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Neuro-epitheliomuscular cell and neuro-neuronal gap junctions in *Hydra*

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Summary

Gap junctions have been described ultrastructurally between neurons and epitheliomuscular cells and between neurons and their processes in the hypostome, peduncle and basal disc of *Hydra*. All gap junctions examined in *Hydra* exhibit two apposed plasma membranes having a 2–4 nm gap continuous with the extracellular space. The gap junctions are variable in length from 0.1–1.6 μm and appear linear or V-shaped in section. Neuronal gap junctions in *Hydra* occur infrequently as compared to chemical synapses. Electron microscopy of serial sections has demonstrated the presence of adjacent electrical and chemical synapses (neuromuscular junctions) formed by the same neuron. In addition, multiple gap junctions were present between two neurons. This is the first ultrastructural demonstration of electrical synapses in the nervous system of *Hydra*. Such synapses occur in neurons previously characterized as sensory-motor-interneurons on the basis of their chemical synapses; these neurons appear to represent a type of stem cell characterized by having both electrical and chemical synapses.

Introduction

Recent reviews on the structural diversity, functional morphology and distribution of gap junctions within the animal kingdom indicate the widespread distribution of these intercellular connections, which are characterized by two apposed plasma membranes exhibiting a 2–4 nm intermembrane gap continuous with extracellular space (Larsen, 1977; Bennett & Goodenough, 1978; Varon & Somjen, 1979). It is generally agreed that gap junctions in excitable tissues function as electrotonic synapses (Bennett & Goodenough, 1978). Gap junctions have only recently been demonstrated ultrastructurally in the nervous systems of coelenterates (Singla, 1978; Westfall, 1978; Spencer, 1979). They are common between epitheliomuscular cells in *Hydra* where they have been postulated to function in both adhesion and electrical coupling (Wood, 1977, 1979). Neuro-epitheliomuscular cell gap junctions have not been reported to date in coelenterates. Previous work in our laboratory has demonstrated ultrastructurally the presence of chemical synapses in *Hydra* (Westfall

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et al., 1971; Westfall, 1973; Westfall & Kinnamon, 1978). The present study provides a description of the ultrastructure of heterocellular (neuro-epitheliomuscular cell) and homocellular (neuro-neuronal) gap junctions in the nervous system of two species of *Hydra*.

Materials and methods

Specimens of *Hydra littoralis* and *Pelmatohydra pseudoligactis* (Carolina Biological Supply) were prepared for electron microscopy by relaxation in urethane (Macklin, 1976), followed by fixation in 2.5% glutaraldehyde in 0.05 M sodium cacodylate (350 mosmol) at pH 7.3. Postfixation in 1% buffered OsO_4 was followed by ethanolic dehydration, acetone rinsing and infiltration of tissue in 1:1 acetone–Epon/Araldite under light vacuum before the tissue was cured in fresh resin (Westfall & Kinnamon, 1978). Serial thin sections were cut on a diamond knife, stained in ethanolic uranyl acetate followed by triple lead stain (Sato, 1968), then carbon coated and examined in a Philips EM 301 at 100 kV.

Observations

We have searched extensively for neuronal gap junctions in three regions of *Hydra* (hypostome, peduncle and basal disc) by examining selected tissues from ten different adult animals representing two species, *Hydra littoralis* and *Pelmatohydra pseudoligactis*. The numbers and types of gap junctions found and their regional distribution are summarized in Table 1.

Table 1.

	Neuro-epitheliomuscular		*Neuro-neuronal*		
	Somatic	*Axonal*	*Somato-somatic*	*Somato-axonal (axo-somatic)*	*Axo-axonal*
Hypostome	6	2	1	1	3
Peduncle				1	1
Basal disc		1		2	2

Eleven of the 20 gap junctions observed were associated with the somata of ganglionic sensory-motor-interneurons (Figs. 1–5). They were both heterocellular

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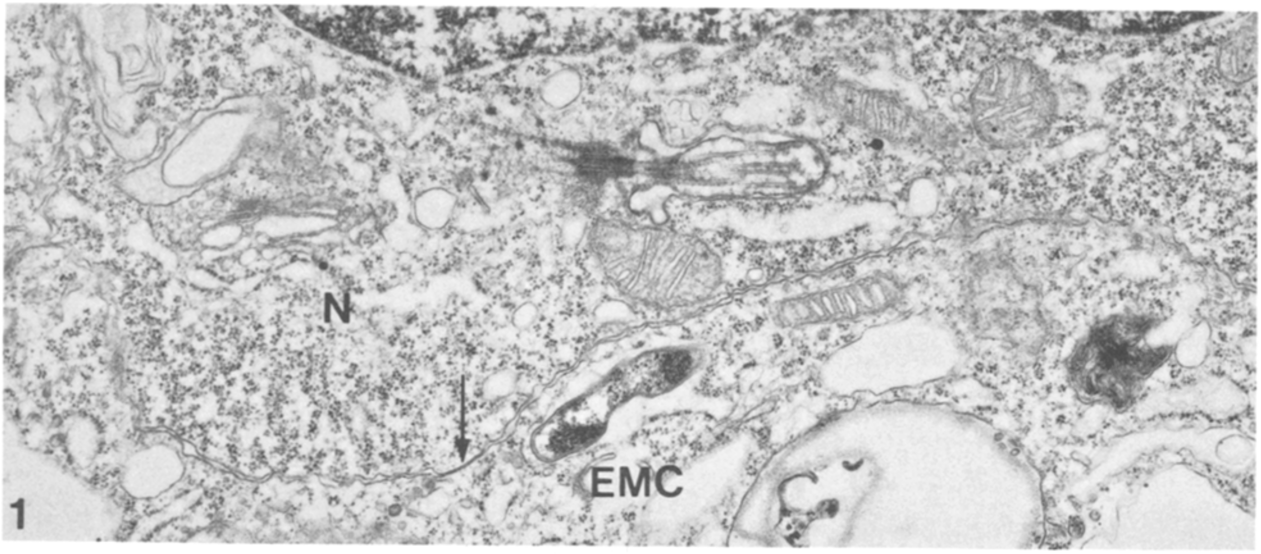
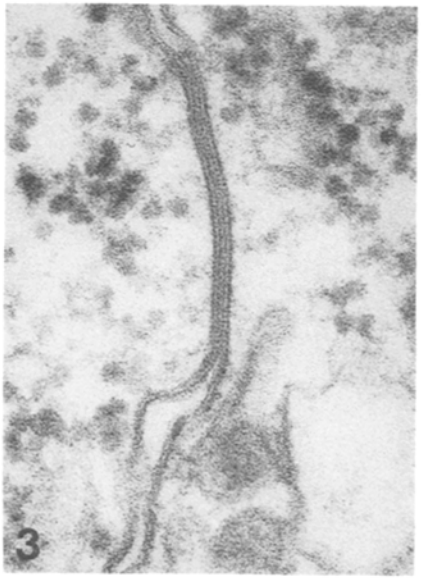
Fig. 1. Sensory-motor-interneuron in hypostome of *Pelmatohydra pseudoligactis* showing gap junction (arrow) between neuronal perikaryon (N) and epitheliomuscular cell (EMC). × 21 000.

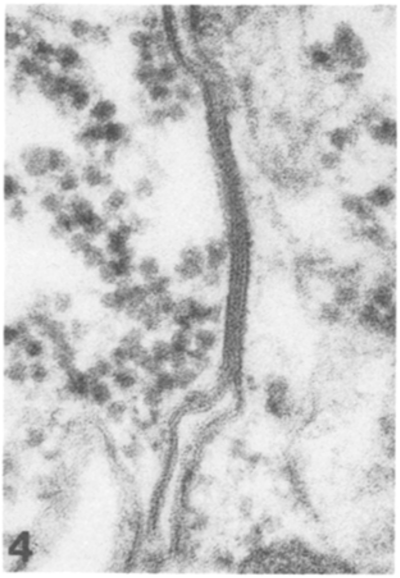
Figs. 2–4. Three serial sections through the neuro-epitheliomuscular cell gap junction in Fig. 1 showing proximity of ribosomes to the pair of closely apposed membranes. Note peripheral dense-cored vesicular invagination into the epitheliomuscular cell. × 118 000.

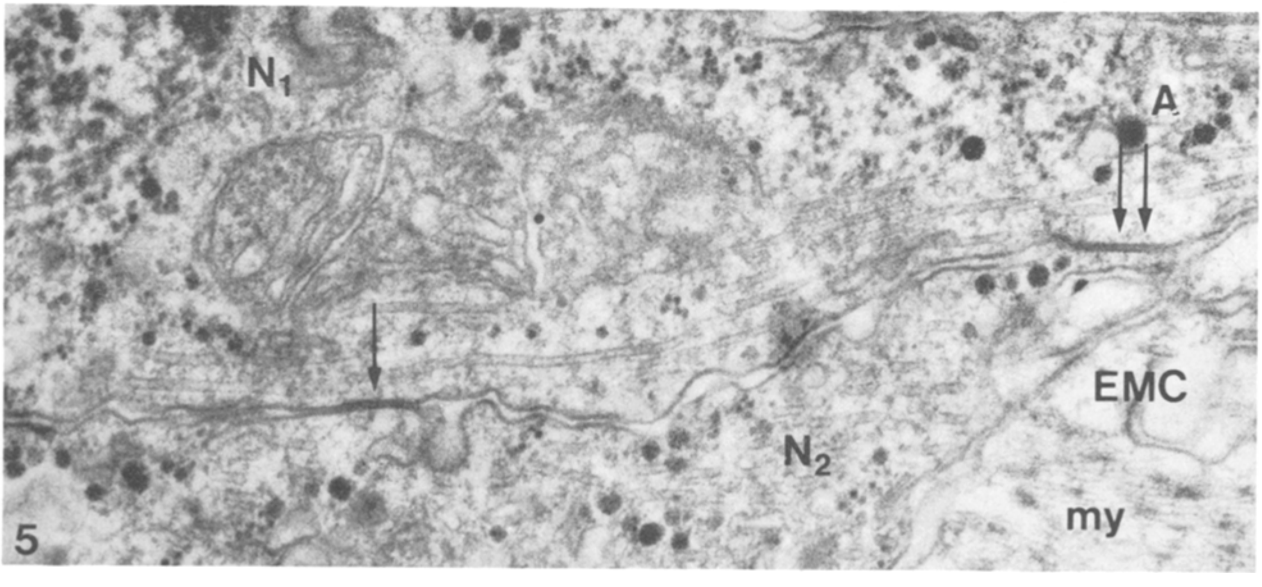
Fig. 5. Origin of axon (A) from perikaryon (N_1) of sensory-motor-interneuron of basal disc showing two of a series of three gap junctions connecting the soma (single arrow) and axon (double arrows) to the process of a second neuron (N_2) above the myoneme (my) of an epitheliomuscular cell (EMC). × 62 700


N
EMC
1


2


3


4


N1
A
EMC
N2
my
5

(neuro-epitheliomuscular cell) and homocellular (neuro-neuronal) in nature. Four of the neuro-neuronal junctions were between the perikarya and other neuronal processes (axons). Each perikaryon bears a sensory cilium (Fig. 1), occasionally seen at the external surface of the animal, which establishes a polarity to the ganglion cell. Hence, all neuronal processes identified by the presence of dense-cored vesicles and bundles of microtubules have been termed axons. In one instance three gap junctions occurred in a series extending from the nuclear region of the perikaryon into the axon. Nine of the gap junctions were between axons and epitheliomuscular cells (Fig. 6) or between two axons (Figs. 7–10). The axo-axonal gap junctions in *Hydra* occurred *en passant*.

All the gap junctions observed in the nervous system resembled those commonly found between epitheliomuscular cells in *Hydra*. The lengths were variable, ranging from 0.1–1.6 μm, but the gaps were uniformly 2–4 nm in width. Cross-sectional views showed mainly short linear or curved junctions (Figs. 1–6). One serially examined gap junction, however, was V-shaped with a slight expansion in the gap at the acute angle (Figs. 7–10).

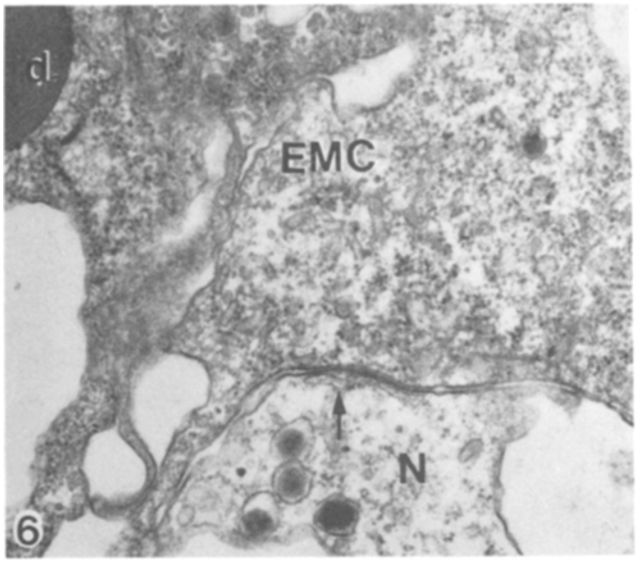
The junctional regions often contained clusters of ribosomes and in one instance a dense-cored vesicular invagination was adjacent to the periphery of the gap (Figs. 1–4). Neuro-epitheliomuscular cell gap junctions occurred near the bases of epitheliomuscular cells; none, however, were in direct contact with the myonemes. The most interesting observation was the close relationship between two neuromuscular chemical synapses and an adjacent axo-axonal gap junction (Figs. 7–10). Serial sections revealed that a chemical synapse, characterized by a row of dense-cored vesicles associated with paramembranous densities and a 15–20 nm wide cleft with transverse filaments, was 0.13 μm from the gap junction. The second axon also established a chemical synapse about 0.25 μm from the gap junction.

Fig. 6. Neuro-epitheliomuscular cell gap junction in basal disc of *Hydra littoralis* showing cisterna (arrow) in neuronal process (N) and dense cytoplasmic region of epitheliomuscular cell (EMC). Note secretory droplet (d) in an adjacent vacuolate glandulomuscular cell. × 29 500.

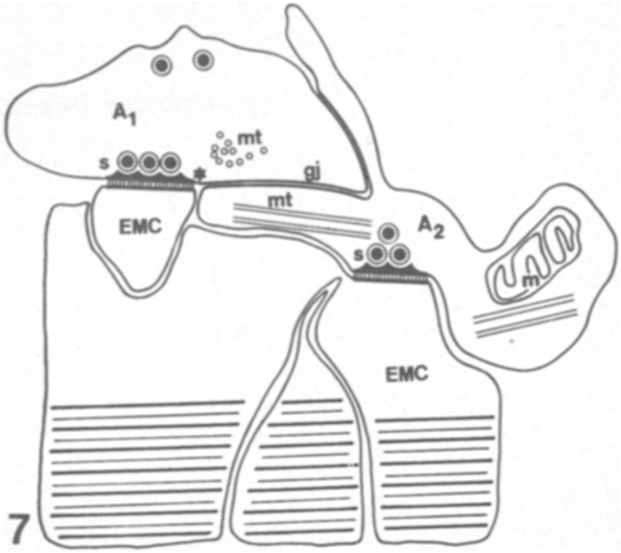
Fig. 7. Diagrammatic representation of V-shaped gap junction (gj) shown in Figs. 8–10 with chemical neuromuscular synapses (s) established by axons (A_1 and A_2) containing bundles of microtubules (mt), dense-cored vesicles and a mitochondrion (m). Note close proximity of two different types of junctions (*) and the apparent innervation of two different epitheliomuscular cells (EMC).

Fig. 8. Axo-axonal gap junction (gj) and parts of two chemical neuromuscular synapses (s) in hypostome of *Hydra littoralis*. Note proximity of electrical and chemical junctions (*) in A_1, mitochondrion (m) and synaptic vesicles (arrow) in A_2, and myoneme (my) of an epitheliomuscular cell (EMC). × 39 300.

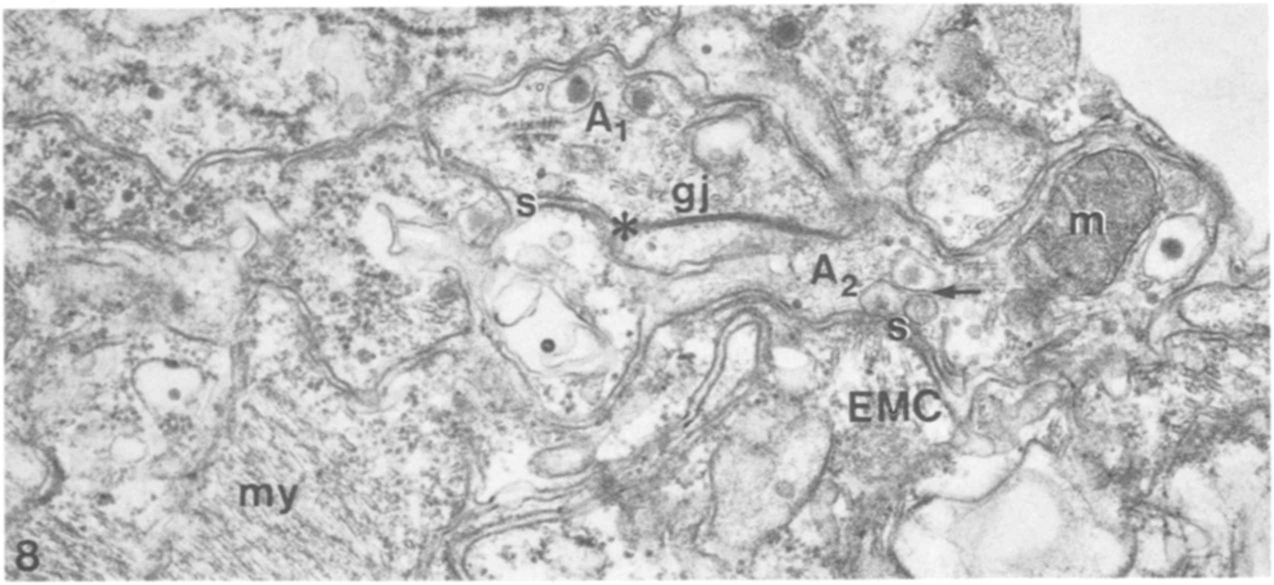
Figs. 9–10. Serial sections of gap junction in Fig. 8 showing long length and V-shape of closely apposed axonal membranes. Note microtubules (mt) in cross-section in A_1 (Fig. 9) and in longitudinal section in A_2 (Fig. 10). The sites of neuromuscular synapses (s) associated with EMC_1 and EMC_2 are faintly visible in Fig. 9 and have disappeared in Fig. 10. × 62 700.


d
EMC
N

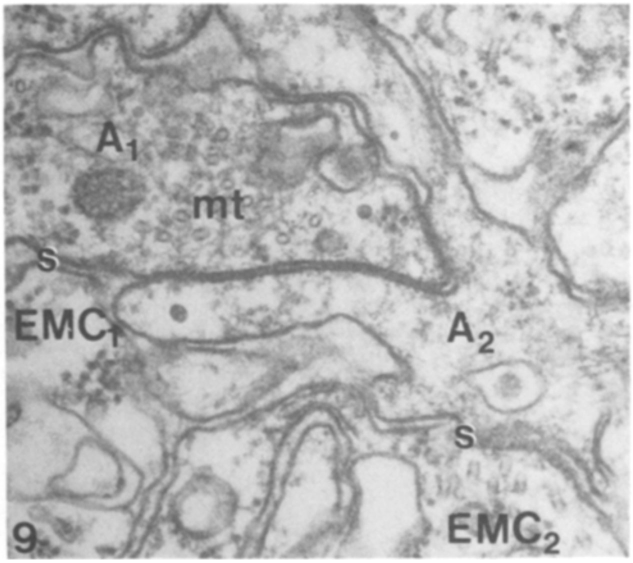
6


A_1
mt
s
gj
EMC
mt
A_2
s
m
EMC

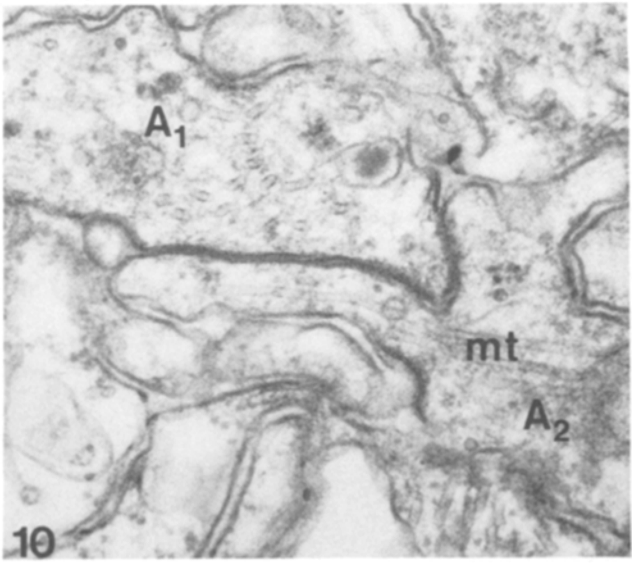
7


A_1
s
*
gj
m
A_2
s
EMC
my

8


A_1
mt
s
EMC_1
A_2
s
EMC_2

9


A_1
mt
A_2

10

Discussion

The functional importance of neuro-epitheliomuscular cell gap junctions in *Hydra* is not known. Those that we have observed are located just above the myonemes. The enveloping nature of the epitheliomuscular cells around *Hydra* neurons, which are naked and lack myelin sheaths, suggests that the gap junctions could be either neuro-glial (neuro-epithelial) or neuromuscular. At present there is no known electrophysiological evidence of neuro-glial gap junctions or examples of electrical neuromuscular junctions in adult animals (Bennett, 1973). The occurrence of gap junctions in nerve–muscle cultures (see Varon & Somjen, 1979) suggests that a temporary coupling may exist during animal tissue development. Neurons in *Hydra*, unlike those in higher animals, are constantly being sloughed and replaced (Davis, 1973; Campbell, 1974); thus the presence of neuro-epitheliomuscular cell gap junctions in *Hydra* might be associated with a temporary coupling and uncoupling of cells during growth and regeneration.

Neuro-neuronal gap junctions in the hypostome, peduncle and basal disc of *Hydra* may be significant in relation to mechanisms underlying behaviour. It has been difficult to explain behaviour in coelenterates entirely on the basis of either non-neuronal conduction between epithelia or solely on the basis of a nerve net. Campbell *et al.* (1976) found that elimination of nerve cells in *Hydra* stopped pacemaker activity, suggesting a role for discrete neurons. On the other hand, nerve-free epithelia are capable of propagating behaviourally meaningful signals (Mackie, 1965). It has also been demonstrated that such epithelial conduction is important in transmitting the three major pulse types in *Hydra* (Rushforth, 1973; Kass-Simon & Passano, 1978). Electrically coupled neurons are involved in producing pacemaker activity in other invertebrates (Getting & Willows, 1973; Berry & Pentreath, 1979); therefore, one might postulate a role for neuro-neuronal gap junctions in generating pacemaker activity in *Hydra*.

There are few neuro-neuronal gap junctions in *Hydra* compared to chemical synapses previously described (Westfall, 1973; Westfall & Kinnamon, 1978). The gap junctions are located on neuronal somata as well as between axons. Neuro-neuronal and intraneuronal (reflexive) gap junctions have recently been reported in a hydromedusan, *Polyorchis*, where they are associated with the giant axons of the inner nerve ring (Singla, 1978; Spencer, 1979). To date we have not observed reflexive gap junctions in *Hydra*.

Mackie (1970) suggested that low resistance intercellular pathways are a primitive feature. As evolution of the nervous system proceeds, there would occur simultaneously a need for increased specificity in conduction and response; thus, he suggested that the original coupling might be replaced by specialized synapses. The ultrastructural demonstration of a variety of chemical synapses in the nervous system of *Hydra* in our laboratory (Westfall *et al.*, 1971; Westfall, 1973; Westfall & Kinnamon, 1978) suggested a complex involvement of the nervous system in behaviour. In another coelenterate, *Polyorchis*, giant axons which initiate swimming behaviour have been ultrastructurally demonstrated to have both neuro-neuronal gap junctions and chemical neuromuscular junctions (Anderson & Mackie, 1977; Spencer, 1979). Now, with the ultrastructural demonstration of neuro-neuronal gap junctions and adjacent chemical and electrical synapses between cells in *Hydra*, there are possibilities for even more complex neural control of behaviour in a structurally simple representative of the phylum having the most primitive nervous system.

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References

ANDERSON, P. A. V. & MACKIE, G. O. (1977) Electrically coupled, photosensitive neurons control swimming in jellyfish. *Science* **197**, 186–8.

BENNETT, M. V. L. (1973) Function of electronic junctions in embryonic and adult tissues. *Federation Proceedings* **32**, 65–75.

BENNETT, M. V. L. & GOODENOUGH, D. A. (1978) Gap junctions, electrotonic coupling and intercellular communication. *Neurosciences Research Program Bulletin* **16**, 377–486.

BERRY, M. S. & PENTREATH, V. W. (1979) Electrotonic coupling among postsynaptic neurons of the characterized dopamine neuron in *Planorbis*. *Journal of Comparative Physiology* **131**, 267–75.

CAMPBELL, R. D. (1974) Cell movements in *Hydra*. *American Zoologist* **14**, 523–35.

CAMPBELL, R. D., JOSEPHSON, R. K., SCHWAB, W. E. & RUSHFORTH, N. B. (1976) Excitability of nerve-free hydra. *Nature* **263**, 388–90.

DAVIS, L. E. (1973) Ultrastructure of neurosensory cell development. In *Biology of Hydra* (edited by BURNETT, A. L.), pp. 271–298. New York, London: Academic Press.

GETTING, P. A. & WILLOWS, A. O. D. (1973) Burst formation in electrically coupled neurons. *Brain Research* **63**, 424–9.

KASS-SIMON, G. & PASSANO, L. M. (1978) A neuropharmacological analysis of the pacemakers and conducting tissues of *Hydra attenuata*. *Journal of Comparative Physiology* **128**, 71–9.

LARSON, W. J. (1977) Structural diversity of gap junctions. A review. *Tissue and Cell* **9**, 373–94.

MACKIE, G. O. (1965) Conduction in the nerve-free epithelia of Siphonophores. *American Zoologist* **5**, 439–53.

MACKIE, G. O. (1970) Neuroid conduction and the evolution of conducting tissues. *Quarterly Review of Biology* **45**, 319–32.

MACKLIN, M. (1976) The effect of urethane on *Hydra*. *Biological Bulletin* **150**, 442–52.

RUSHFORTH, N. B. (1973) Behavioral modifications in coelenterates. In *Invertebrate Learning*, Vol. 1 *Protozoans through Annelids* (edited by CORNING, W. C., DYAL, J. A. and WILLOWS, A. O. D.), pp. 123–169. New York, London: Plenum.

SATO, T. (1968) A modified method for lead staining of thin sections. *Journal of Electron Microscopy (Tokyo)* **17**, 158–9.

SINGLA, C. L. (1978) Fine structure of the neuromuscular system of *Polyorchis penicillatus*. (Hydromedusae, Cnidaria). *Cell and Tissue Research* **193**, 163–74.

SPENCER, A. N. (1979) Neurobiology of *Polyorchis*. II. Structure of effector systems. *Journal of Neurobiology* **10**, 95–117.

VARON, S. S. &. SOMJEN, G. G. (1979) Neuron–glia interactions. *Neurosciences Research Program Bulletin* **17**, 1–239.

WESTFALL, J. A. (1973) Ultrastructural evidence for a granule-containing sensory-motor-interneuron in *Hydra littoralis. Journal of Ultrastructure Research* **42**, 268–82.

WESTFALL, J. A. (1978) Gap junctions between neurons of *Hydra. American Zoologist* **18**, 578.

WESTFALL, J. A. & KINNAMON, J. C. (1978) A second sensory-motor interneuron with neurosecretory granules in *Hydra. Journal of Neurocytology* **7**, 365–79.

WESTFALL, J. A., YAMATAKA, S. & ENOS, P. D. (1971) Ultrastructural evidence of polarized synapses in the nerve net of *Hydra. Journal of Cell Biology* **51**, 318–23.

WOOD, R. L. (1977) The cell junctions of *Hydra* as viewed by freeze fracture replication. *Journal of Ultrastructure Research* **58**, 299–315.

WOOD, R. L. (1979) The fine structure of the hypostome and mouth of *Hydra* II. Transmission electron microscopy. *Cell and Tissue Research* **199**, 319–38.