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CENTRAL CIRCUITRY IN THE JELLYFISH *AGLANTHA DIGITALE*

I. THE RELAY SYSTEM

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Summary

1. The relay system is an interneuronal pathway in the margin of the jellyfish *Aglantha digitale*. It excites a second interneuronal pathway, the carrier system, and is itself excited by pacemaker neurones concerned with slow swimming. It also excites a slow conduction pathway in the tentacles causing graded, tonic contractions of all the tentacles during slow swimming.

2. The pacemakers, the carrier system and the relay system all contribute to the production of excitatory postsynaptic potentials (EPSPs) in a giant axon that runs in the outer nerve ring (ring giant axon). These EPSPs may cause the latter to spike during slow swimming. If it does so, it will fire tentacle giant axons, producing twitch contractions of the tentacles. Such contractions probably help to contract the tentacles rapidly at the start of slow swimming. This is an unusual case of a giant axon that normally mediates escape behaviour being appropriated for use during a non-escape activity.

3. The relay system can conduct impulses on its own but their conduction velocity is greatly increased when preceded by either pacemaker or ring giant spikes. This phenomenon, termed the 'piggyback effect', may be due to extracellular field effects rather than to actions mediated by chemical or electrical synapses.

4. Recordings from the epithelial cells that ensheath the ring giant and outer nerve ring neurones show miniature synaptic potentials and other events that seem to reflect events in the nervous system, but no functions can be assigned to them.

5. There is no obvious counterpart to the relay system in medusae lacking escape circuitry.

Key words: Cnidaria, jellyfish, hydromedusan behaviour, escape swimming, nervous system, nerve ring, giant axon, intracellular recording, relay system, carrier system, pacemaker, central circuitry, *Aglantha digitale*.

Introduction

The existence of bundles of neurones in the margins of hydromedusae has been known since 1850 when Louis Agassiz described them in *Bougainvillea superciliaris* (see Mackie, 1989*a*), and the physiological analysis of hydromedusan behaviour, launched by Romanes (1876), has continued to the present day. It was Passano (1965) who first applied electrophysiological techniques to hydromedusae, uncovering a wealth of conduction pathways and pacemaker systems in the marginal nerve bundles. These 'nerve rings' are now understood to be the animal's central nervous system in which all the major pathways interface and interact synaptically as in the ganglia of higher invertebrates. As the physiological analysis proceeded, it became possible to devise increasingly comprehensive wiring diagrams of the central circuitry in forms such as *Stomotoca atra* (Mackie and Singla, 1975; Mackie, 1975) and *Polyorchis penicillatus* (Spencer, 1978, 1979; Spencer and Arkett, 1984; Arkett and Spencer, 1986). The *Polyorchis* work has gone furthest in this direction, synthesizing evidence from electron microscopy, immunocytochemistry, dye injection and extra- and intracellular recordings.

We are now in a position to start filling in the picture for another jellyfish, *Aglantha digitale*. Along with other members of the Family Rhopalonematidae (Mills *et al.* 1985), this medusa has neuronal pathways specialized for escape swimming and rapid, twitch-type tentacle contractions in addition to systems driving normal 'slow' swimming and slow, graded tentacle responses. Escape responses are conducted around the margin by a 'ring giant' axon located in the outer nerve ring (Roberts and Mackie, 1980). The ring giant elicits large excitatory postsynaptic potentials in eight 'motor giant' axons located in the subumbrellar myoepithelium (Meech and Mackie, 1995). EPSPs that exceed threshold give rise to a large overshooting sodium-dependent action potential which is rapidly conducted to the swimming muscles, causing a strong escape contraction. The ring giant also excites giant axons in the tentacles ('tentacle giants'), causing them to contract rapidly

during the escape response. The anatomy of the central nervous system of *A. digitale*, based on earlier histological studies (Roberts and Mackie, 1980; Mackie, 1989*a*,*b*; Mackie *et al.* 1989; Bickell-Page and Mackie, 1991), is depicted in Fig. 1.

Slow swimming is initiated by rhythmically active pacemaker neurones in the inner nerve ring. These pacemaker neurones also excite the motor giant axons but, unlike the ring giant, their slower, low-amplitude EPSPs (Meech and Mackie, 1995) give rise to a calcium-based action potential that propagates relatively slowly to the swimming muscles and produces a weaker muscle response. Thus, the ability of the motor giants to conduct two sorts of action potential (Mackie and Meech, 1985) is matched by the existence of two sorts of EPSP (Meech and Mackie, 1995). The role of a family of potassium channels in regulating the excitability of the axon membrane so as to allow separate sodium and calcium spikes has been explored by Meech and Mackie (1993*a*,*b*).

Each of the two clearly defined forms of swimming has its own specialized neuronal pathway, but the two systems are not completely independent and we show in this paper that subtle interactions occur between them. A key mediator of these interactions, and the main focus of the present paper, is a ring of interneurones we call the 'relay' system because it relays excitation from the pacemaker neurones to the ring giant axon. Another interneuronal system involved in exciting the ring giant, the 'carrier' system, will be discussed in the companion to this paper (Mackie and Meech, 1995). These systems have no known counterparts in hydromedusae that lack the specialized escape circuitry. By providing a pathway linking the neurones responsible for escape and non-escape swimming, they allow the animal to draw upon an escape component in its normal swimming behaviour.

Materials and methods

Specimens of *Aglantha digitale* Müller were caught off the dock at the Friday Harbor Laboratories, Washington, USA, during May, June and July and were kept in the laboratory at 7 °C. They were dissected in sea water containing about 115 mmol l^{-1} Mg^{2+}, which relaxes the tentacles and prevents swimming. As even small *A. digitale* (height 0.9 cm) may have as many as 80 tentacles, each up to 7 mm long, the tentacles were cut short close to their bases to prevent them from getting in the way of the recording electrodes. Preparations, which consisted of a section of margin about 0.6 cm long (one-quarter of the circumference of the animal) and about 0.4 cm in the radial direction, were pinned out in small Petri dishes lined with Sylgard 184 (Dow-Corning Corp.) using spines of the cactus *Opuntia*. The most useful spines are about 0.5 mm long. Glass microelectrodes were used to cut or isolate nerves by scoring the neighbouring myoepithelium. Experiments were conducted in sea water unless otherwise stated.

Extracellular recordings were made with suction electrodes pulled from heated polyethylene tubing. The amplified signals were filtered and displayed on a storage oscilloscope equipped with a wave-form digitizer. They were recorded on Polaroid

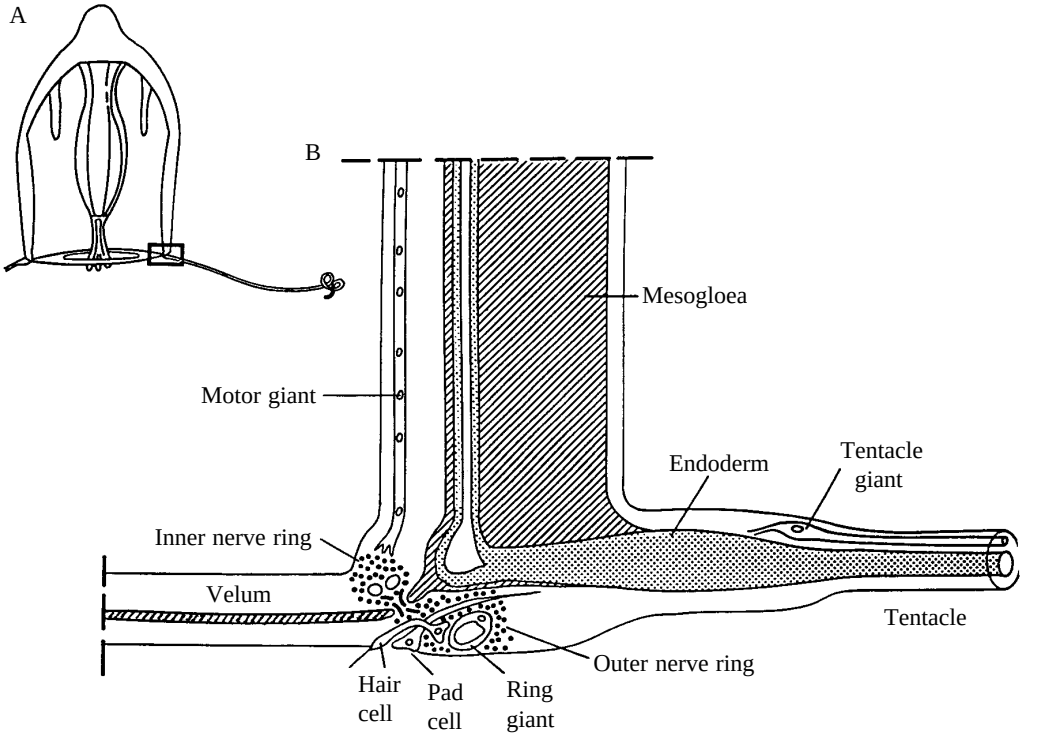

Fig. 1. (A) Outline drawing of a medium-sized *Aglantha digitale*, 1.2 cm bell height. The proportions change somewhat as the animal grows. (B) Perradial section through the area boxed in A to show marginal nerve centres. The inner and outer nerve rings are bundles of neurones running in the ectoderm at the base of the velum. There are about 800 neurones in a typical cross section, most of them less than 1.0 μm in diameter. The motor giant, ring giant and tentacle giant axons are conspicuously larger than other neurones running in the same regions. Both the ring giant and the tentacle giants have prominent central vacuoles. Nerves are shown which cross the mesogloea at the base of the velum and connect the outer and inner nerve rings. The basal processes of the hair cells make contact with the ring giant axon directly. The pad cell sends a long process under the outer nerve ring, contributing to the epithelial ensheathment of groups of neurones. To simplify the picture, the connections passing around the sides of the tentacle that connect the tentacle giant axon to the outer nerve ring have been omitted along with the small nerve bundle that runs beside the motor giant and the small-diameter nerve plexuses running in the outer velar ectoderm and tentacle ectoderm.

film. Most events recorded extracellularly were captured within the 1 Hz to 1 kHz waveband. Signals were also recorded on an instrumentation tape recorder and/or a chart recorder. Unless otherwise stated, negative potentials are in the upward direction in the extracellular records. It should be noted that an electrode on one side of the margin can pick up events from both nerve rings.

For intracellular recordings, glass capillary micropipettes, filled with 3 mol l^{-1} potassium chloride, were mounted on Zeiss (Jena) manipulators fitted with magnetic bases. Amplification of the intracellular signal was conventional and provided for intracellular current injection *via* a bridge circuit. Stimuli were also applied externally through small co-axial bipolar metal electrodes (Clark Electromedical Instruments, SNE-100).

The preparation was viewed with a stereoscopic binocular microscope mounted over a special transillumination base incorporating a double mirror system (Mackie, 1976*a*). Preparations were kept cool (10–12 °C) during recordings using a Cambion thermoelectric cooling unit with a doughnut-shaped cooling platform that allowed transillumination of the specimen during recordings.

Elevated Mg^{2+} concentrations, used to reduce muscle contractions and epithelial after-potentials associated with nervous activity, were obtained by adding 7 % $MgCl_2{\cdot}6H_2O$ to the sea water containing the preparation. Octanol and heptanol were used at concentrations between 0.2 and 1.0 mmol l^{-1} to reduce the spread of epithelial depolarizations. Their efficacy as gap junction blockers (Johnston *et al.* 1980) was checked on the exumbrellar epithelium of *A. digitale*, a nerve-free conducting epithelium (Mackie, 1980). At 0.5 mmol l^{-1}, octanol and heptanol both blocked conduction in this epithelium but had no effect on the production or propagation of sodium spikes in the motor giant axons.

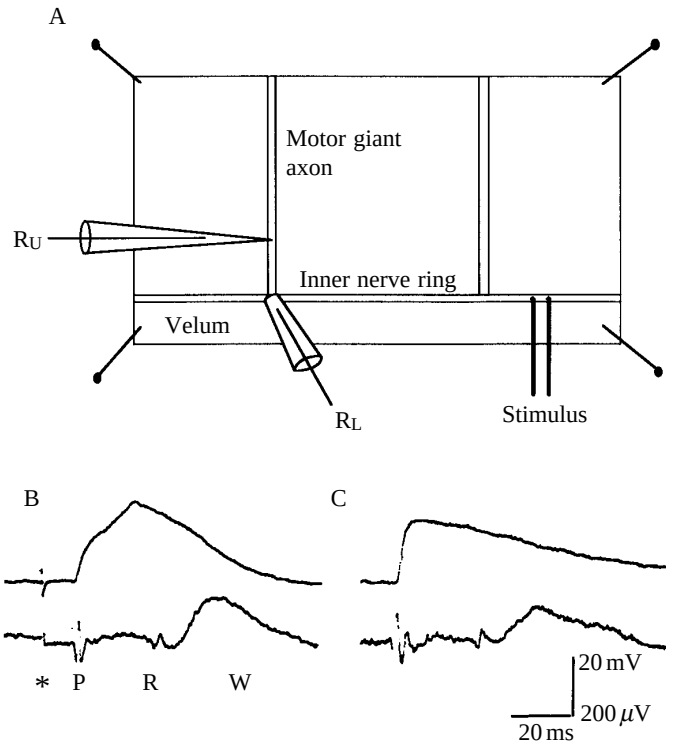

Fig. 2. (A) A drawing of the preparation pinned with its subumbrellar side up. An intracellular recording electrode (R_U) is shown in the motor giant axon close to its junction with the inner nerve ring and an extracellular electrode (R_L) is shown on the inner nerve ring at the junction with the motor giant axon. Stimulating electrodes are situated on the inner nerve ring further along the margin. Total length of preparation, 0.6 cm. (B) A shock (asterisk) on the inner nerve ring evokes a sequence of pulses and a slow wave (P, R and W) recorded by the extracellular electrode (lower trace). The intracellular electrode in the motor giant axon shows a calcium spike generated by a slow postsynaptic potential coincident with the P wave (upper trace). (C) Spontaneous P–R–W sequence obtained after treatment with 84 mmol l^{-1} Mg^{2+} which has blocked the calcium spike, leaving the postsynaptic potential (fictive slow swimming).

Results

General characteristics of the relay system

Spontaneous slow swimming is driven by pacemaker neurones in the inner nerve ring (Meech and Mackie, 1995). Extracellular recordings from the nerve ring during slow swimming reveal trains of pacemaker impulses and, as shown in Fig. 2, similar events can be obtained by stimulating the inner nerve ring electrically. In recordings where stimulation of the inner nerve ring evokes a single pacemaker impulse (P), the extracellular recording (lower trace) shows that the P impulse is followed by a second event representing an impulse in the relay system (R). This is invariably followed by a slowly rising and falling, negative-going after-potential, typically lasting 50–70 ms, termed the slow wave (W). The sequence P–R–W was seen consistently both in preparations bathed in sea water (where the P impulse evoked a slow postsynaptic potential and calcium spike in the motor giant axons; upper trace in Fig. 2B) and in solutions containing excess Mg^{2+}, which suppresses the calcium spike, exposing the slow postsynaptic potential (PSP) that generated it ('fictive slow swimming'; upper trace in Fig. 2C). Thus, R and W events follow P impulses regardless of whether the motor giants and swimming muscles are actually excited and regardless of whether the pacemaker system fired spontaneously (Fig. 2C) or as a result of stimulation (Fig. 2B). There was no trace of postsynaptic events in the motor giants related to the R and W events. We will show that the W event represents a depolarization of the ring giant axon and of the epithelial cells ensheathing the outer nerve ring, while the R impulse arises in a system which relays excitation from the pacemaker neurones to the ring giant.

In certain stimulating positions and with rather weak shocks, it was sometimes possible to obtain propagated R–W sequences without preceding P impulses. In such recordings, the R event conducted very slowly. Although conduction velocity increased with repetitive stimulation, it never exceeded 10 cm s^{-1}. In Fig. 3A, with stimulation at 0.4 Hz, the R event evoked by the first shock conducted at 2.9 cm s^{-1}

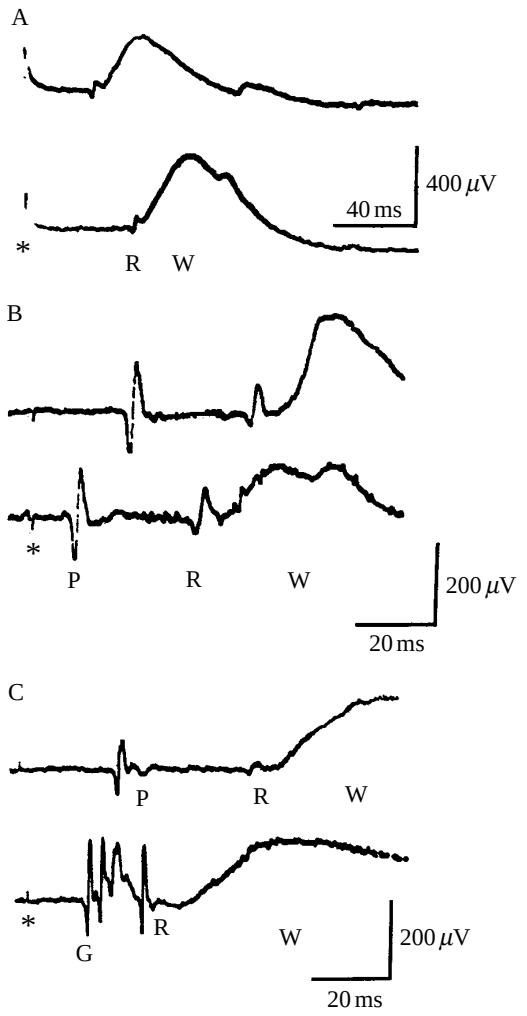

Fig. 3. Extracellular recordings made with a single recording electrode placed on the inner nerve ring following shocks (asterisk) further along the margin. (A) Small shocks that fail to excite the pacemaker system evoke a propagated event consisting of a coupled pulse (R) and slow wave (W) whose conduction velocity increases with repetitive stimulation (upper trace; see text). Small events following the W depolarization here and elsewhere are miniature synaptic potentials. (B) P–R–W sequences evoked by shocks at different distances from the recording electrode; the P–R latency remains constant. (C) A shock evokes a typical P–R–W sequence (upper trace) while a slightly stronger shock (lower trace) fires the ring giant axon (G) as well, causing a burst of three spikes to overlap the P event. The arrival time of the R–W sequence is determined by the piggyback relationship. Octanol (0.5 mmol l^{-1}) was used to reduce ring giant after-potentials that would otherwise obscure the P event.

(lower trace) while the one following the fourth shock conducted at 5.7 cm s^{-1} (upper trace).

Piggyback effect

Although capable of propagating on its own, the relay system has never been observed to be spontaneously active but is apparently always triggered by direct stimulation of the margin or by activity in other systems. When triggered by a P event, the R impulse followed the P event within 25–40 ms regardless of the length of the conducting pathway. In Fig. 3B, the conduction pathway was 11.7 mm in the upper recording and 6 mm in the lower one, but the P–R interval was virtually the same in both (about 30 ms). Thus, the relay system was not only triggered by the P event but was carried along by it at a velocity considerably greater than its velocity when propagating alone. A similar case, where events generated in a slowly conducting system somehow 'hitch a ride' on the back of a faster system, has been termed the 'piggyback effect' (Mackie, 1976*b*).

Relay (R) events can be piggybacked not only by P impulses but also by ring giant impulses (Fig. 3C), where the effect is very pronounced. In Fig. 3C, the piggybacked conduction velocity of the R event was 24 cm s^{-1} in the upper recording (P-assisted) and 41 cm s^{-1} in the lower one (ring-giant-assisted). Piggybacking by the ring giant system was not blocked by 0.5 mmol l^{-1} octanol (Fig. 3C), 6.0 mmol l^{-1} Mn^{2+} or 129 mmol l^{-1} Mg^{2+} (not shown), suggesting that it may be mediated neither by gap junctions nor by chemical synapses.

Three-step depolarization of the ring giant axon

Following electrical stimulation of the inner nerve ring, P–R–W sequences in the extracellular record can be matched with depolarizations in the ring giant axon (Fig. 4A). Both P and R impulses produce small postsynaptic potentials (PSPs) and, as the two normally fire in sequence, their PSPs sum to depolarise the ring giant axon by up to 16 mV. These summed PSPs together with similar depolarizations of the epithelial cells around the outer nerve ring (see below) are responsible for the W component of the extracellularly recorded P–R–W sequence. Similar two-step summing depolarizations are seen during spontaneous fictive swimming. As shown in Fig. 4B, the first depolarization of the ring giant axon coincides with the slow PSP recorded in the motor giant, both being due to pacemaker input. The second step, representing relay input, follows after an interval determined by the P–R piggyback relationship.

Under our recording conditions, the two-step depolarization of the ring giant axon due to summed pacemaker and relay inputs was never sufficient to bring the ring giant to spike threshold. Spiking often occurred, however, during P–R–W sequences, the spikes typically occurring on or near the peak of the W event. Analysis of such cases shows that a third system, the 'carrier' (C) system, contributed to the depolarization of the ring giant axon, bringing the total change to about 20 mV (Fig. 4C,D). Impulses in the carrier system were presumably triggered by impulses in the relay system, as a carrier impulse was never seen without a preceding R event. We show in the companion paper (Mackie and Meech, 1995) that the carrier system normally fires in fairly close synchrony with the ring giant system, producing composite signals in extracellular recordings in which the two components cannot be separated. In intracellular recordings such as Fig. 4D, however, the carrier postsynaptic potential could be clearly distinguished

as a separate entity, labelled as step 3. Production of ring giant spikes during slow swimming always required three depolarization steps, representing input from three presynaptic systems, the pacemaker, relay and carrier systems, firing in that order. Carrier pulses were never seen without a preceding R pulse, nor R pulses without a P event, suggesting that the P–R–C cascade represents triggering of the relay system by the pacemaker and of the carrier system by the relay.

Although, as noted above, ring giant spikes generated in this way usually occurred on or near the peak of the W event, they sometimes occurred earlier or later. The time relationships presumably depend on (a) the P–R triggering interval, (b) the R–C triggering interval and (c) the distance from the recording electrode at which spike threshold was first reached. We are dealing with a linear preparation representing the cut and straightened margin of a circular animal. Several systems run along the strip in parallel and interact at numerous points along their length. A ring giant spike, once initiated, can presumably travel at its own high conduction velocity and, by the time it reaches the recording electrode, can catch up with or even overtake events in the presynaptic systems that triggered it. Again, with impulses generated by a stimulating electrode near the recording site and propagating both towards and away from it, the ring giant may fail to reach spike threshold except at a point remote from the recording electrode, allowing impulses in the presynaptic systems to arrive at the recording electrode well in advance of the ring giant spike, despite the more rapid conduction velocity of the latter.

Triggering of tentacular contractions

P–R–W sequences in the marginal nerves were typically followed by electrical events in the system previously referred to as the 'small tentacle pulse system' (Bickell-Page and Mackie, 1991), and here termed the 'slow tentacle conduction system' or 'slow tentacle system'. A typical example of a pulse in this system is shown in Fig. 5B labelled TS (upper trace). Impulses in the slow tentacle system excite the longitudinal muscle of the tentacle, causing slow graded contractions. The postsynaptic muscle potential is seen as a large upward after-potential following the smaller presynaptic nervous event. What sets off the slow tentacle pulse is not definitely known, but triggering probably involves the relay system, as P impulses alone failed to excite a slow tentacle pulse and triggering often occurred in the absence of C events. In traces where the ring giant axon fired following a three-step depolarization (as described above), the ring giant spike was followed by an impulse in the tentacle giant axon, which caused a twitch contraction of the tentacle. This is shown in Fig. 5C, where the tentacle giant spike was superimposed on the slow tentacle pulse and its after-potential. Thus, activity in the pacemaker neurones can be relayed by the R system to the tentacles during slow swimming, causing both graded and twitch-type contractions.

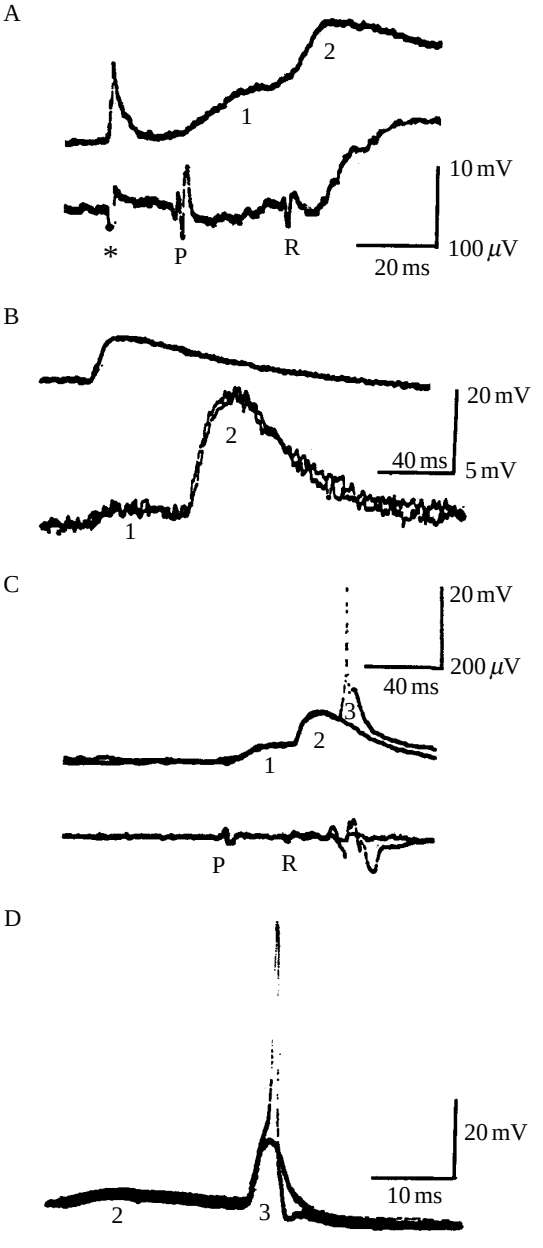

Fig. 4. (A) A shock (asterisk) on the inner nerve ring evokes a typical P–R–W sequence recorded extracellularly (lower trace). A simultaneous intracellular recording from the ring giant axon (upper trace) shows depolarizations due to pacemaker (1) and relay (2) system inputs. (B) Two superimposed traces showing simultaneous intracellular recordings from a motor giant axon (upper trace) and an adjacent ring giant axon (lower trace) during a P–R–W sequence (not shown, but as in A). The slow postsynaptic potential (PSP) in the motor giant corresponds in time to depolarization 1 in the ring giant, both being due to pacemaker input. (C) Two successive sweeps superimposed showing a three-step depolarization of the ring giant axon (upper trace) representing successive input from the pacemaker, relay and carrier systems and culminating in a ring giant spike (3) on the second sweep. The extracellular correlate of the carrier system is lost within the complex deflection representing the ring giant spike and its after-potential (lower trace). (D) Intracellular recordings from the ring giant axon at the peak of three-step depolarizations. Supra- and subthreshold sweeps are superimposed, showing the production of a ring giant spike by a carrier system postsynaptic potential (3).

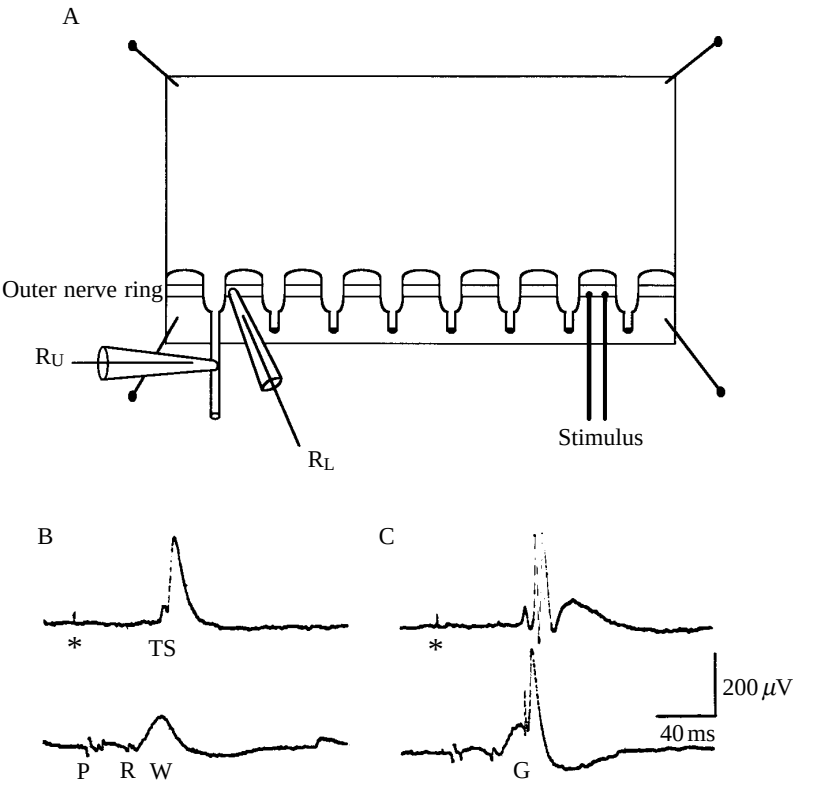

Fig. 5. (A) Drawing to show a preparation pinned with its exumbrellar side up. Most of the tentacles have been truncated, leaving one (left-hand side) intact. An extracellular recording electrode (R_U) is shown attached to it. A second extracellular electrode (R_L) is sited on the outer nerve ring close to the tentacle. Stimuli are delivered on the outer nerve ring further along the margin. (B) Extracellular recordings from a tentacle (upper trace) following shocks (asterisk) on the margin evoking P–R–W sequences, recorded from the outer nerve ring adjacent to the tentacle (lower trace). A slow tentacle pulse (TS, with its large upward after-potential) was triggered by the R event. (C) As for B except that a ring giant spike (G) occurred following the R event and resulted in a tentacle giant impulse in the tentacle, seen superimposed on the slow tentacle pulse and its upward after-potential.

Epithelial depolarizations

Attempts to penetrate the ring giant often gave an initial resting potential of −72 to −75 mV. In such cases, the electrode tip appeared to be located in the epithelium covering the ring giant rather than in the ring giant itself, and this was verified by ionophoresis of carboxyfluorescein. The dye spread diffusely through a patch of epithelium around the injection point. During these intracellular recordings, the epithelium was observed neither to spike nor to conduct action potentials although it showed a great deal of spontaneous background activity, presumably reflecting activity in the neurones it ensheaths. The spontaneous potentials showed no clear effector correlates and were strictly local, although two electrodes placed close together could pick up the same events (Fig. 6A). Though not truly 'miniature' events (because they reached 5 mV in intracellular and 150 μV in extracellular recordings), we tentatively regard them as the equivalent of miniature end-plate potentials and suggest that they represent spontaneous transmitter release by the ensheathed neurones. Similar events recorded in the subumbrellar myoepithelium were termed 'miniature synaptic potentials' by Kerfoot *et al.* (1985), a term we adopt here. Miniature synaptic potentials (MSPs) usually occurred in fairly regular patterns, although phases of high activity (approximately 3 Hz) often alternated with phases of low activity (approximately 1 Hz). Similar patterns of MSPs were also seen in the pad cell, a specialized epithelial cell associated with hair cell clusters (Arkett *et al.* 1988; Mackie, 1989*b*). Like other epithelial cells showing MSPs, the pad cell contributes to the ensheathment of the outer nerve ring.

Patterns of MSPs can be affected by events in at least two nervous sub-systems. They were frequently seen to be blocked for several seconds by spikes in the ring giant axon (Fig. 6B) and they appeared to be induced by both spontaneous (Fig. 6C) and shock-induced P–R–W sequences (Fig. 6D). In several recordings, shocks delivered more than 500 ms after a spontaneous MSP evoked a P–R–W sequence that consistently generated an MSP on the descending slope of the W component.

Intracellular recordings from epithelial cells show that the latter depolarize during each R–W event. In fact, as noted earlier, the W event is in part the extracellular correlate of this depolarization. In Fig. 7A, an R–W sequence piggybacked by a shock-evoked ring giant event corresponds in time to a 17 mV long-lasting depolarization of the epithelium. The extracellular record captured only the early part of this depolarization owing to capacitative coupling in the amplifiers. The epithelial cell was also depolarized following a simple ring giant spike (Fig. 7B). In Fig. 7C, stimulation evoked a P–R–W sequence where the W event again corresponded to a 17 mV depolarization in the epithelium. In the same preparation (Fig. 7D), a P–R–W–ring giant sequence also occurred, and a PSP due to the ring giant event is seen mounted on the top of the epithelial depolarization.

Discussion

The findings reported here show that the swimming pacemaker neurones, whose most obvious function is to fire the motor giant axons in the slow swimming response, also produce PSPs in the ring giant axon and trigger events in

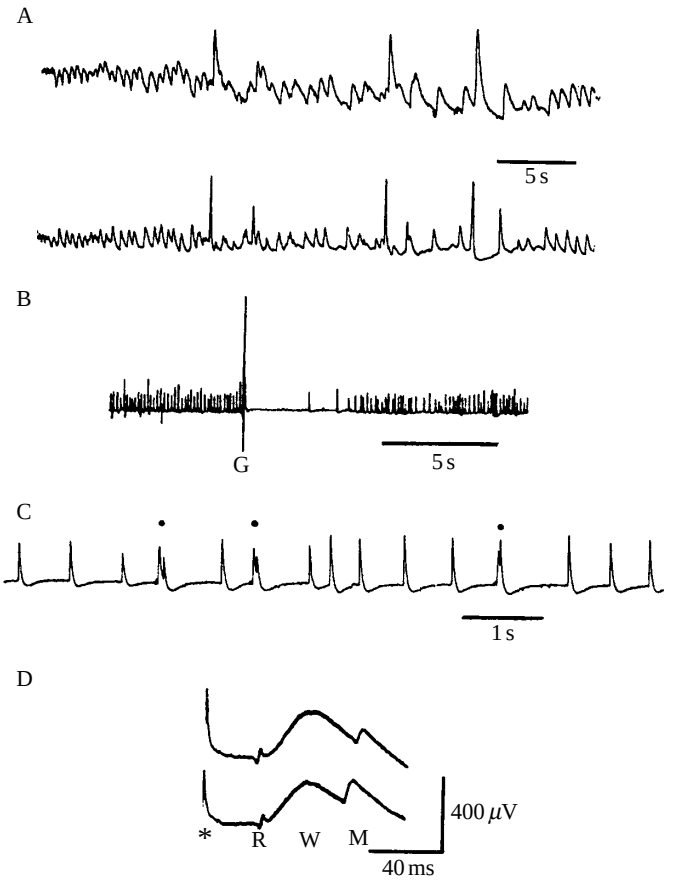

Fig. 6. Miniature synaptic potentials (MSPs). (A) Simultaneous intracellular (upper trace) and nearby extracellular (lower trace) recordings of spontaneous MSP patterns showing alternating high- and low-activity phases. (B) In an extracellular recording, a regular pattern of MSPs is blocked for several seconds following a burst of spikes in the ring giant axon (G). (C) In an extracellular recording from the epithelium, spontaneous P–R–W sequences (dots) occurring during rhythmic MSP activity induce precocious MSPs, briefly altering the rhythm. (D) Induction of MSPs (M) following shock-induced R–W sequences (shock artefact marked with an asterisk); two similar extracellular recordings on an expanded time scale (see also Fig. 3A).

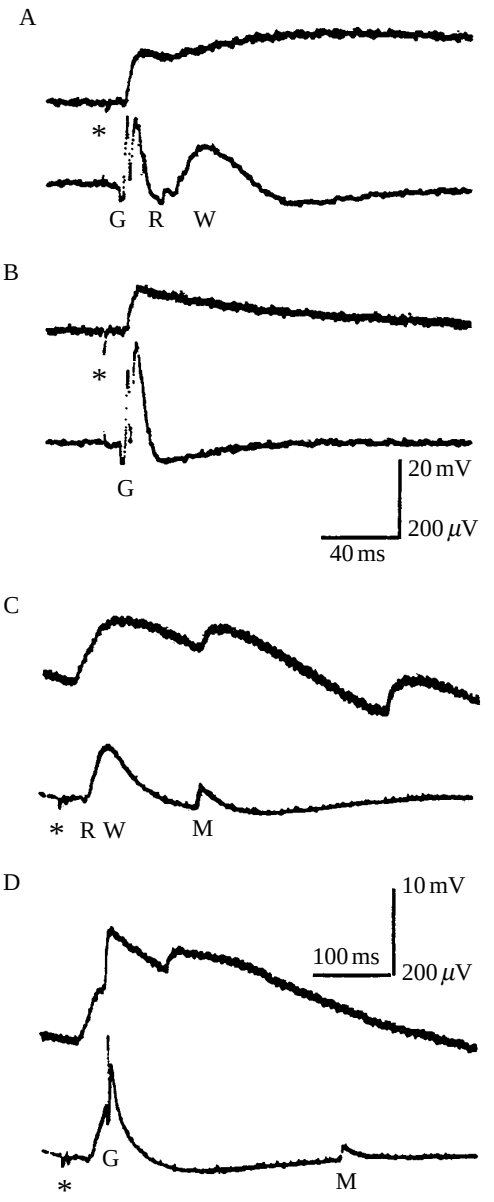

Fig. 7. Intracellular recordings from the epithelium overlying the outer nerve ring (upper traces) during nervous activity recorded extracellularly in the same vicinity (lower traces). (A) A shock-evoked ring giant spike (G) and piggybacked R–W sequence are both accompanied by epithelial depolarizations. In B, the ring-giant-evoked depolarization is seen alone. In C, a P–R–W sequence (the P following very closely upon the shock artefact, marked with an asterisk) gives rise to an epithelial depolarization corresponding to the W event. In D, epithelial depolarizations are associated with both W and ring giant events (G) in a P–R–W–ring giant sequence. In C and D, some miniature synaptic potentials events (M) are seen on both channels, but the two electrodes were not close enough for both electrodes to pick up the same events all the time.

another system of interneurones, the relay system. The relay system similarly produces PSPs in the ring giant and triggers events in a third system, the carrier system, which in turn generates PSPs in the ring giant. The result is a sequential, three-step depolarization of the ring giant that may bring it to spike threshold. These interactions are shown diagrammatically in Fig. 8.

Whenever the ring giant fires, the tentacles contract together in a twitch response. Thus, twitch contractions of the tentacles frequently accompany slow swimming even though we think of such contractions as ‘belonging’ to the escape response. By virtue of the relay and carrier systems, the animal has bridged the gap between its escape and non-escape circuits and can utilize an escape component in its normal, slow swimming behaviour.

The ring giant is an enormous axon, and the three-step depolarization cascade described here may be necessary to bring it to spike threshold, given its presumed high membrane capacitance. The pacemaker–relay–carrier triggering sequence can therefore be seen as a way of amplifying the

initial pacemaker impulse to the level needed to fire the ring giant.

In Fig. 8 we show the ring giant and carrier system as lying immediately adjacent to one another, connected by bidirectional excitatory links and with the various inputs and outputs entering at or exiting from the interface between the two. The reason for this ambivalence is that the two systems usually fire in synchrony and it is hard to know which system is primarily involved in any given interaction. This question is further addressed by Mackie and Meech (1995).

Twitch contractions of the tentacles, mediated by the tentacle giant axon, are not the only way in which the tentacles can contract. The animal has a mechanism for graded, tonic contractions that is also activated during slow swimming. At each slow swimming event, the relay system directly excites the slow tentacle system (Fig. 8). The tentacle longitudinal muscles contract incrementally with each slow tentacle pulse. It might seem that twitch contractions of the tentacles are redundant, given the existence of tonic contractions, but it seems likely that the twitch contractions are needed to bring about the requisite degree of shortening at the start of a slow swimming bout. The firing frequency of the tentacle system triggered through the relay pathway cannot exceed the frequency of slow swimming, which is only about 0.5 Hz and, given the incremental, graded nature of the tonic response, such a low frequency would probably be insufficient to contract the tentacles early enough and strongly enough to prevent excessive hydrodynamic drag or the danger of entanglement. We suggest, therefore, that the twitch contractions serve to bring about a rapid, high degree of contraction which can then be maintained tonically by the graded input. Here, however, physiological speculation has gone some way ahead of behavioural observations and there is a need to go back to the animal in nature and observe its behaviour more closely.

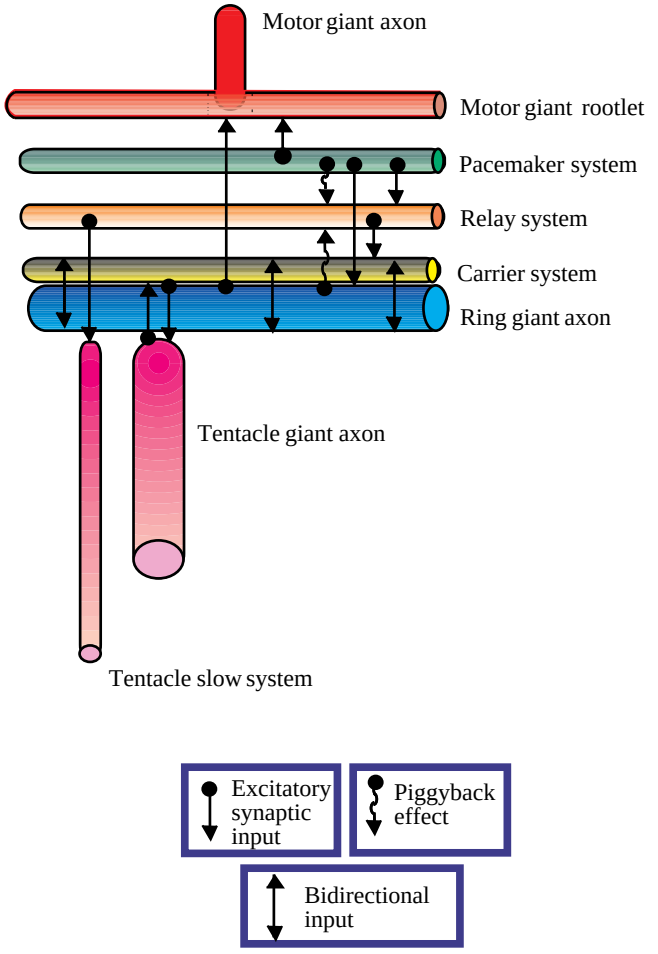

Fig. 8. Diagrammatic ‘side-on’ view of the central nervous system summarizing the interactions discussed in the text. There are three kinds of synaptic connection. Some have an excitatory synaptic input, some are bidirectional and some show the piggyback effect.

If, following a pacemaker impulse, the ring giant axon is depolarized in a three-step cascade and reaches spike threshold, the resulting action potential should not only cause the tentacles to twitch but should also fire the motor giant axons, causing sodium spikes in them and leading to escape swimming. This does not happen, however, and it appears that the ring giant axon to motor giant axon excitation pathway is delicately adjusted so that the occurrence of a slow swimming event, whether real or fictive, somehow blocks transmission from the ring giant to the motor giant or prevents the generation of a sodium spike. How it does so is unknown at present.

We know of no other case where a giant axon mediating escape behaviour can be ‘borrowed’ to augment a non-escape activity. The tail-flip response of crayfish can be mediated by giant axons or by non-giants. There are separate pathways to the muscles in the two cases, and one pathway (the fast flexor motor neurones) is used in both responses. In addition, certain non-giant interneurones that innervate the fast flexor motor neurones in non-giant tail-flipping are ‘commandeered’ during giant-mediated flips (Krasne and Wine, 1984). These examples of shared circuitry recall the situation in *A. digitale* in so far as the motor giant axons are concerned, for the latter provide the final common pathway for both escape and non-escape swimming, but the crayfish offers no counterpart to the commandeering by *A. digitale* of its ring giant axons during non-escape locomotion.

The piggyback effect

Our findings show that the relay system is capable of conducting slowly on its own but that, when impulses in it are preceded by impulses in the pacemaker system or the ring giant axon, it shows a much higher conduction velocity. The mechanism for this ‘piggyback’ effect (shown as crooked arrows in Fig. 8) is unclear, but the drug effects reported here suggest that piggybacking may not be due to conventional junctional transmission but may involve extracellular spread of action currents between faster-conducting and slower-conducting elements. Such ‘field effects’ are, as noted by Bullock (1984), hard to prove or disprove, and a lengthy discussion of the problem would be inappropriate at this juncture, but it should be noted that cnidarian neurones are not individually ensheathed (Horridge *et al.* 1962). As shown for

Polyorchis (Spencer and Arkett, 1984), hydrozoan conduction systems consist of clusters of neurones functioning more or less in synchrony and lying close to, and probably intermingling with, other such clusters. In *A. digitale*, epithelial processes do appear to subdivide the nerve rings into separate territories to some extent, but the evidence for this is tentative, and it would not be surprising if cross-talk between different systems sometimes occurred. Indeed, Spencer (1981) has suggested that inhibition of the swimming motor neurones in *Polyorchis penicillatus* may be due to field effects from surrounding epithelial cells. Whatever the mechanism, piggybacking of relay system impulses by the pacemaker system is clearly of functional importance because it ensures that relay impulses follow pacemaker impulses within the fairly short time interval necessary for summing of their postsynaptic potentials in the ring giant during P–R–C sequences. Piggybacking of relay impulses by ring giant spikes is not so clearly advantageous. Its effect would be to prolong the state of tentacle contraction slightly.

When no other systems are excited and the relay system is stimulated with a series of shocks, its conduction velocity increases following the first shock. A similar phenomenon, termed 'action potential facilitation', has been described in oligochaetes (Bullock, 1951; Drewes *et al.* 1978), in which a second impulse following the first within about 6 ms shows a velocity increase of up to 20 %. In *A. digitale*, the velocity increase could, like the piggyback effect, be a phenomenon in the field effect category if stimulating or action currents are stored capacitatively in nearby excitable tissues long enough to affect the excitability level of the relay system at its next impulse.

Interactions between nerves and epithelial cells

The origin and functional significance of the events recorded from the epithelial cells ensheathing the neurones of the outer nerve ring are largely unknown at present, but some understanding of their basis is essential for the interpretation of the somewhat complicated extracellular recordings from the margin. If the events interpreted as miniature synaptic potentials (MSPs) do indeed represent spontaneous transmitter release, neuro-epithelial synapses must occur, but as yet we have no ultrastructural evidence of such junctions in the immediate vicinity of the outer nerve ring. The blocking of MSP patterns by ring giant spikes (Fig. 6B) suggests that carrier neurones may be the source of MSPs, as they fire when the ring giant fires, which would deplete their vesicular content. Gap junctions occur between the ring giant and surrounding epithelial cells (Mackie, 1989*b*) and might mediate the depolarization of the epithelium by ring giant spikes. Their presence might also help to account for the observation that the ring giant and the enveloping epithelial cells depolarize in synchrony following R impulses, producing the W potential. If the two systems are coupled, however, the coupling must be fairly weak because MSPs are recorded only in the epithelium, and the background oscillations and synaptic activity characteristic of the ring giant axon (Mackie and Meech, 1995) are not picked up in the epithelium. Processes of the epithelial cells ensheath bundles of neurones and could function actively in distributing electrical field effects or passively as insulating barriers. Thus, they could play some part in mediating piggyback interactions between neuronal subsets.

Comparisons with other medusae

We noted earlier that the relay system has no obvious counterpart in other medusae. Coordination of the tentacles in other medusae is carried out by a system originally termed the marginal pulse system (Passano, 1965) and represented in *Polyorchis penicillatus* by the 'B' system (Spencer, 1978; Spencer and Arkett, 1984; Arkett and Spencer, 1986). This system runs around the margin in the outer nerve ring and down each tentacle. Its rate of firing determines the degree of tentacular contraction. It is excited when the tentacles are stimulated, but also fires spontaneously, the pattern of firing being the same all round the margin. Bursts of B pulses are seen prior to swimming, so that by the time swimming starts, the tentacles are already contracted. The B system receives input from the ocelli in the form of excitatory PSPs following shadowing of the ocelli and responds with bursts of spikes. These in turn produce excitatory PSPs in the swimming motor neurones (equivalent to the pacemaker system of *A. digitale*) and in the epithelium covering the outer nerve ring.

The relay system of *A. digitale*, like the B system, runs around the margin, but it does not run down the tentacles. Tentacle posture is controlled by another system, the slow tentacle system, which is fired by the relay system. There is no evidence for transmission in the reverse direction. Tentacle stimulation produces bursts of tentacle pulses that remain localized within that tentacle and do not enter the relay system or spread around the margin to other tentacles. Unlike the B system, the relay system is not spontaneously active and it does not fire prior to swimming. On the contrary, impulses in it are triggered by the same events that produce swimming. It shows no response to shadowing or illumination and although R impulses evoke epithelial depolarizations (W events) there is no effect on the pacemaker system. If *A. digitale* has a counterpart to the B system, it could only be the slow tentacle system, but this would require that the system has become restricted to each tentacle rather than interconnecting all the tentacles.

It would therefore appear that the relay system has evolved in conjunction with escape circuitry in rhopalonematid medusae as a mechanism for bringing about concerted tentacle contractions during slow swimming. If this function was originally performed by the B system, the latter has either been lost or has become restricted to each tentacle forming the tentacle system. The relay system coordinates the tentacles in two ways: (a) by directly exciting the tentacle system, producing tonic contractions, and (b) by helping to depolarize the ring giant axon to spike threshold, the resulting ring giant spikes causing excitation of the tentacle giant axons

and twitch contractions. Contraction of the tentacles during swimming is clearly advantageous in terms of drag reduction and because it prevents tangling. It is doubtful if the necessary degree of contraction could be achieved by activating the slow tentacle system alone as the relay input frequency cannot exceed the rather low value set by the slow swimming pacemaker system. The animal has therefore made use of part of its escape circuitry (the ring giant and tentacle giants) to enhance tentacle contractions during its normal slow swimming behaviour.

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