

THE INVOLVEMENT OF NERVES IN THE EPITHELIAL CONTROL OF CRUMPLING BEHAVIOUR IN A HYDROZOAN JELLYFISH

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SUMMARY

1. The excitation pathways mediating the protective crumpling behaviour of *Polyorchis penicillatus* were studied with electrophysiological and ultra-structural techniques.

2. Stimulating the subumbrellar endoderm consistently resulted in a complex crumpling potential when recorded with suction electrodes from radial muscle (the prime effector). The potential represents the summation of a quick radial muscle potential (RMP) and a slow endodermal canal pulse (ECP).

3. The latencies of ECPs recorded from radial muscle during crumpling were directly proportional to the distance between the recording electrode and the subumbrellar stimulating electrode. Conversely, the latencies of RMPs, which were not tightly time-coupled to ECPs, were more directly related to the distance of the recording and stimulating electrodes from the marginal or apical termini of the radial muscle.

4. Stimulating the exumbrellar ectoderm resulted in a variable crumpling response, typically occurring after facilitation of numerous exumbrellar pulses (EPs). Since exumbrellar stimulation did not usually excite endoderm, the response recorded from radial muscle normally involved a simple RMP, unassociated with an ECP.

5. Typical synaptic junctions were observed between radial muscle processes and marginal neurites and between radial muscle and neurites of the radial nerve bundles along the length of the muscle.

6. The independence of the ECP and RMP conducting pathways demonstrates that endoderm does not provide the direct source of radial muscle excitation and the initiation of RMPs at points of known (marginal) and suspected (apical) nerve-muscle contact suggests the involvement of nerves in the control of crumpling behaviour.

7. These results are discussed in the light of other examples of active neuronal-epithelial interaction.

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INTRODUCTION

Hydrozoan jellyfish have provided the most fruitful preparations for the study of epithelial conduction; for these animals much is now known about the behavioural correlates (Mackie & Passano, 1968; Mackie, 1975; Mackie & Singla, 1975; Spencer, 1975, 1978), the cellular physiology (Mackie, 1976; Spencer, 1978; Josephson & Schwab, 1979) and the ultrastructural basis (King, 1979; King & Spencer, 1979) of this mechanism of communication. However, although it is known that epithelial conduction mediates the protective crumpling response in hydromedusae (Mackie & Passano, 1968), little effort has been directed at elucidating the underlying excitation pathways. It was originally proposed that crumpling is activated by an entirely epithelial pathway, involving the spread of excitation from exumbrellar ectoderm to endoderm at the margin and subsequently to the subumbrellar ectodermal radial muscles, which are the prime effectors of the response (Mackie & Passano, 1968). Despite the lack of direct corroborative evidence, this hypothesis has been unchallenged and reaffirmed (Mackie, 1975). Although the presence of transmesogleal epithelial bridges between ectoderm and endoderm at the margin and along the perradial subumbrellum (Mackie & Singla, 1975; Spencer, 1979) supports this hypothesized pathway, this evidence is circumstantial in that it provides only a possible anatomical basis.

The rapidly expanding list of non-coelenterate systems in which impulse-propagating epithelia have been demonstrated includes the skin of frog tadpoles (Roberts & Stirling, 1971), the skin of larval (Mackie & Bone, 1976) and adult (Bone & Mackie, 1975; Anderson *et al.* 1979) urochordates, several mammalian endocrine glands (Matthews & Saffran, 1973; Matthews & Sakamoto, 1975; Brandt *et al.* 1976; Taraskevich & Douglas, 1977), the gastropod salivary gland (Kater, Rued & Murphy, 1978) and the elytra of a polychaete worm (Herrera, 1979). In those systems in which the excitable epithelium serves as a conductive field for the activation of an escape behaviour (Roberts & Stirling, 1971; Bone & Mackie, 1975; Mackie & Bone, 1976; Anderson *et al.* 1979) there is an intermediate nervous pathway which relays the epithelial excitation to the effector muscle. Thus, if the hypothesis of Mackie & Passano (1968) is correct, the control of crumpling in hydromedusae would represent an unusual condition in which there is direct epithelial excitation of muscle.

It was the purpose of this study to determine the precise pathways involved in translating epithelial excitation into a crumpling response through electrical recordings from the radial muscle.

MATERIAL AND METHODS

Medusae of *Polyorchis penicillatus* were collected from eel-grass beds in Bamfield and Grappler inlets (west coast of Vancouver Island) and held in a flow-through aquarium at about 11 °C.

Electrophysiology

Extracellular recordings were made with flexible polyethylene suction electrodes (Pt or Ag/AgCl) with tip diameters between 25 and 100 μm . Electrical signals were

amplified and displayed in a conventional manner. Ag/AgCl suction electrodes were also used for tissue stimulation with 1–2 ms square pulses. A WPI 140A Scope Raster/Stepper allowed display of multiple consecutive oscilloscope tracings for analysing the effect of repetitive stimulation.

The standard preparation used was an isolated radius (1 quadrant) of *Polyorchis* which consisted of a single radial muscle with intact connexions with the margin and the manubrium. For certain experiments the radial muscle (with radial nerves) was severed without damaging the underlying endoderm. Demarginate preparations are those in which the marginal radial muscle connexion was cut and depedunculate preparations are those in which the apical end of the radial muscle, at the base of the peduncle, was cut. For recordings, the preparation was pinned to the Sylgard (Dow Corning) base of the recording dish and kept at about 13 °C using a glass coil with circulating sea water.

In the figures, potentials above the baseline are negative. For clarity, diagrams are given adjacent to these recordings to show the exact location of the recording (R) and stimulating (S) electrodes.

Light and electron microscopy

Jellyfish were anaesthetized in a 1:1 mixture of sea water and isotonic (0.33 M) $MgCl_2$ and fixed for 1½ h at 4 °C in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer at pH 7.4 containing 10 mM- $CaCl_2$ and 0.18 M-NaCl. After rinsing in 0.2 M cacodylate buffer with 10 mM- $CaCl_2$ and 0.3 M-NaCl, the tissue was post-fixed for 2 h at 4 °C in 1% OsO_4 in 0.2 M cacodylate buffer with 10 mM- $CaCl_2$ and 0.28 M-NaCl, rinsed as before, dehydrated in graded alcohols and embedded in Araldite. Thick sections (0.5 µm) were stained with a 1:1 mixture of 1% aqueous azure II and 1% methylene blue in 1% sodium borate (Richardson, Jarett & Finke, 1960). Thin sections were stained in a 50% ethanol-saturated uranyl acetate solution for 20 min and in lead citrate for 5 min.

RESULTS

Gross anatomy

The anatomy of *Polyorchis* is typical of anthomedusae as described by Spencer (1979). The relationships between tissue layers are illustrated in Fig. 1 to aid interpretation of the electrophysiological data. The bell-shaped body consists of two ectodermal surfaces, an outer exumbrellum, which is free of muscular processes and nerves, and an inner subumbrellum. These surfaces join at the velum which is an annular flap at the base of the bell or margin. The manubrium is attached to the apex of the subumbrellum by the peduncle.

The endoderm is separated from the ectoderm by a layer of acellular mesogloea which is very thick on the exumbrellar side. The endoderm consists of a contiguous canal system, represented by the marginal ring canal, the four radial canals, which pass to the bell apex, and the cavity of the manubrium, and an interradian lamella interconnecting the radial canals and the ring canal. Overlying each radial canal the subumbrellar ectoderm is present as longitudinal radial muscles (the perradius) whereas

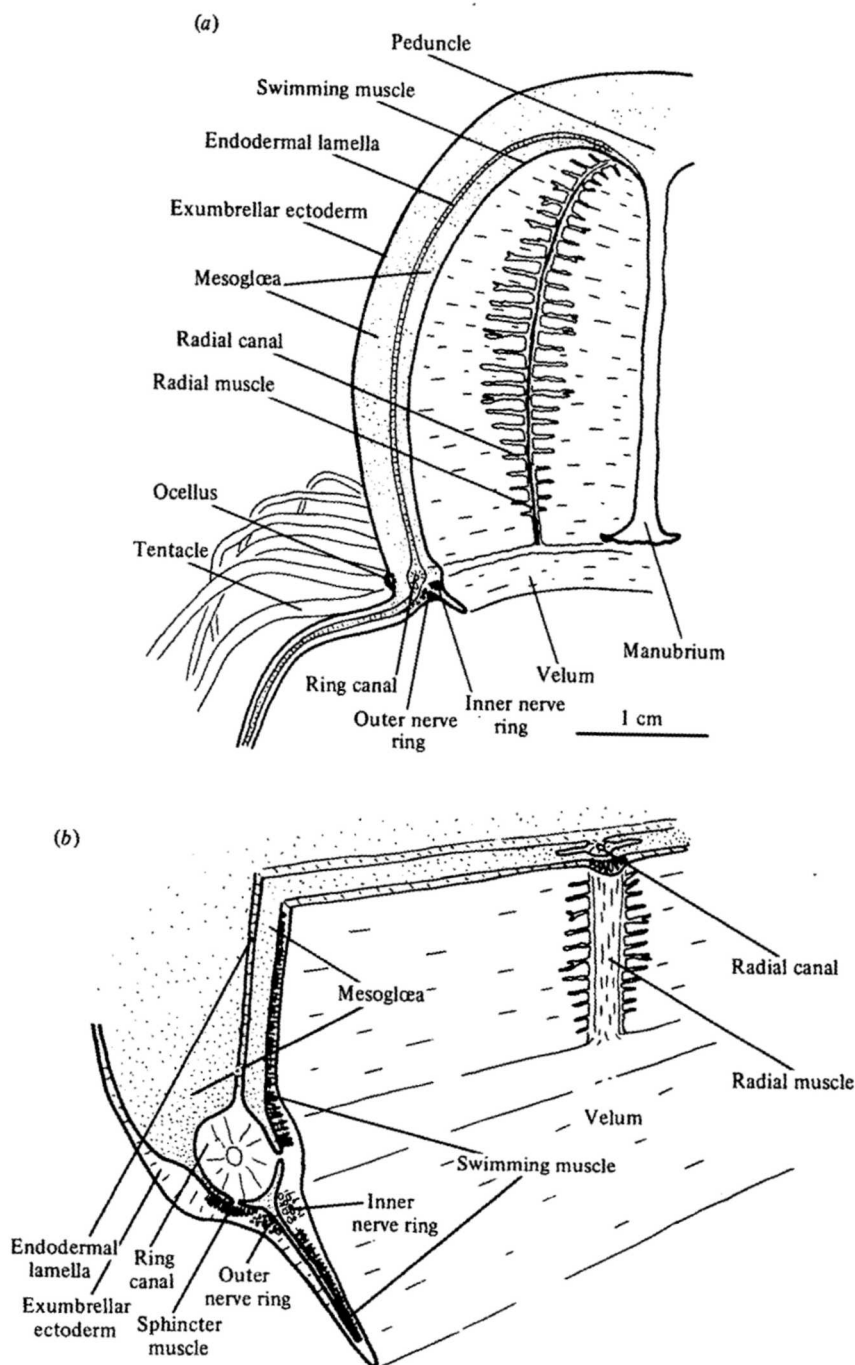


Fig. 1. Diagrams of *Polyorchis penicillatus* showing the relationships between tissue layers. (a) Animal with radial cuts through the margin and bell. (b) Enlargement of radially sectioned margin and transversely sectioned radial muscle.

between canals the subumbrellar ectoderm forms the circular swimming muscle (the interradius).

The primary nervous 'centre' consists of the inner (subumbrellar) and the outer (exumbrellar) marginal ectodermal nerve-rings which lie on either side of the base of the velum. In addition, there are radial nerve bundles on either side of each radial muscle.

Behavioural observations

The crumpling response of *Polyorchis* resembled that of other anthomedusae (Hyman, 1940; Mackie & Passano, 1968; Mackie, 1975), as previously reported (King, 1979). Crumpling can be initiated by mechanical or electrical stimulation of any part of the ectoderm, particularly the subumbrellar surface.

A maximal crumpling response involved simultaneous contraction of the four subumbrellar radial muscles, the marginal sphincter muscle and the longitudinal muscles of the tentacles and manubrium. This resulted in an involution of the margin and shortening of the bell so that it enclosed the tentacles, nerve-rings, gonads and manubrium. The bell opening was also narrowed.

Exumbrellar stimulation frequently elicited 'partial' crumpling in which no sphincter muscle contraction was visible. However, marginal or subumbrellar stimulation consistently yielded a 'full' crumpling response.

Electrophysiology

The behavioural observations suggested a complexity not easily reconciled with the entirely epithelial crumpling pathway hypothesized by Mackie & Passano (1968). The precise role of epithelial impulses in the control of crumpling was determined by establishing the temporal relationships between radial muscle potentials (RMPs) and endodermal (subumbrellar) or ectodermal (exumbrellar) impulses in response to various endodermal or ectodermal sites of stimulation. To test the hypothesis that excitation passes directly from endoderm to radial muscle during crumpling (Mackie & Passano, 1968), endodermal lamellae were stimulated directly and the resulting radial muscle activity was recorded with suction electrodes. If this hypothesis is correct, radial muscle potential latency should be directly related to the distance separating the recording and stimulating electrodes, since propagation of epithelial impulses is non-polarized (Mackie & Passano, 1968; Mackie, 1975; Spencer, 1975, 1978).

Activity recorded by electrodes attached to radial muscle was complex due to the juxtaposition of several excitable tissues: radial muscle, radial nerve, swimming muscle, radial canal and endodermal lamella. To simplify recordings the endodermal lamella was stimulated directly, to avoid excitation of swimming muscle, and the tip diameter of the recording electrode was always less than the width of the radial muscle, to reduce the likelihood of recording endodermal lamellar activity. It is assumed, therefore, that recordings from intact radial muscle during crumpling represent the concurrent excitation of radial muscle (perhaps with a component derived from excitation of radial nerves) and endodermal canal. The complex crumpling potential recorded from intact radial muscle (Fig. 2*a*) became simplified when recordings were made from the underlying radial canal after the radial muscle and radial nerves had been removed

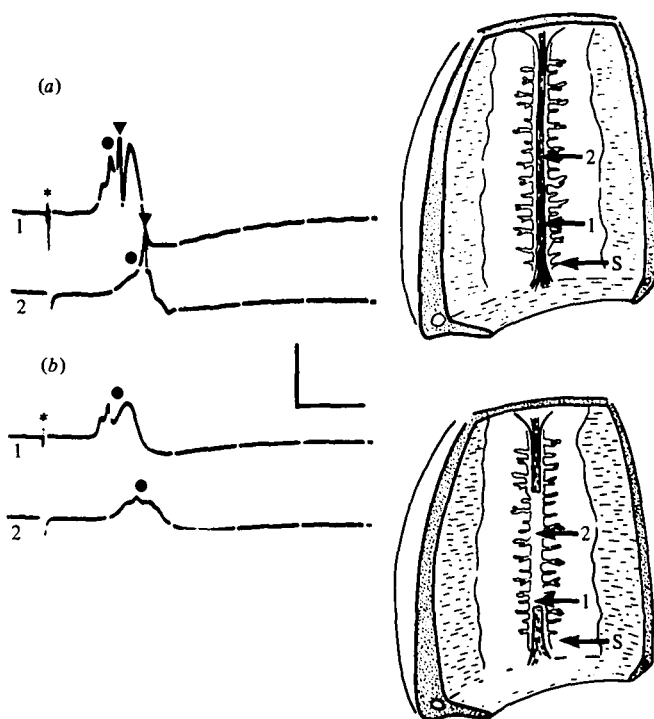


Fig. 2. (a) Complex crumpling potentials recorded from intact radial muscle resulting from endodermal lamellar stimulation. (b) Recording from radial canal after radial muscle is peeled off shows that the slow component (circle) is the endodermal canal pulse (ECP) and suggests that the quick component (triangle in *a*) is the radial muscle potential (RMP). Scale bars: 50 ms, 2 mV. In this and subsequent figures, trace 1 is a recording from electrode 1, trace 2 is a recording from electrode 2, asterisks indicate stimulus artifacts, S is the stimulating electrode, 1 is recording electrode 1 and 2 is recording electrode 2.

by stripping them off with forceps (Fig. 2*b*). This demonstrates that the slow, long duration component of the crumpling response is the endodermal canal pulse (ECP). The complementary experiment was performed by recording from radial muscle before (Fig. 3*a*) and after (Fig. 3*b*) it had been peeled from the radial canal, leaving the marginal attachment intact. This shows that the fast, large-amplitude component of the crumpling response is the radial muscle potential (RMP).

The role of endodermal impulses in exciting radial muscle was determined by stimulating the endodermal lamella and comparing the conduction time of RMPs recorded at two sites. The latency of RMPs was dependent upon the exact position of the stimulating electrode in relation to the recording electrodes and upon the distance of the electrodes from the bell margin and apex. Although stimulating the endoderm near the margin at a point equidistant between recording sites resulted in an ECP which arrived simultaneously at the two sites, the RMP was recorded with different latencies (Fig. 4*a*). This verifies that propagation of endodermal impulses is unpolarized, and the lack of a constant temporal relationship between ECPs and RMPs suggests that endodermal canal pulses do not directly excite radial muscle. Furthermore, the record in Fig. 4(*a*) suggests polarized RMP conduction originating at the

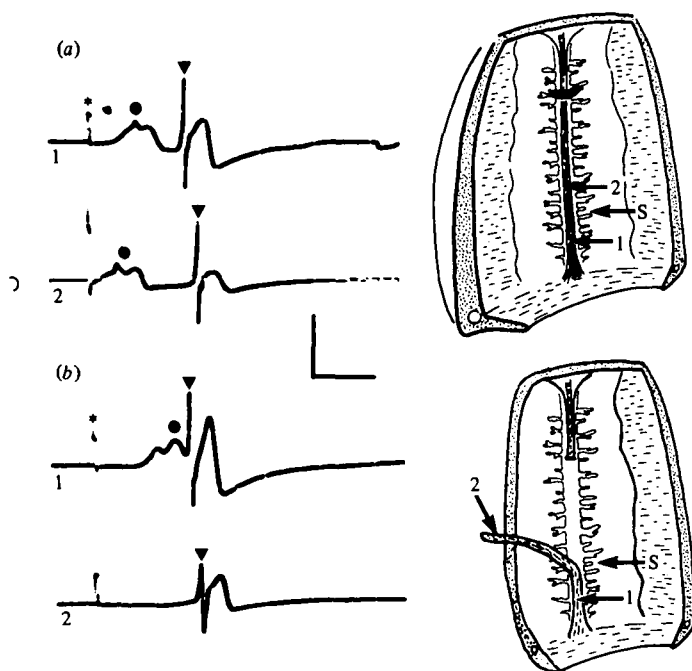


Fig. 3. (a) Complex crumpling potentials recorded from intact radial muscle resulting from endodermal lamellar stimulation. The apical connexion of the muscle has been cut (semicircle). (b) Comparison between a complex crumpling potential recorded from intact radial muscle, with slow (circle) and fast (triangle) components, (R₁) and a discrete radial muscle potential (RPM) recorded from isolated muscle (R₂). Scale bars: 50 ms, 2 mV.

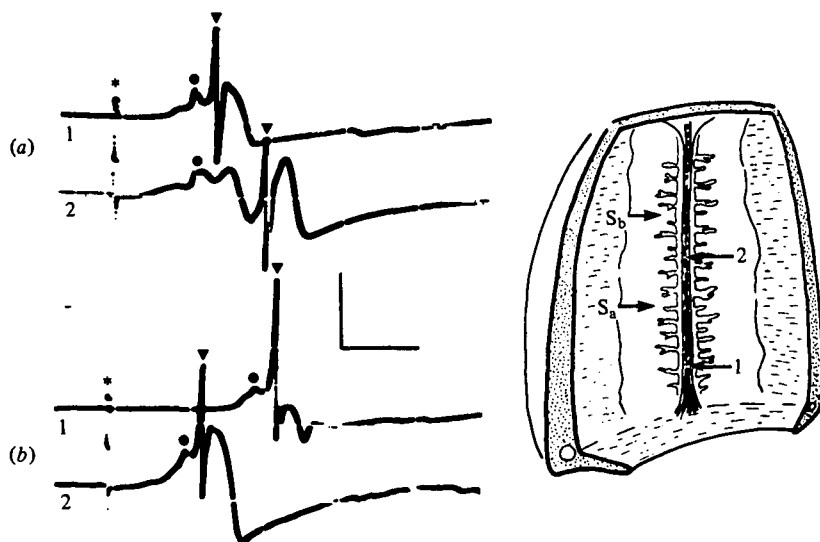


Fig. 4. (a) Stimulating the endodermal lamella equidistant between R₁ and R₂ and between margin and peduncle (S_a) excites an ECP (circle), recorded simultaneously at R₁ and R₂, and an RPM (triangle), arriving first at R₁. (b) Stimulating apical endodermal lamella (S_b), with R₁ and R₂ as in (a), excites an ECP and RPM which both arrive first at R₂. Scale bars: 50 ms, 2 mV.

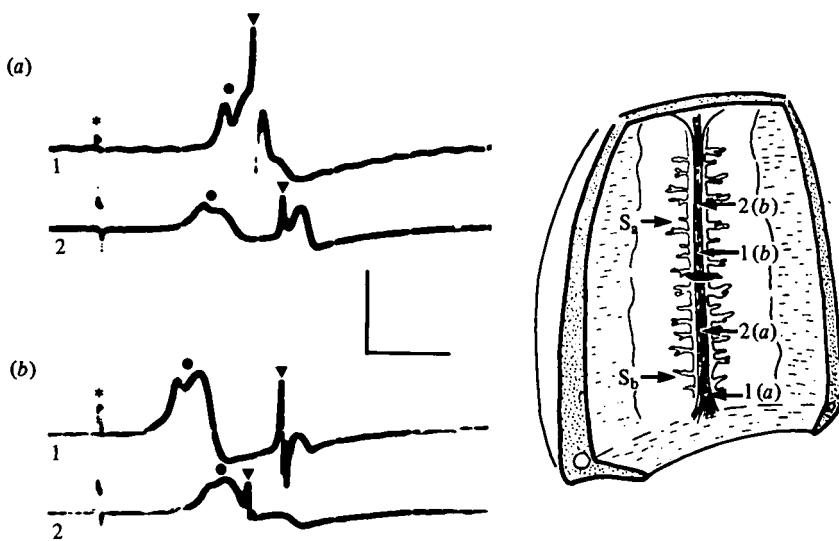


Fig. 5. When a radial muscle is cut (semicircle) between margin and apex, (a) apically directed RMPs (triangles) are recorded from the marginal portion of the muscle when endodermal lamella is stimulated near the apex (S_a) and (b) marginally directed RMPs are recorded from the apical portion of muscle when endodermal lamella is stimulated near the margin (S_b). ECPs (circles) are recorded with latencies proportional to the distance separating the stimulating and recording electrodes. Scale bars: 50 ms, 2 mV.

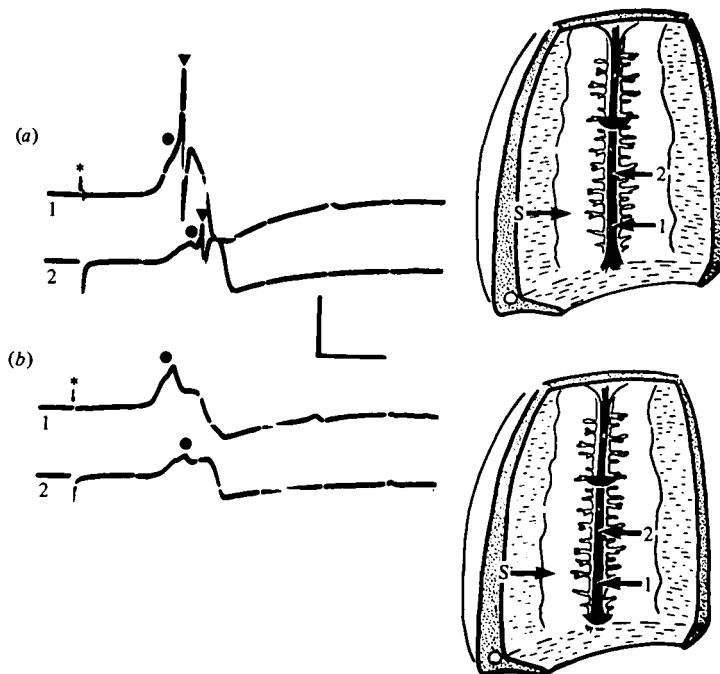


Fig. 6. (a) RMPs (triangles) conducted from the margin and ECPs (circles) recorded from radial muscle in a depedunculate (semicircle) preparation with endodermal lamellar stimulation. (b) ECPs but not RMPs are recorded when the marginal attachment of the muscle is cut (semicircle). Scale bars: 50 ms, 2 mV.

margin. This apically directed conduction along the radial muscle is dependent upon the point of stimulation since stimulating the endoderm near the apex reverses the apparent direction of RMP conduction (Fig. 4*b*). There is some ambiguity in this result since R2 in Fig. 4(*b*) is situated much closer than R1 to S (demonstrated by the delayed arrival of the ECP at R1), consequently, RMP conduction is consistent with endodermal excitation of radial muscle at both the margin and apex. However, severing a radial muscle midway between the margin and apex allowed an investigation of RMP conduction in muscle segments attached only at the margin (Fig. 5*a*) or at the apex (Fig. 5*b*) in the same preparation. Since RMPs were recorded first by the electrodes furthest from S (R1 in Fig. 5*a*; R2 in Fig. 5*b*), resulting in a wide separation of the ECP and RMP recorded by R2 in Fig. 5(*a*) and by R1 in Fig. 5(*b*), the endoderm cannot have directly excited radial muscle. Furthermore, since the direction of RMP conduction depends upon the point of intact radial muscle attachment and not upon the site of stimulation, there are two distinct crumpling pathways in the subumbrellum. In the experiment shown in Fig. 5(*a*) the RMP originated at the margin whereas in Fig. 5(*b*) it originated at the apex.

To unequivocally demonstrate that RMPs are initiated only at the termini of radial muscle, the effect of severing terminal radial muscle was tested. In a preparation in which the radial muscle was cut at the peduncle, only marginal excitation of RMPs occurred (Fig. 6*a*). When the radial muscle was also severed at the margin, while being careful not to damage the underlying endoderm, no radial muscle excitation occurred when the endodermal lamella is stimulated (Fig. 6*b*). Similar results were obtained in preparations where radial muscle was severed at the margin first and then cut at the peduncle.

Conduction pathways were also identified on the basis of rate of fatigue, since it was found that this differed for marginal and peduncular pathways during repetitive stimulation. Repetitive stimulation of endoderm in which an RMP appeared at the apical electrode with the first stimulus, consistently yielded marginally initiated RMPs with subsequent stimuli (Fig. 7*A*). This suggested a greater lability of the apical pathway which was verified by the effect of repetitive stimulation on conduction in depedunculate (Fig. 7*B*) and demarginate (Fig. 7*C*) preparation. Note that fatigue of the apical pathway involved an increased initiation delay of RMPs with each stimulus although the conduction velocity remained constant (Fig. 7*C*). Virtually no increase in initiation delay was discernible throughout a series of responses mediated by the marginal RMP route (Fig. 7*B*).

Since crumpling is more likely to be evoked by irritation of the outer exumbrellar surface in nature, it is important to establish the pathway travelled by exumbrellar pulses (EPs) in evoking radial muscle contraction.

As mentioned previously, the crumpling behaviour elicited by exumbrellar stimulation was quite variable. This seemed to be correlated with the frequent lack of a one-for-one correspondence between exumbrellar pulses and RMPs (Fig. 8). The apparent facilitatory effect of EPs is not easily explained by the direct epithelial pathway to radial muscle as suggested by Mackie & Passano (1968). Furthermore, the waveform of crumpling responses recorded by a suction electrode covering the radial muscle and underlying canal when the exumbrellum was stimulated appeared less

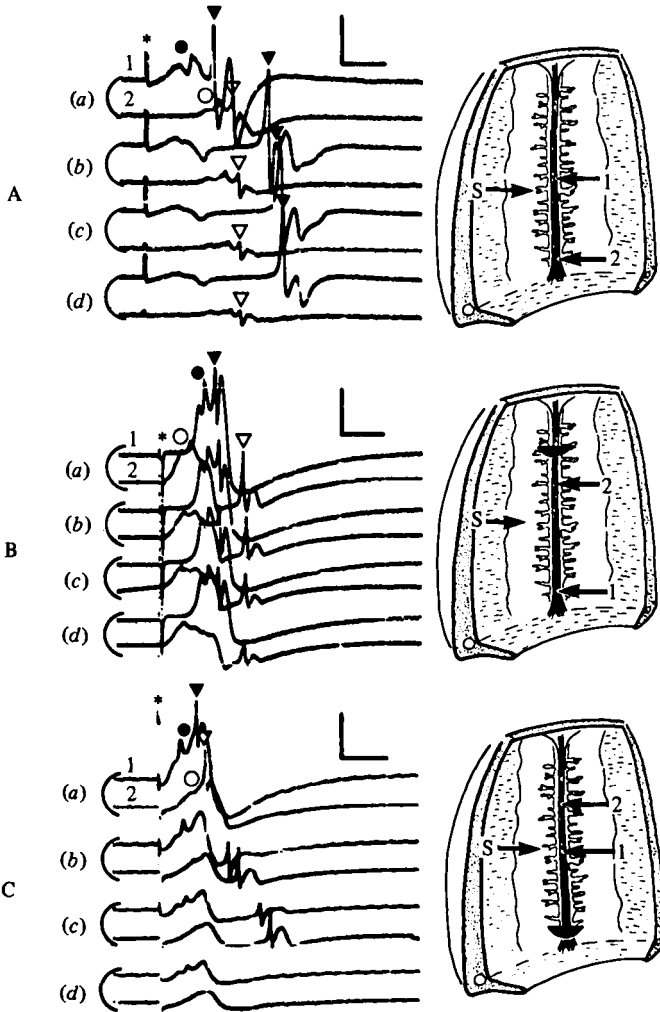


Fig. 7. Raster tracings involving consecutive sweeps (a)–(d) of R1 and R2 each separately triggered by a stimulus. Repetitive stimulation of the endodermal lamella at 0.2 pulses/s results in (A) a differential fatigue of the two RMP pathways, in an intact preparation, such that apical RMP (triangles) initiation is preferentially blocked, (B) a minimal fatigue of the marginal RMP pathway seen in a depedunculate (semicircle) preparation and (C) substantial fatigue, involving increased initiation delay and eventual failure, of the apical pathway seen in a demarginate (semicircle) preparation. Note that ECP (circle) conduction is minimally affected. Solid symbols, trace 1; open symbols, trace 2. Scale bars: 50 ms, 1 mV.

complex than subumbrellar stimulated responses. This difference was most obvious in direct comparisons of exumbrellar (Fig. 9a) and subumbrellar (Fig. 9b) evoked potentials recorded from radial muscle. Note that exumbrellar stimulation did not usually excite the endoderm (Fig. 9a) whereas, subumbrellar stimulation, with the same recording electrode positions, yielded the usual endodermal response recorded from the radial muscle and the endodermal lamella (Fig. 9b). Therefore, the typical radial muscle response resulting from exumbrellar stimulation was a simple RMP without a summated ECP. In the infrequent cases in which endoderm was excited by

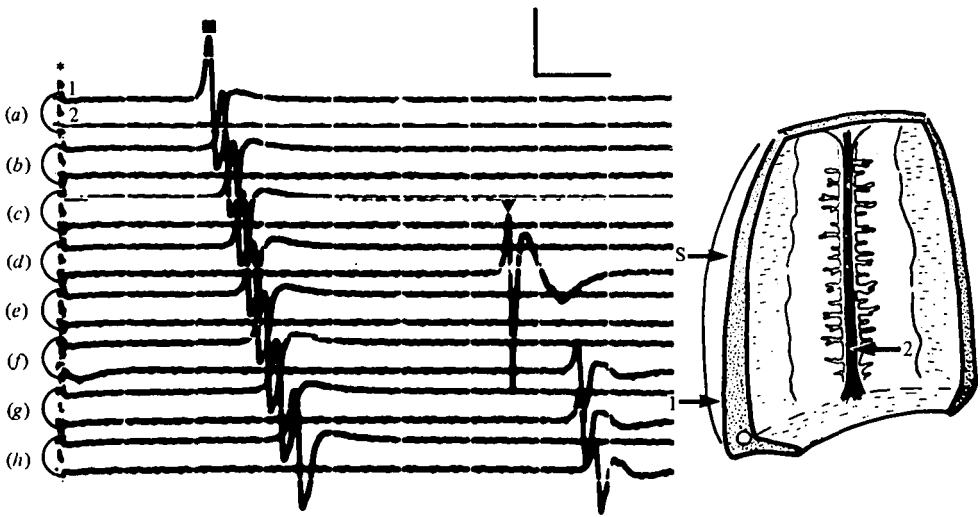


Fig. 8. Raster tracing of consecutive sweeps (a)–(h) of R1 and R2 each triggered by a separate stimulus. Excitation of an RMP (triangle) requires 4 exumbrellar ectoderm stimuli at 1.5 pulses/s, each of which excites an exumbrellar pulse (EP, square). Scale bars: 50 ms, 1 mV.

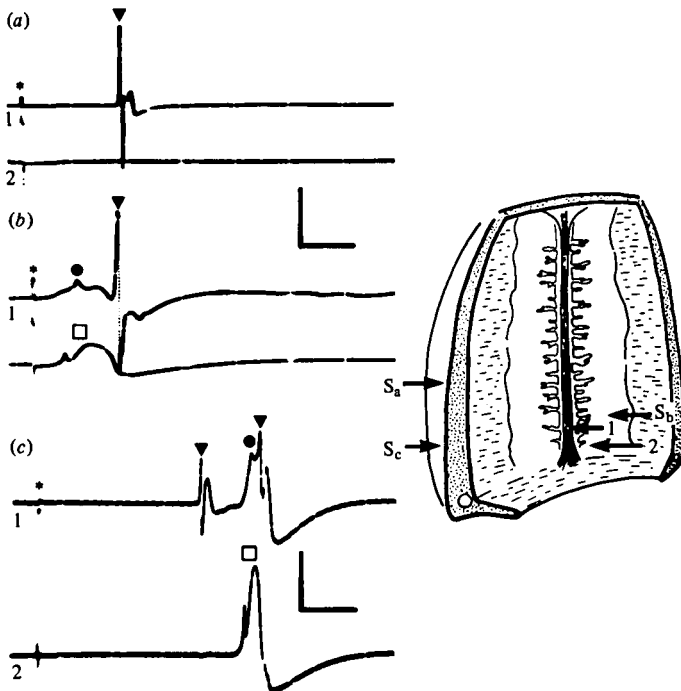


Fig. 9. (a) RMP (triangle) recorded from radial muscle, in the absence of endodermal activity, resulting from stimulation of the exumbrellar ectoderm (S_a). (b) Associated ECP (circle) and RMP in R1 and endodermal lamellar pulse (ELP, open square) in R2 recorded from electrodes as in (a) upon endodermal lamellar stimulation (S_b). (c) RMP followed by compound RMP/ECP in R1 and ELP in R2 recorded from electrodes as in (a) upon exumbrellar stimulation (S_c). Scale bars: (a) 100 ms, 2 mV; (b) 50 ms, 2 mV; (c) 50 ms, 1 mV.

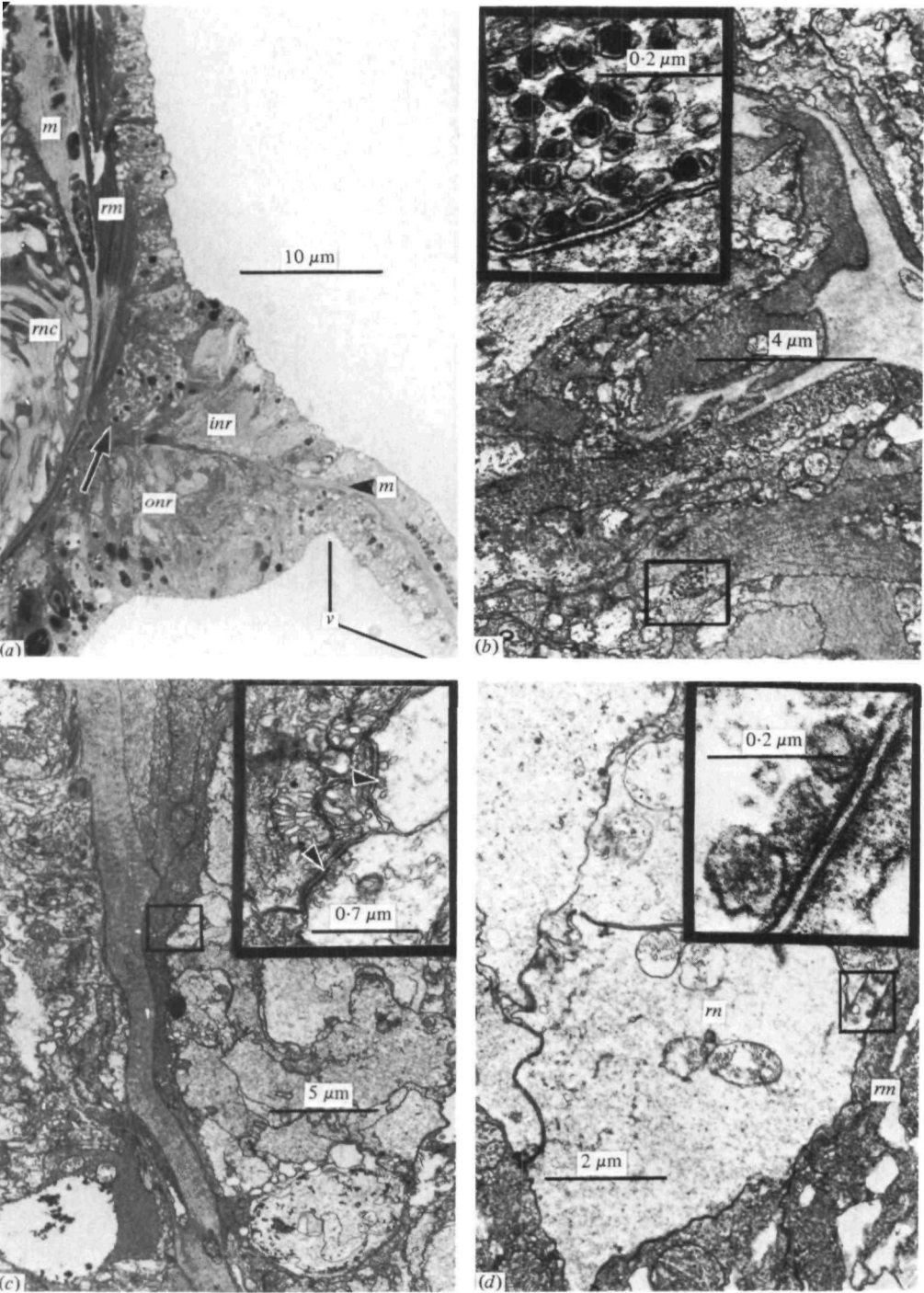
exumbrellar stimulation (Fig. 9c), the endodermal excitation was usually subsequent to radial muscle activity, thus demonstrating that endodermal impulses cannot have a causal relationship to radial muscle excitation. Cutting experiments (as above) showed that an intact marginal RMP pathway was normally required to excite radial muscle by exumbrellar stimulation unless endoderm was excited, in which case, the apical pathway elicited a normal radial muscle response. Behavioural observations have shown that excitation of endoderm via exumbrellar or subumbrellar stimulation was correlated with the 'full' crumpling response. Alternatively, radial muscle activity occurring in the absence of endodermal excitation was associated with the 'partial' response, in which no sphincter muscle contraction occurred (King, 1979).

Morphology

In an attempt to find morphological correlates of the physiologically defined crumpling pathways, sections through the margin at the perradius were examined. The tissue was specifically scrutinized for the presence of specialized contacts between marginal nerve and radial muscle which would explain marginal initiation of radial muscle activity. At the margin the longitudinal processes of radial muscle divide into two halves which cross the mesogloea separating the subumbrellar and exumbrellar ectoderm (Fig. 10a) and eventually merge with the circular sphincter muscle on either side of the perradius (King, 1979). There is extensive apposition of radial muscle processes with neurites of the inner nerve-ring but only infrequent synaptic contacts (Fig. 10b). The rarity of synapses between inner nerve-ring neurites and radial muscle is likely correlated with the primary role of the inner nerve-ring in controlling the swimming musculature (Anderson & Mackie, 1977; Spencer, 1978). In contrast, numerous synapses occur between outer nerve-ring neurites and smooth muscle processes at the perradius (Fig. 10c). The position of these synapses along the velar mesogloal radial muscle connexions suggests that this could represent functional radial muscle innervation.

In addition, frequent synapses occur between radial muscle and radial nerves throughout the length of the muscle (Fig. 10d). This may represent another source of neuronal activation of radial muscles relevant to crumpling since these radial nerves are continuous with the inner nerve-ring (A. N. Spencer & R. A. Satterlie, unpublished observations).

Fig. 10. (a) Light micrograph of radial section through the margin at the perradius, with subumbrellum up and exumbrellum down, showing extensive ectodermal connexions between subumbrellum and exumbrellum (arrow) in the form of radial muscle processes (*rm*). *inr*, Inner nerve-ring; *m*, mesogloea; *onr*, outer nerve-ring; *rc*, ring canal; *v*, velum. $\times 2000$. (b) Radial section through the perradial margin showing a synapse (box) between a neurite of the inner nerve-ring and radial muscle (enlarged in the inset). $\times 6000$, inset $\times 90000$. (c) Radial section through perradial margin with subumbrellum to the left and exumbrellum to the right. Box indicates the position of 2 synapses (enlarged in inset) between neurites of the outer nerve-ring and smooth muscle along the velar mesogloal face. $\times 4000$, inset $\times 22000$. (d) Transverse section through radial muscle showing a synapse (box) between a radial neurite (*rn*) and radial muscle (*rm*), enlarged in the inset. $\times 9000$, int $\times 100000$.



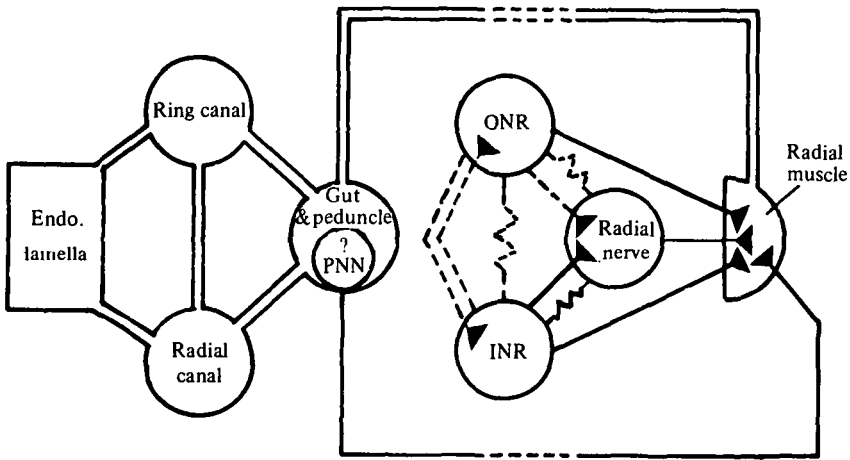


Fig. 11. Wiring diagram of radial muscle excitation pathways which summarizes the established (solid lines) and hypothesized (dashed lines) tissue connexions.?, Presence of ectodermal plexus in the peduncle is unproven; resistors, electrotonic connexions; triangles, chemical synapses; INR, inner nerve-ring; ONR, outer nerve-ring; PNN, peduncular nerve-net.

DISCUSSION

Figure 11 outlines the anatomical connexions in *Polyorchis* revealed by the electrophysiological and ultrastructural observations of this study. Note that the connexions denoted by dashed lines are those which are consistent with the physiology but for which no firm structural data exist.

The easiest explanation for the control of the crumpling response, in light of these tissue interrelationships, is that nervous activity controls the excitation of radial muscle and thus crumpling behaviour. The marginal pathway can be visualized as follows. Subumbrellar stimulation results in the excitation of endodermal impulses which conduct freely between endodermal lamella and radial and ring canal. Upon reaching the margin these impulses are presumed to pass to marginal nerves (within the inner and/or outer nerve-ring) via transmesogloal, electrotonic bridges from the ring canal. Neurones of the inner nerve-ring would then excite radial muscle at its marginal terminus or along its length via radial nerve. Alternatively, activity of neurones of the outer nerve-ring could excite the proximal processes of radial muscle. Exumbrellar stimulation similarly excites ectodermal impulses which upon conduction to the margin could directly excite marginal nerves. Explaining the apical pathway of radial muscle excitation is more speculative since the relevant histological relationships have not been described in *Polyorchis*. It is possible that there are epithelial connexions between radial (endodermal) canal and ectoderm of the peduncle. Furthermore, it is likely that there is an ectodermal peduncular nerve plexus in *Polyorchis* analogous to that described in the closely related hydromedusan *Stomatoca* (Mackie & Singla, 1975). Given these assumptions, the apical initiation of radial muscle contraction could be explained as a consequence of synaptic output of peduncular nerves resulting from electrotonic endodermal spread. In comparing the marginal and apical excitation pathways, the greater lability of the apical route, as seen by its greater sensitivity

to repetitive stimulation, suggests a different sequence of excitation for these two pathways. It is conceivable that apical excitation involves the activation of radial muscle by radial nerves (endoderm \rightarrow peduncular nerve net \rightarrow radial nerves \rightarrow muscle) whereas the marginal pathway proceeds through the more direct excitation of muscle via marginal nerves (endoderm \rightarrow marginal nerves \rightarrow muscle).

This hypothesis is counter to the prevalent theory that radial muscle excitation is mediated through an entirely epithelial pathway in *Sarsia* and *Stomatoca* (Mackie & Passano, 1968; Mackie, 1975; Mackie & Singla, 1975). These authors suggest that ectodermal impulses resulting from exumbrellar stimulation pass to the endoderm at the margin via epithelial bridges from the ectoderm. Histological evidence of transmesogloal ectoderm–endoderm processes in *Stomatoca* (Mackie & Singla, 1975) has given credence to this hypothesis. In *Polyorchis* there are also numerous epithelial bridges along the lengths of the radial canals (Spencer, 1979). If an entirely epithelial pathway controls crumpling in *Polyorchis* then it is necessary to propose that only the apical and marginal ends of the radial muscles have functional bridges to the underlying radial canals. Thus, despite the apparent structural equivalence of the transmesogloal connexions along the entire radial muscle, only the marginal and apical bridges would have the characteristics which allow sufficient current to flow from the endoderm to depolarize radial muscle beyond spiking threshold. If indeed this were the case, it would represent a novel circumstance in which excitation flow is from an epithelium to muscle, contrary to the well-established dogma of neuronal excitation of effector cells.

There is other circumstantial evidence for implicating nervous activity in effecting the crumpling behaviour of *Polyorchis*. It is evident that epithelial excitation in itself is an insufficient condition for eliciting a crumpling response. Thus, severing the marginal and apical attachments of radial muscle inhibited an exumbrellar or a subumbrellar stimulated radial muscle contraction although the other crumpling effectors responded normally. Furthermore, the exact form of the crumpling response depended upon whether epithelial conduction originates from the exumbrellum or the subumbrellum. Several exumbrellar pulses were usually needed to excite radial muscle and only occasionally did they conduct to the endoderm, in which case they also excited the sphincter muscle. The consistent association of sphincter muscle activity with transfer of epithelial impulses from exumbrellar ectoderm to endoderm suggests a common excitation pathway for these events. The lability of the conduction pathway between exumbrellar ectoderm and subumbrellar endoderm suggest that nerves are involved. Since exumbrellar stimulation can excite radial muscle in the absence of endodermal activity, there must be an independent, more direct excitation pathway from ectoderm to radial muscle. The above observations in *Polyorchis* are difficult to explain in terms of direct epithelial pathway from exumbrellar ectoderm to subumbrellar endoderm and subsequently to radial muscle. Furthermore, excitable epithelial systems are generally considered as providing non-polarized, through-conduction pathways (reviewed by Mackie, 1970; Spencer, 1974; Anderson, 1980), presumably mediated through the intercellular low-resistance channels at gap junctions (King & Spencer, 1979). Consequently, the observed facilitation and lability of conducted impulses cannot be readily attributed to a simple epithelial pathway, but seems to necessitate the presence of neuronal relays within the circuit.

Although hypothesizing an epithelial excitation of nerves invokes a novel, undefined mechanism of information flow, epithelial impulses have been shown to excite neurones (Roberts, 1971; Roberts & Stirling, 1971; Bone & Mackie, 1975; Bassot *et al.* 1978) and to inhibit them (Mackie, 1975). It should be pointed out that systems in which a behaviour directly results from epithelial excitation (Matthews & Sakamoto, 1975; Brandt *et al.* 1976; Mackie, 1976; Taraskevich & Douglas, 1977; Douglas & Taraskevich, 1978; Herrera, 1979) differ from those above in that the epithelium serves both as the conducting substrate and effector of the response.

In nature, severe stimulation of the subumbrellum is probably required to activate crumpling since the endodermal lamella is situated beneath a layer of ectoderm and mesogloea. Considering the height of the bell in *Polyorchis* it is likely that the manubrium, which during feeding extends well below the margin, might be frequently agitated; the apical pathway thus becomes significant. Endodermal impulses arising from manubrial stimulation would rapidly activate crumpling. The latency of this response would be considerably greater if excitation had to pass to the margin. The low sensitivity of the exumbrellar ectoderm and the need for facilitated epithelial impulses in activating a full crumpling response may also be significant to the animal. It can additionally be speculated that the relative insensitivity of the general exumbrellar surface is adaptive in conserving muscular output for times of real potential harm and for performing other essential behaviour patterns, such as feeding and swimming, which cannot occur concurrent with crumpling.

In conclusion, it is suggested that although *Polyorchis* is distinguished from most higher animals in possessing excitable epithelia, which serve as sensory and conductile fields mediating the protective crumpling response, the activation of this response involves neuronal mechanisms similar to those of higher animals.

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REFERENCES

- ANDERSON, P. A. V. (1980). Epithelial conduction: its properties and functions. *Prog. Neurobiol.* **15**, 161-203.
- ANDERSON, P. A. V., BONE, Q., MACKIE, G. O. & SINGLA, C. L. (1979). Epithelial conduction in salps. II. The role of nervous and non-nervous conduction system interactions in the control of locomotion. *J. exp. Biol.* **80**, 241-250.
- ANDERSON, P. A. V. & MACKIE, G. O. (1977). Electrically coupled, photosensitive neurons control swimming in a jellyfish. *Science, N.Y.* **197**, 186-188.
- BASSOT, J.-M., BILBAUT, A., MACKIE, G. O., PASSANO, L. M. & PAVANS DE CECCATTY, M. (1978). Bioluminescence and other responses spread by epithelial conduction in the siphonophore *Hippopodius*. *Biol. Bull. mar. biol. Lab. Woods Hole* **155**, 473-498.
- BONE, Q. & MACKIE, G. O. (1975). Skin impulses and locomotion in *Oikopleura* (Tunicata: Larvacea). *Biol. Bull. mar. biol. Lab. Woods Hole* **149**, 267-286.
- BRANDT, B. L., HAGIWARA, S., KIDOKORO, Y. & MIYAZAKI, S. (1976). Action potentials in the rat chromaffin cell and effects of acetylcholine. *J. Physiol., Lond.* **263**, 417-439.
- DOUGLAS, W. W. & TARASKEVICH, P. S. (1978). Action potentials in gland cells of rat pituitary pars intermedia: inhibition by dopamine, an inhibitor of MSH secretion. *J. Physiol., Lond.* **285**, 171-184.
- HERRERA, A. A. (1979). Electrophysiology of bioluminescent excitable epithelial cells in a polynoid polychaete worm. *J. comp. Physiol.* **129A**, 67-78.
- MAN, L. H. (1940). Observations and experiments on the physiology of medusae. *Biol. Bull. mar. biol. Lab. Woods Hole* **79**, 282-296.

- JOSEPHSON, R. K. & SCHWAB, W. E. (1979). Electrical properties of an excitable epithelium. *J. gen. Physiol.* **74**, 213-236.
- KATER, S. B., RUED, J. R. & MURPHY, A. D. (1978). Propagation of action potentials through electrotonic junctions in the salivary glands of the pulmonate mollusc, *Helisoma trivolis*. *J. exp. Biol.* **72**, 77-90.
- KING, M. G. (1979). Epithelial conduction and the control of crumpling behavior in *Polyorchis penicillatus*. M.Sc. thesis, University of Alberta, pp. 183.
- KING, M. G. & SPENCER, A. N. (1979). Gap and septate junctions in the excitable endoderm of *Polyorchis penicillatus* (Hydrozoa, Anthomedusae). *J. Cell Sci.* **36**, 391-400.
- MACKIE, G. O. (1970). Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.* **45**, 319-332.
- MACKIE, G. O. (1975). Neurobiology of *Stomatoca*. II. Pacemakers and conduction pathways. *J. Neurobiol.* **6**, 357-378.
- MACKIE, G. O. (1976). Propagated spikes and secretion in a coelenterate glandular epithelium. *J. gen. Physiol.* **68**, 313-325.
- MACKIE, G. O. & BONE, Q. (1976). Skin impulses and locomotion in an ascidian tadpole. *J. mar. biol. Ass. U.K.* **56**, 751-768.
- MACKIE, G. O. & PASSANO, L. M. (1968). Epithelial conduction in hydromedusae. *J. gen. Physiol.* **52**, 600-621.
- MACKIE, G. O. & SINGLA, C. L. (1975). Neurobiology of *Stomatoca*. I. Action systems. *J. Neurobiol.* **6**, 339-356.
- MATTHEWS, E. K. & SAFFRAN, M. (1973). Ionic dependence of adrenal steroidogenesis and ACTH-induced changes in the membrane potential of adrenocortical cells. *J. Physiol., Lond.* **234**, 43-64.
- MATTHEWS, E. K. & SAKAMOTO, Y. (1975). Electrical characteristics of pancreatic islet cells. *J. Physiol., Lond.* **246**, 421-437.
- RICHARDSON, K. C., JARETT, L. & FINKE, E. H. (1960). Embedding in epoxy resins for ultrathin sections in electron microscopy. *Stain Technol.* **35**, 313-322.
- ROBERTS, A. (1971). The role of propagated skin impulses in the sensory system of young tadpoles. *Z. vergl. Physiol.* **75**, 388-401.
- ROBERTS, A. & STIRLING, C. A. (1971). The properties and propagation of a cardiac-like impulse in the skin of young tadpoles. *Z. vergl. Physiol.* **71**, 295-310.
- SPENCER, A. N. (1974). Non-nervous conduction in invertebrates and embryos. *Am. Zool.* **14**, 917-929.
- SPENCER, A. N. (1975). Behavior and electrical activity in the hydrozoan *Proboscoidactyla flavicirrata* (Brandt). II. The medusa. *Biol. Bull. mar. biol. Lab. Woods Hole* **149**, 236-250.
- SPENCER, A. N. (1978). Neurobiology of *Polyorchis*. I. Functions of effector systems. *J. Neurobiol.* **9**, 143-157.
- SPENCER, A. N. (1979). Neurobiology of *Polyorchis*. II. Structure of effector systems. *J. Neurobiol.* **10**, 95-117.
- TARASKEVICH, P. S. & DOUGLAS, W. W. (1977). Action potentials occur in cells of the normal anterior pituitary gland and are stimulated by the hypophysiotropic peptide thyrotropin-releasing hormone. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4064-4067.