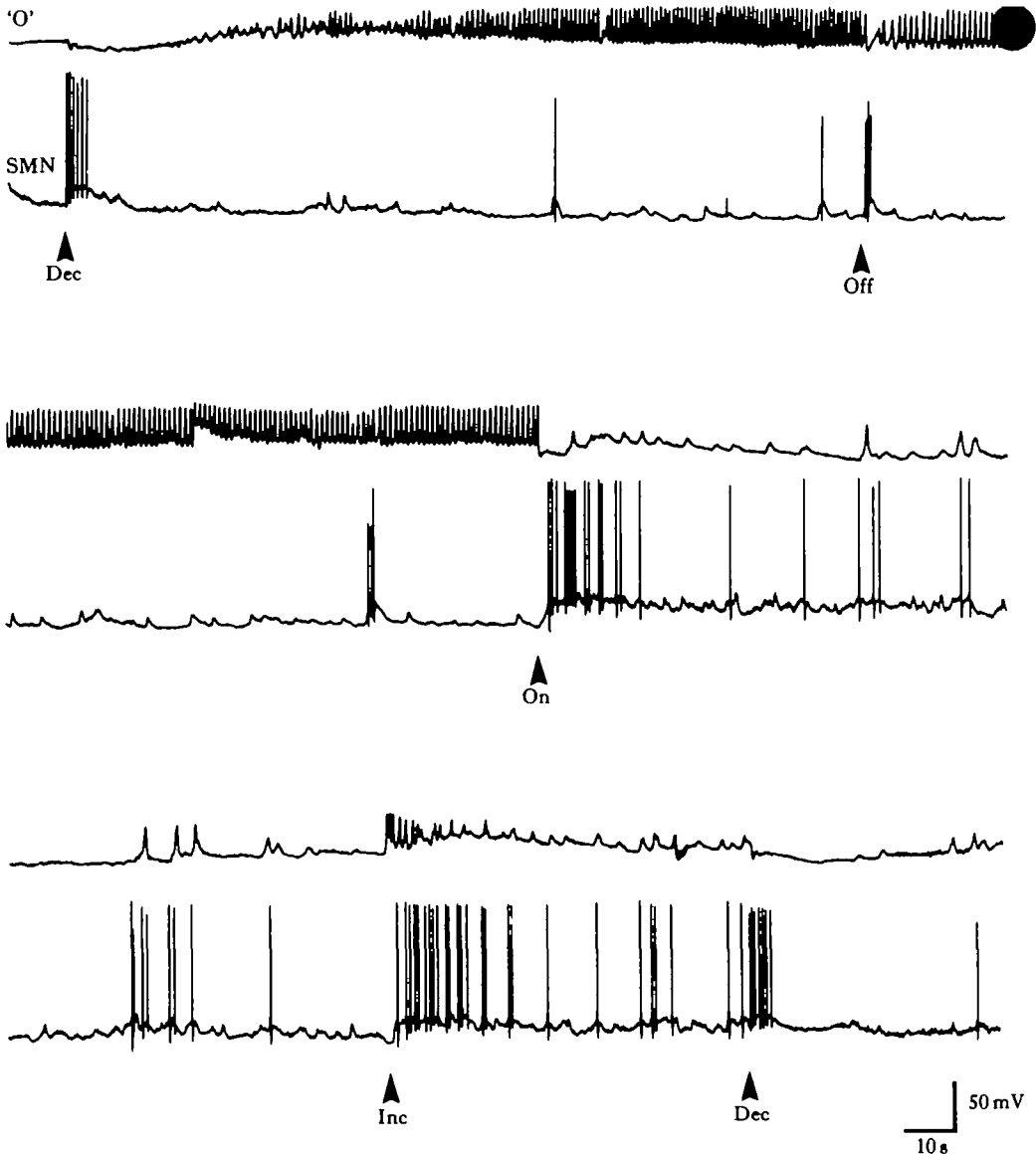


Fig. 9. Light sensitivity of 'O' neurones and the accompanying changes in swimming motor neurone activity. These are continuous records of intracellularly recorded activity in an 'O' neurone (upper traces) with a simultaneous intracellular record of SMN activity (lower traces). The light intensity was altered either by adjusting the rheostat on the illuminating lamp or by turning it off. Off = less than $0.1 \mu\text{E m}^{-2} \text{s}^{-1}$; Dec and On = $15 \mu\text{E m}^{-2} \text{s}^{-1}$; Inc = $120 \mu\text{E m}^{-2} \text{s}^{-1}$.

beginning of the next period. There is often an arrhythmic period preceding or following rhythmic periods (Fig. 9). Oscillations in this system can also be recorded by suction electrodes applied to the outer nerve-ring provided the rates of change of potential are rapid enough to be detected by a.c.-coupled amplifiers. Although modifications to the established pattern of oscillation do occur spontaneously, most alterations are associated with rapid changes in the intensity of the ambient light.

In a preparation which is producing regular oscillations when illuminated, ar

Rapid reduction in light intensity will normally hyperpolarize the 'O' neurones and spontaneous oscillations of the membrane potential will stop (Fig. 9). Even if there are no oscillations when the light intensity is reduced, a hyperpolarization can still be seen. However, after a period of between 10 s and 2 min in the reduced light, small, irregular oscillations begin to appear which rapidly return to their original amplitude and frequency (Fig. 9). If regular oscillations are being produced in the dark or at low intensities, then a sudden increase in light intensity may disrupt the rhythm (Fig. 9). At moderate to low light intensities, a rapid increase in the light intensity will cause a temporary (3–8 s) increase in the amplitude and frequency of oscillations if the system is not producing strong oscillations at that time (Fig. 9). Cutting off tentacles so as to remove the ocelli does not abolish the responses of the 'O' system to changes in light intensity. In a 1 : 1 mixture of sea water and isotonic magnesium chloride, the oscillations become very irregular and are of low amplitude. The response of the 'O' system to changes in light intensity, however, remains essentially unchanged from that of the unanaesthetized system.

Like the 'B' system, the 'O' system is composed of electrically coupled neurones, as can be demonstrated by passing current into one neurone and recording the resulting potential change in a second neurone (Fig. 10). Dual electrode recordings of spontaneous activity show that member neurones in large sections of the system have synchronized membrane potential oscillations (Fig. 10). Electrode separations of up to 0.6 mm give a maximum latency of 30 ms in the appearance of the peak depolarization.

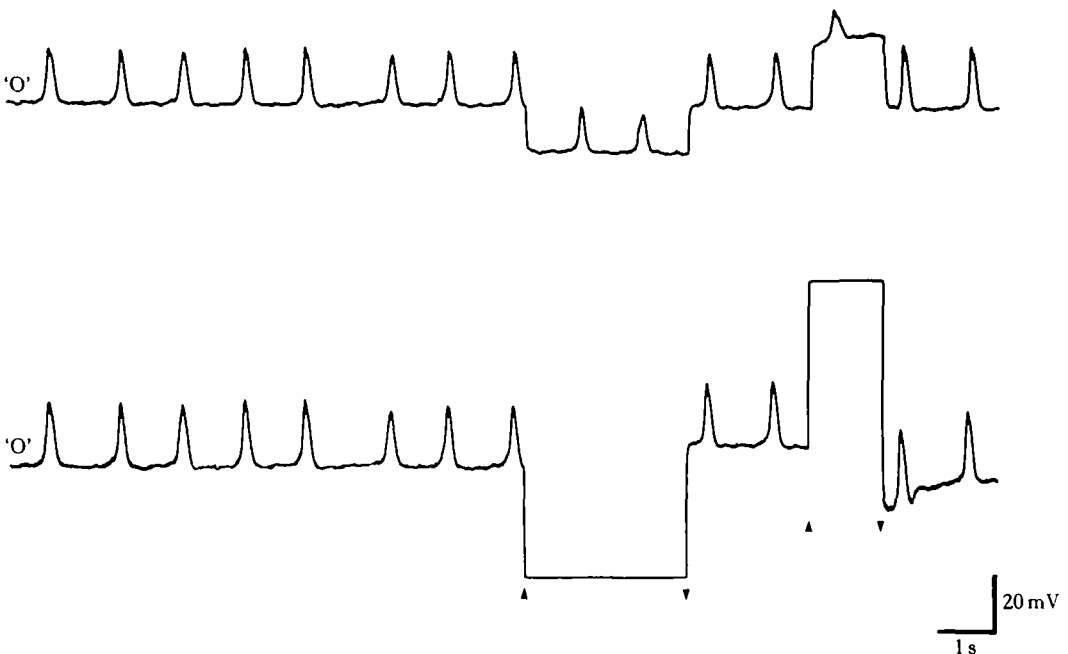
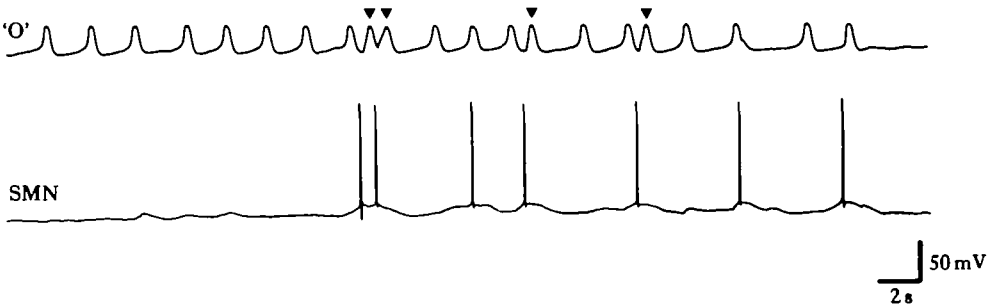


Fig. 10. Electrical coupling between 'O' neurones. Simultaneous recording of spontaneous activity in two 'O' neurones separated by $314 \mu\text{m}$. One hyperpolarizing and one depolarizing current pulse (3 nA each) were injected into one neurone (lower trace) and the voltage change in the second neurone recorded. The bridge circuit could not be balanced in the injected neurone. Note the synchrony and similarity of the voltage oscillations in both neurones.

It is not known if oscillations are synchronized at opposite sides of the nerve-ring. In some, but not all, preparations it is possible to alter the frequency of oscillations by passing current. Hyperpolarizing current tends to decrease the frequency whereas depolarizing current increases it.

Although it is not necessary to infer that some direct interaction or connection exists between the 'O' and swimming motor neurones, many preparations show strong correlations in the outputs of the two systems. In general, regular large amplitude depolarizations in the 'O' system are associated with swimming bouts and hence with SMN spikes. This is particularly true at the beginning of a period of swimming. Later during a bout of swimming, the oscillations may cease completely. On other occasions, regular, strong oscillations can be recorded while there is no swimming; nevertheless, there is usually an associated increase in the frequency of oscillations when swimming recommences. In many recordings, it is apparent that spikes in

A



B

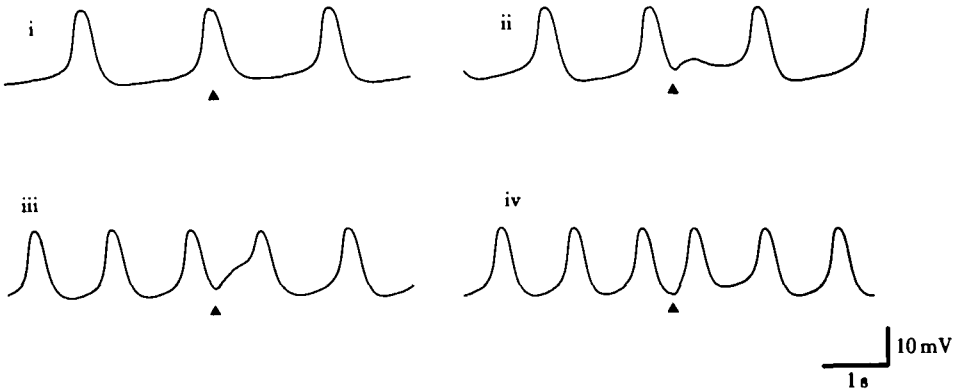


Fig. 11. Oscillations interpolated into the 'O' system rhythm by SMN spikes. (A) An intracellular recording from an 'O' neurone (upper trace) shows that if an SMN spike (lower trace) occurs when the 'O' neurone is hyperpolarized, then a depolarization is interpolated into the rhythm. 'Extra' oscillations are not seen if SMN spikes coincide with a depolarization. Separation between electrodes was $118 \mu\text{m}$. (B) Interpolated depolarizations only occur if the 'O' neurone is hyperpolarized when an SMN spike occurs (arrows). The amplitude of the depolarization is influenced by the degree of hyperpolarization of an 'O' neurone when the SMN spike occurs. (i) No interpolated spike. (ii) Small interpolated depolarization on falling phase. (iii) Larger depolarization interpolated later on falling phase. (iv) Full amplitude depolarization interpolated when 'O' neurone was fully hyperpolarized.

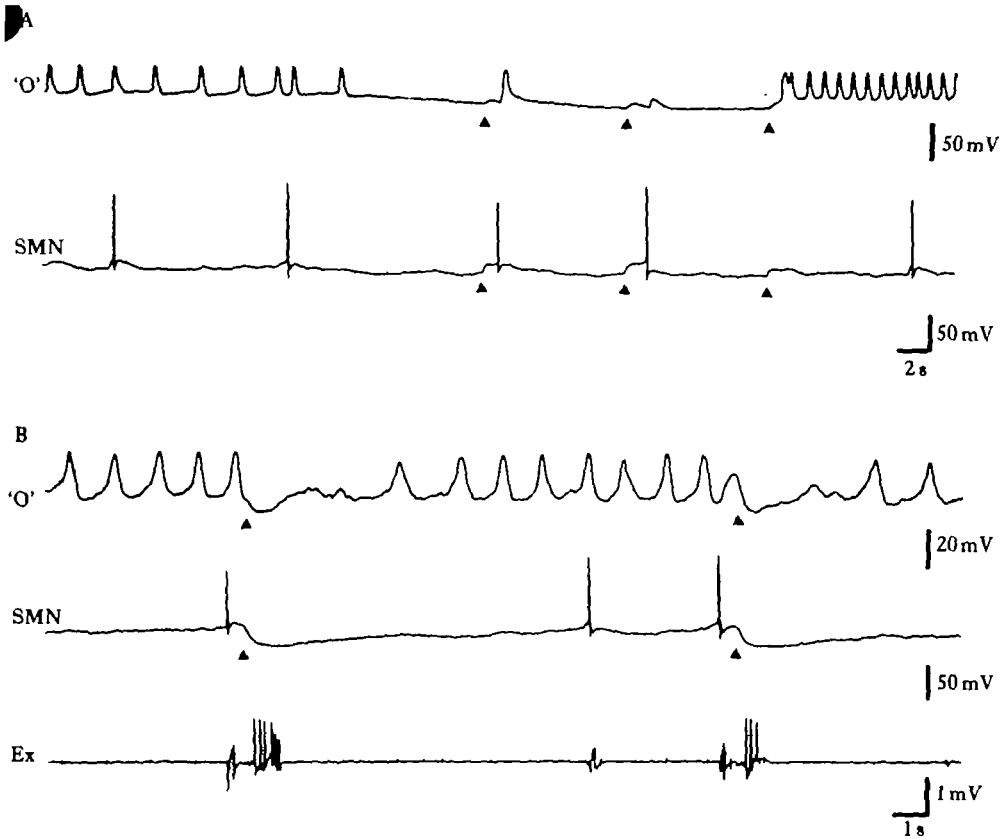


Fig. 12. 'O' neurones and SMNs receive common excitatory and inhibitory input. (A) Simultaneous intracellular recordings from an 'O' neurone (upper trace) and an SMN (lower trace). Spontaneous EPSPs (arrows) appear simultaneously in both neurones, on two occasions they lead to spikes in the SMN and on another occasion an EPSP appears to initiate an oscillatory period in the 'O' system. Three EPSPs are only recorded in 'O' neurones when they are hyperpolarized and not showing oscillations. Interelectrode distance is $118 \mu\text{m}$. (B) Simultaneous intracellular recordings from an 'O' neurone (upper trace) and an SMN (middle trace). IPSPs (arrows) appear simultaneously in both neurones. Each EPSP is associated with a burst of epithelial spikes which is recorded extracellularly with a suction electrode applied to the outer nerve-ring (Ex). Note that 'O' system membrane potential oscillations are detected by the suction electrode. The suction electrode is some 4 mm distant from the micropipettes, which are separated by $120 \mu\text{m}$.

swimming motor neurones can interpolate an oscillation in the predicted rhythm (Fig. 11A). Whether an 'extra' oscillation appears depends on the timing of the SMN spike in the 'O' rhythm. If it coincides with an 'O' system depolarization, then no additional oscillation is seen; however, if an SMN spike occurs while the 'O' neurones are fully hyperpolarized, then an oscillation is interpolated (Fig. 11B). If the SMN spike occurs when the membrane potential of the 'O' neurones is not fully hyperpolarized, then partial depolarizations can often be seen with the amplitude of the depolarization proportional to the degree of hyperpolarization (Fig. 11B). Signal averaged recordings have demonstrated the strong temporal correlation between SMN spikes and the resulting depolarization. This does not necessarily imply that the SMNs are presynaptic to the 'O' neurones, indeed, there is strong evidence to suggest that there

is common synaptic input to the SMNs and 'O' neurones. While the 'O' system is hyperpolarized and not oscillating, it is sometimes possible to see EPSPs arriving synchronously at 'O' neurones and swimming motor neurones (Fig. 12A). This excitatory input may be sufficient to initiate both SMN spiking and oscillations in the 'O' system. The source of these EPSPs has not been clearly established.

As has already been described, the ectodermal epithelium in the vicinity of the outer nerve-ring can generate bursts of action potentials that are superimposed on a slow depolarization. Besides their interactions with SMN and 'B' neurone activity, these epithelial spikes will hyperpolarize 'O' neurones (Fig. 12B).

Morphology of the 'O' system

Lucifer Yellow injected into a neurone of the 'O' system rapidly diffuses through neighbouring neurones, so that after a few minutes an extensive network becomes filled with the dye (Fig. 7B). A portion of the 'O' network is shown in Fig. 8 which was synthesized from several negatives. In contrast to the distribution of the 'B' system neurones, the 'O' network extends across the full width of the outer nerve-ring. Neurones of this network also project up both sides of each tentacle as discrete bundles of neurones, with at least five neurites in each bundle. These tentacular neurones appear to be connected to most parts of the network within the nerve-ring. It has not been possible to trace the tentacular portions of the network to their termini. The majority of 'O' neurones are bipolar, though multipolar neurones are also present, particularly where the tentacular branches arise. Another feature of this network is the numerous small neurites which project perpendicularly onto the velum for a short distance. This was only seen when it was possible to pass a large current (10 nA) for many minutes.

DISCUSSION

Electrical coupling between motor neurones or command neurones is common where synchronous innervation of a set of muscles or other effectors is required (Furshpan & Potter, 1959; Lent, 1973; Bennett, Nakajima & Pappas, 1967). Thus, it is not surprising that the motor neurones responsible for innervating the swimming muscles of hydromedusae are electrically coupled to one another (Spencer, 1981; Satterlie & Spencer, 1983), since efficient jet-propulsion demands that the contractions be both synchronous and symmetrical (Gladfelter, 1972; Spencer & Satterlie, 1981). These swimming motor neurones are located both in the inner nerve-ring and along each side of the radial canals (Fig. 1A, B). Since the subumbrella and velar muscle cells are electrically coupled, a network of motor fibres throughout the 'swimming' muscle sheets is not required. This restriction in the distribution of motor neurones puts them close to other central neurones with which they can potentially interact; for example, the 'B' and 'O' systems and individual sensory neurones which are common throughout the epithelial covering of the outer nerve-ring (Fig. 1B). The SMN network is able to integrate this sensory input and distinguish between general and local input. This is achieved because of the SMN network's low-pass filtering properties and long space constant (Spencer, 1981). In that study, it was shown that identical EPSP activity could be recorded in neurones separated by up to half a spa

Instant. This occurs despite the dominant frequency components of the EPSPs being well above the 1 Hz cut-off of the network filter. At that time it was suggested that the only neuroanatomical arrangement that could explain this paradox would be other neuronal systems that form numerous synapses onto the SMNs throughout the margin and which release a transmitter synchronously. The 'B' system fits the above description precisely. It consists of a network of electrically coupled neurones in the outer nerve-ring which is concentric with and presynaptic to the SMN network. Since EPSPs which follow 'B' system spikes with a constant, short latency can be recorded simultaneously at many pairs of sites around the SMN network, it can be assumed that there is synchronous release of transmitter at the presynaptic endings of 'B' neurones (Fig. 5). Obviously the strong electrical coupling between 'B' neurones is responsible for this synchrony (Fig. 3A). The effectiveness of any one active synapse in depolarizing a postsynaptic neurone will depend on the electrical loading this cell experiences from neighbouring coupled neurones (Getting, 1974). Thus, if neighbouring cells are themselves experiencing a synaptically induced depolarization, then less current will flow into neighbours and more will be available to depolarize any one postsynaptic cell, resulting in larger EPSPs. Additionally, such EPSPs will sum temporally and spatially in the SMN network (Figs 2, 4, 5, 6). The previous synonymous descriptions of the 'B' system from extracellular recordings in a number of anthomedusae (Passano, 1965; Mackie, 1975; Spencer, 1978) have suggested to these authors that it warranted the label 'pacemaker system'. Although not conclusive, two lines of evidence indicate that the firing rhythm of 'B' neurones is endogenously driven. First, hyperpolarizations following each spike (Fig. 2) are reminiscent of those seen in other endogenously rhythmic neurones in which competing Ca^{2+} currents and Ca^{2+} -activated K^{+} currents create the underlying membrane potential oscillations (Meech & Standen, 1975; Smith, Barker & Gainer, 1975). Second, an essentially unaltered spiking rhythm continues in Mg^{2+} -rich solutions.

The bursting response to shadows is mediated by the ocellar photoreceptors or by other photosensitive neurones that synapse onto the 'B' system in the region of the ocelli (Fig. 3B). This is deduced from the observation that removal of the tentacles at their bases, and hence the ocelli, obliterates the 'shadow response'. The location of these excitatory synapses onto 'B' neurones has not been determined. It can be assumed that they are chemical in nature as the bursting response is blocked by excess Mg^{2+} .

Besides exciting the swimming motor neurones, the 'B' system simultaneously controls tentacle length by direct or perhaps indirect innervation of the longitudinal muscles in the tentacle ectoderm. The diffuse network of multipolar neurones seen at the bases of the tentacles in Lucifer-filled networks is a potential site for this innervation. In addition, the 'B' system may receive its shadow-induced excitatory input from the ocelli through this portion of the network rather than in the outer nerve-ring. It is not known how far the 'B' system extends up the tentacles, but immunohistochemical studies (C. J. P. Grimmelikhuijzen & A. N. Spencer, in preparation) show an identical network extending throughout the tentacles. The double excitatory input onto the tentacle muscles and SMNs ensures that a strong simultaneous contraction of all the tentacles occurs during a bout of swimming, so reducing drag.

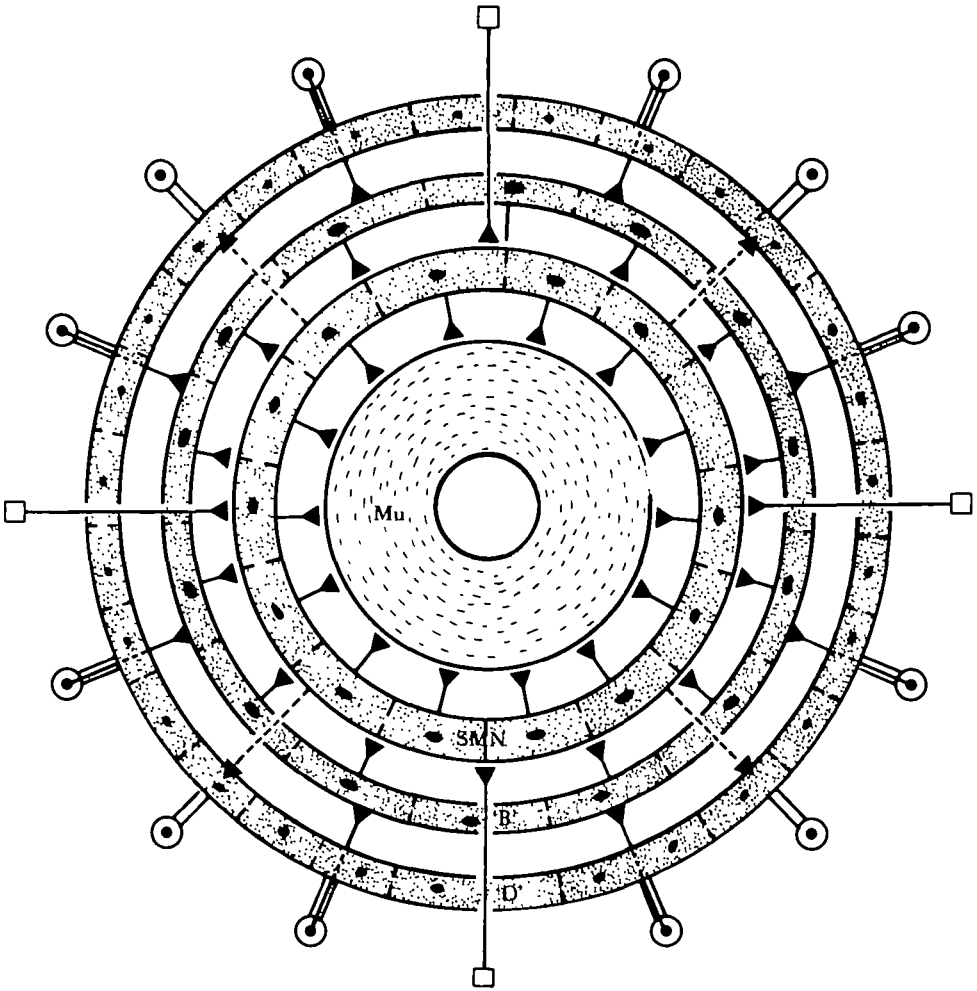


Fig. 13. Diagrammatic representation of the organization of identified neuronal networks in the nerve-rings of *Polyorchis penicillatus* to show the demonstrated pathways of connectivity. The swimming motor neurones, SMN, are located in the inner nerve-ring, while the 'B' and 'O' networks are primarily located in the outer nerve-ring. All three of these networks consist of electrically-coupled member neurones. Excitatory chemical synapses are shown by the symbol, ▲. The excitatory synapses onto the 'B' network are from the vicinity of the ocelli. The dotted lines with arrowheads represent an unidentified excitatory pathway from the SMNs to the 'O' network. The swimming muscle sheets, Mu, are shown in the centre of the diagram. This diagram does not illustrate the known inhibitory connections between epithelial cells and SMNs or 'O' neurones. Note that the excitatory input from the unicellular receptors, open circles, is very localized.

Presumably the EPSPs resulting from 'B' spikes, that can be recorded in ectodermal epithelial cells (Figs 4, 6), are in some way related to the contraction of tentacle longitudinal muscle, or perhaps sphincter muscle (cf. Spencer, 1978). Because spikes have not been seen arising from these EPSPs, it is possible that the EPSPs are in fact graded epithelio-muscular depolarizations that have spread electronically from the sites where 'B' neurones innervate the tentacle ectoderm.

The function of the 'O' system is enigmatic. Nevertheless, we propose two ro

Based on the distribution of the network and on its electrophysiology. There is little question that the neurones of the 'O' system that can be seen passing up either side of every tentacle form a part or all of the ocellar nerves described by Spencer (1979) and Singla & Weber (1982). Although we could not trace these neurones to the ocellus, it is likely that they project that far. It is not clear whether they are the second order sensory neurones described by Singla & Weber (1982); indeed, from the forty or so neurite profiles seen in the ocellar nerves, it is probable that there is some other component to these nerves besides the 'O' system. Inasmuch as the 'O' network is photoresponsive even when the ocelli are removed, it is unlikely that the 'O' neurones are the primary photoreceptors of the ocelli. Like many photoreceptor cells and second order sensory neurones, the potentials produced in the 'O' network are graded (Fig. 11). It appears that the oscillations in membrane potential have an endogenous component, since the frequency of the oscillations can be altered by passing current; however, Mg^{2+} anaesthesia reduces both the amplitude of these oscillations and their regularity. Weber (1982) reports 'high-frequency pulses' of 200–300 ms duration that can be recorded by suction electrodes applied to ocelli. It is possible that these are 'O' system oscillations. The oscillations we recorded are of lower frequency, but larger jellyfish were used.

Efferent fibres projecting to the retina are known for a number of types of photoreceptors (e.g. Arechiga & Wiersma, 1969; Eskin, 1971). The possibility that the 'O' neurones are such efferents should be considered. Indeed, Singla & Weber (1982) describe efferent synapses onto receptors from second order neurones. In this regard, it is relevant that the oscillatory rhythm can be modified by spikes in the SMN network. The precise relationship between electrical activity in the 'O' network and the SMN network is not obvious, though they do receive a common excitatory input when the 'O' neurones are hyperpolarized and common IPSPs are associated with epithelial spikes (Fig. 12).

There is some indication that the 'O' system also has a motor output because of the fairly regular pattern of projections onto the velum. The only muscles on the ex-umbrellar side of the velum are radially-orientated bundles of smooth muscle (Fig. 1B) which are probably used to control the shape of the velar aperture during turning (Gladfelter, 1972). Unfortunately we have no physiological evidence to support the assumption that velar radial muscles are used during turning.

Fig. 13 summarizes the organization of those neurones in the two nerve-rings that we have identified. As will become apparent, we are of the view that the nerve-rings constitute the central nervous system of hydrozoan jellyfish and that the condensation of neuronal networks into ring structures is the maximum degree of localization that can be tolerated, bearing in mind the integrative mechanisms used. It is evident that networks of identical neurones are the radiates' equivalent of identifiable, paired neurones in the CNS of bilateral protostomes. Rather than cephalic condensation as a way of forming cerebral ganglia, the hydromedusae have distributed their ganglia throughout the outer nerve-ring so as to match the distributed arrangement of multicellular sense organs such as the ocelli. The motor networks (SMN and 'B') are also circularly distributed to match the radially segmented arrangement of muscles. This contrasts with the linear repetition of units of motor neurones in the segmental ganglia of many protostomes.

The most noticeable feature of these identifiable networks in *Polyorchis* is that they are all formed from electrically coupled populations of neurones. In the case of the swimming motor neurones (S) electrical coupling ensures synchrony, redundancy and the ability to distinguish between general sensory input throughout the nerve-ring and local input from unicellular receptors (open squares). This type of integration requires that the coupled network extends over several space constants, and since the space constant is large (Spencer, 1981), this distance must be several centimetres. A ring-shaped network is one way to achieve this length. We are still ignorant about the control of behaviour that involves local muscle contractions. For example, turning and feeding. It appears that such behaviour is controlled *via* pathways that rely on the filtering properties of marginal networks to restrict the spread of excitation. The 'B' neurones are coupled so that excitatory input onto the SMN network is synchronized, thus amplifying the numerous individual EPSPs by temporal summation. Similar evidence for the significance of electrical coupling in the 'O' system is lacking; however, it would be reasonable to suppose that synchrony is again important. That different networks communicate with each other by chemical means may be to allow for long-term modification of synaptic efficacy. Alterations to the performance of a synapse are important for adaptive behavioural modifications associated with previous experience. Although it is known that electrical synapses are plastic (Harris, Spray & Bennett, 1983), it seems likely that chemical transmission affords greater opportunities for modifying the properties of a synapse (Klein, Shapiro & Kandel, 1980). Of course, it may be that these neuronal systems are ontogenetically so different that gap-junctions are unable to form between them.

We thank Dr R. Foreman for providing facilities at Bamfield Marine Station and NSERC for funding this research.

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