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# Ultrastructure of synapses in the first-evolved nervous systems

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## Summary

The phylum Cnidaria represents the first group of animals to evolve a recognizable nervous system. A comparison of the ultrastructural features of synaptic loci in animals representing all four classes of the cnidaria has provided an overview of the first-evolved synapses that can be compared morphologically to synapses in higher forms. Synapses in these watery jellylike animals with unmyelinated axons are sparse and difficult to fix well. However, we now have sufficient evidence to define an early synapse as one with paired electron dense plasma membranes separated by a 13–25 nm gap containing intracleft filaments and with vesicles on one or both sides of the synaptic cleft. The vesicles are of three types: dense-cored, clear, and opaque. Neuromuscular synapses resemble neuronal synapses and lack the postsynaptic specializations of higher animals. However, some coelenterates, such as the jellyfish *Chrysaora*, have a postsynaptic cisterna in the muscle. Neuromuscular and neuronematocyte synapses can have either clear or dense-cored vesicles. Opaque vesicles at two-way interneuronal synapses and at neuromuscular synapses in the oral sphincter muscle of sea anemones can be labelled with antisera to the neuropeptides Antho-RFamide (Antho-Arg-Phe-NH<sub>2</sub>) and Antho-RWamides (Antho-Arg-Trp-NH<sub>2</sub>) I and II, respectively. That suggests that neuropeptides evolved as neurotransmitters early in the animal kingdom. The basic differences between first evolved synapses and synapses of higher animals are the lack of postjunctional folds at neuromuscular synapses and the presence of fewer and somewhat larger synaptic vesicles, generally containing granular cores, in the more primitive animals.

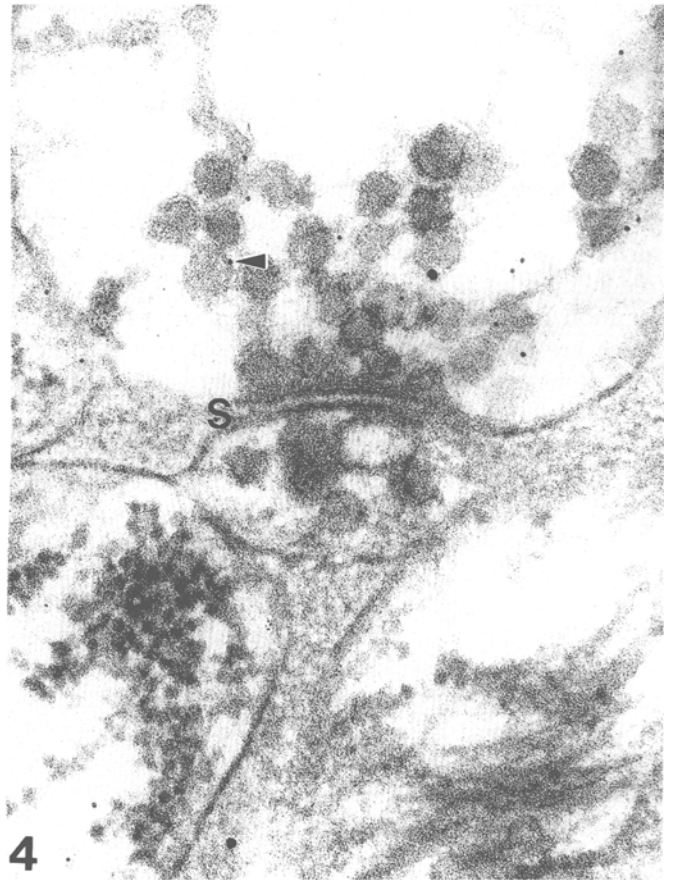
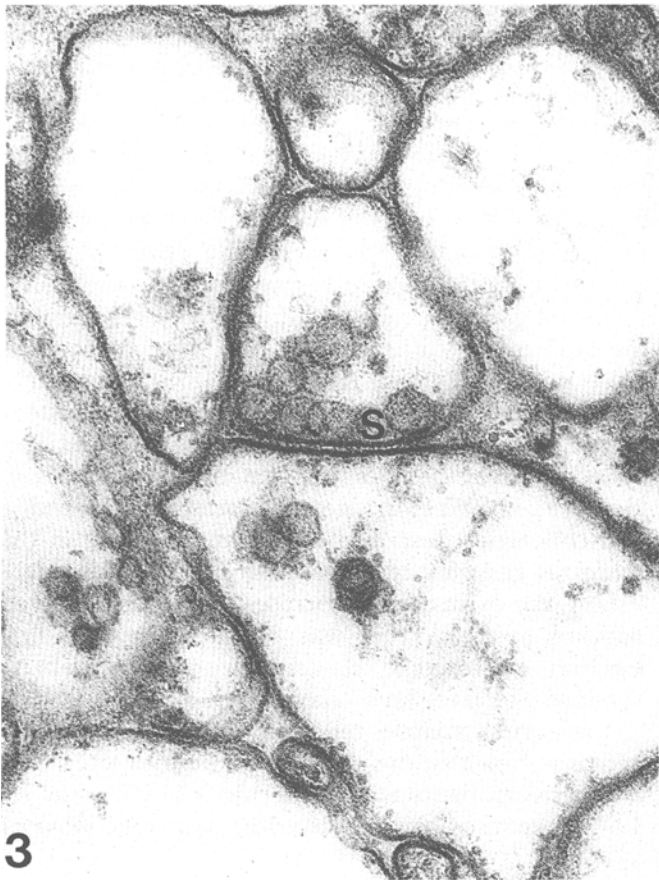
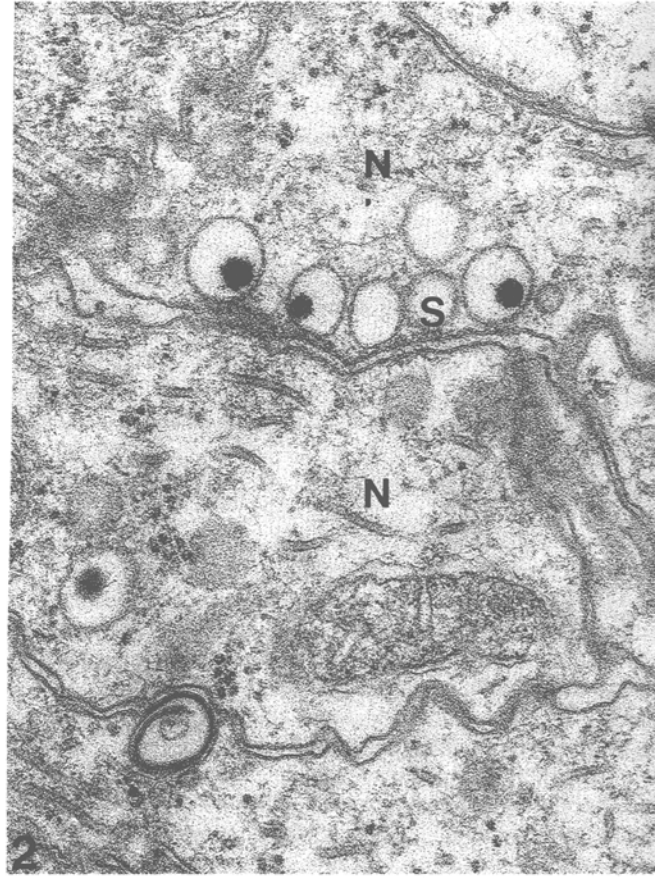
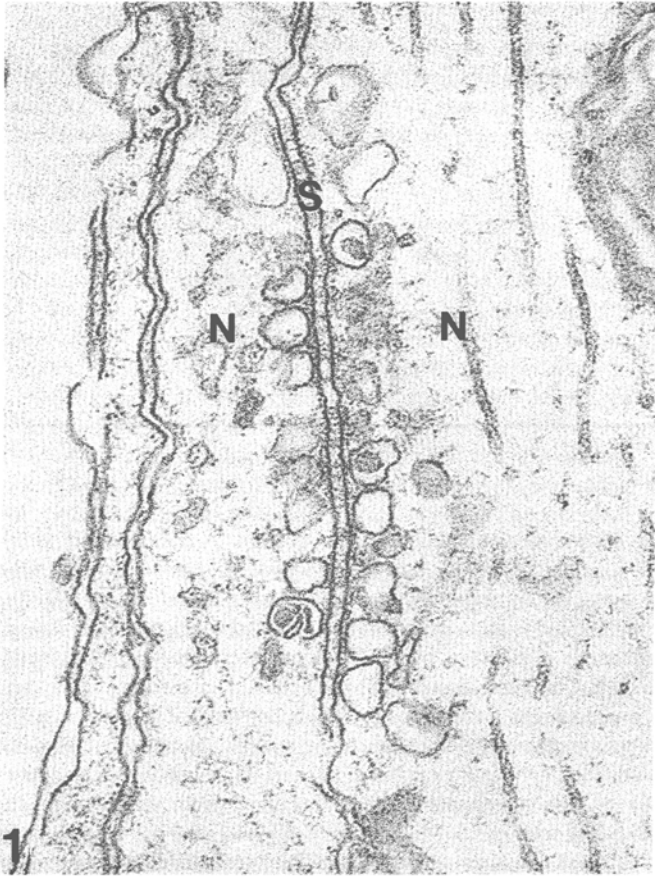
## Introduction

The first recognizable nervous system is present in the phylum Cnidaria, which includes diploblastic forms such as the sessile flowerlike sea anemones and the free-swimming medusae. These animals have in common cnidae or stinging capsules (nematocysts), which are used for defense and for capturing prey. Cnidarians are radially symmetric forms which arose in the Cambrian seas over 500 million years ago. When we study modern representatives of the existing four classes of Cnidaria, we actually may be looking at several examples of the first-evolved nervous systems because of the long history of these animals (Anderson, 1989).

Early workers, who macerated coelenterate tissues to identify the cell types, observed sensory cells attached to the muscular bases of epitheliomuscular cells (Hadži, 1909). Parker (1919) postulated that, early in the evolution of the Cnidaria, some flagellated epithelial cells became separated to become sensory cells, which connected to the muscular bases of the epitheliomuscular cells, and a third cell appeared

later, thus forming a three-cell system for muscle contraction. Two types of nerve cells predominate in the Cnidaria: sensory cells, usually with an apical cilium in contact with the environment, and baso-epithelial ganglion cells with long processes forming the bulk of the diffuse nerve nets that characterize these animals (Hertwig & Hertwig, 1879). Some early investigators believed that this diffuse nervous system lacked a functional separation of nerve cells and was syncytial rather than synaptic in nature (Parker, 1919). However, in 1962 Horridge and Mackay and Horridge and colleagues described a two-way synapse in the marginal ganglion of the jellyfish *Cyanea*, thus establishing the existence of synapses in the first-evolved nervous systems. This was 8 years after the first electron microscopic description of a vertebrate synapse by Palade and Palay (1954).

The term synapse refers to functional contacts between two neurons (Foster & Sherrington, 1897) and between neurons and effector cells (Peters *et al.*, 1991). Synapses can be electrical (gap junctions) or



chemical. Gap junctions will not be discussed here. They play a prominent role in non-neuronal epithelial conduction in the Hydrozoa, but have not yet been reported in the Scyphozoa or Anthozoa (Mackie *et al.*, 1984).

Chemical synapses having vesicles associated with one or both sides of a pair of electron-dense membranes are found in animals representative of all four classes of the Cnidaria (Westfall, 1987). In addition, neuromuscular synapses have been reported in several cnidarians, and a few reports indicate neuronematocyte synapses associated with specific cnidae (nematocysts). In this paper, I will describe and compare the ultrastructure of various types of interneuronal and neuroeffector synapses in modern representatives of the four classes (Hydrozoa, Scyphozoa, Cubozoa, and Anthozoa) of the phylum Cnidaria and briefly comment on their putative neurotransmitters and probable evolution.

## Materials and methods

Transmission electron micrographs from unpublished (Figs 1, 3–5, 12–14) and published (Figs 2, 6–11) work have been combined with diagrammatic drawings to illustrate the types of synapses that are representative of various species of the phylum Cnidaria. Most of the tissues were fixed first in buffered glutaraldehyde and then in osmium tetroxide, except for the tissues used in immunocytochemical experiments for which a combination of 4% paraformaldehyde and 0.1% glutaraldehyde followed by osmium fixation was used (Figs 3, 4, 13, & 14). All tissues were dehydrated in ethanol and embedded in Epon or a mixture of Epon and Araldite.

Measurements were made on synaptic vesicles from 20 species of coelenterates observed in my laboratory and taken from published reports on 16 species studied by other investigators.

## Observations

### Synaptic structure

The first synapses described in the jellyfish were two-way synapses with a long synaptic membrane complex having fairly large and irregular vesicles aligned along both sides of the paired, electron-dense membranes that were separated by a narrow 18–22 nm cleft containing

dense material (Horridge & MacKay, 1962). The very uniform vesicles were 70–100 nm in diameter and contained a small granule (type A), whereas those with no electron-dense contents (type B) were irregular in size with the larger ones ranging in diameter from 150 to 200 nm and the smaller ones from 70 to 100 nm (Horridge & MacKay, 1962). The cored vesicles appeared to arise from the Golgi complex. At that time, no example of two-way chemical transmission was known in coelenterates. However, in 1985, Anderson demonstrated physiologically that bidirectional chemical synapses occur in the motor nerve net of *Cyanea*. The occurrence of morphologically identifiable and functionally active chemical synapses in coelenterates is now established, despite arguments to the contrary by Van Marle (1989).

In 1965, Bullock and Horridge reported that *Hydra* was the exception to the rule that coelenterates have synapses. This report triggered an ultrastructural search for synapses in this tiny fresh water polyp that led to the discovery of polarized axo-axonal synaptic loci with a short row of 120–200 nm dense-cored vesicles associated with a pair of electron-dense membranes that were separated by a 15 nm gap traversed by fine filaments (Westfall *et al.*, 1971). Neuromuscular synapses are also present and have similar dense-cored vesicles associated with the paired synaptic membranes; however, postsynaptic specializations are lacking (Westfall, 1973a). Such junctional specializations also are lacking in *Hydra* neuronematocyte synapses, which may have clear or dense-cored vesicles (Westfall 1973a; Westfall & Kinnamon, 1984).

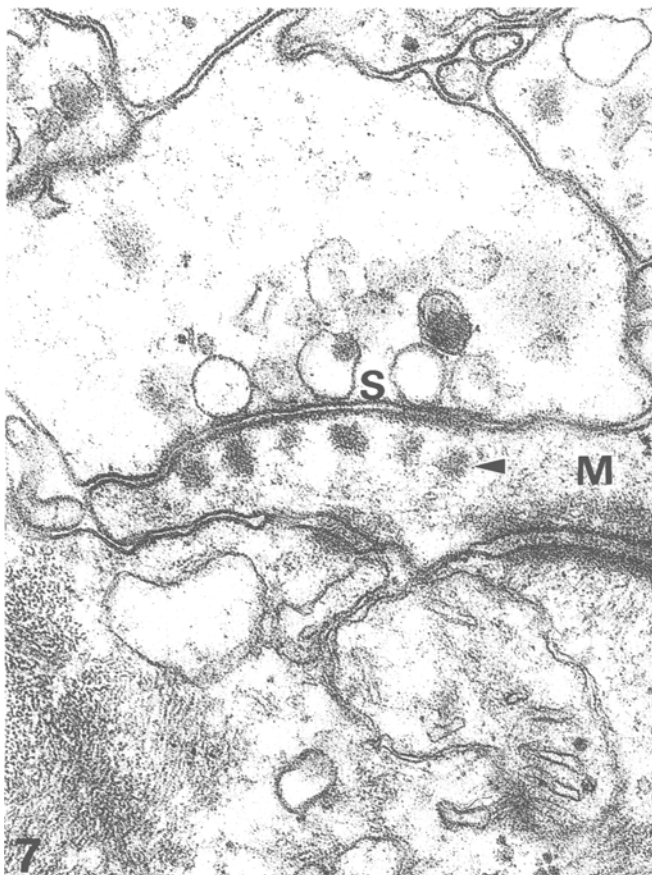
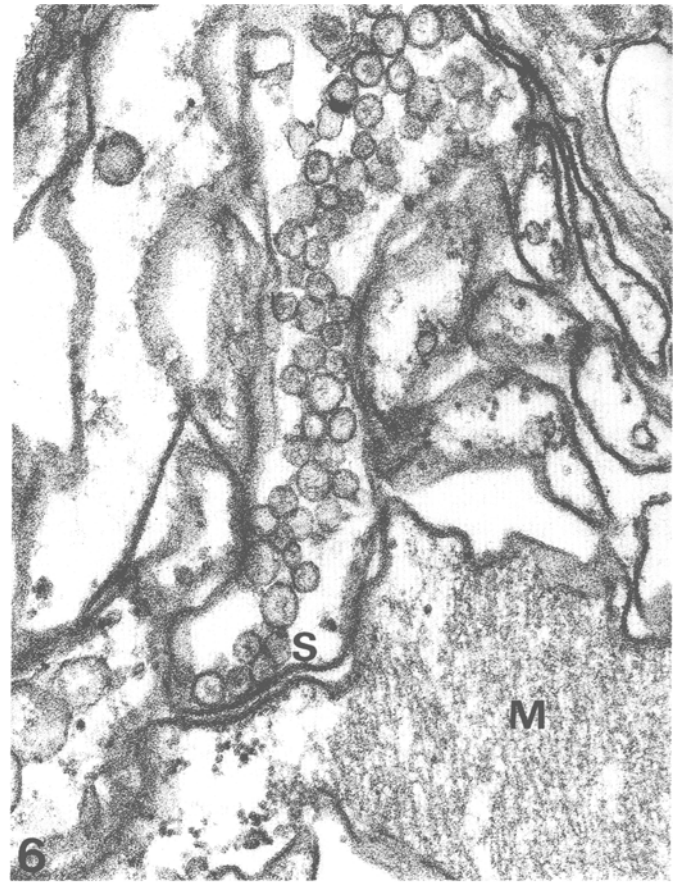
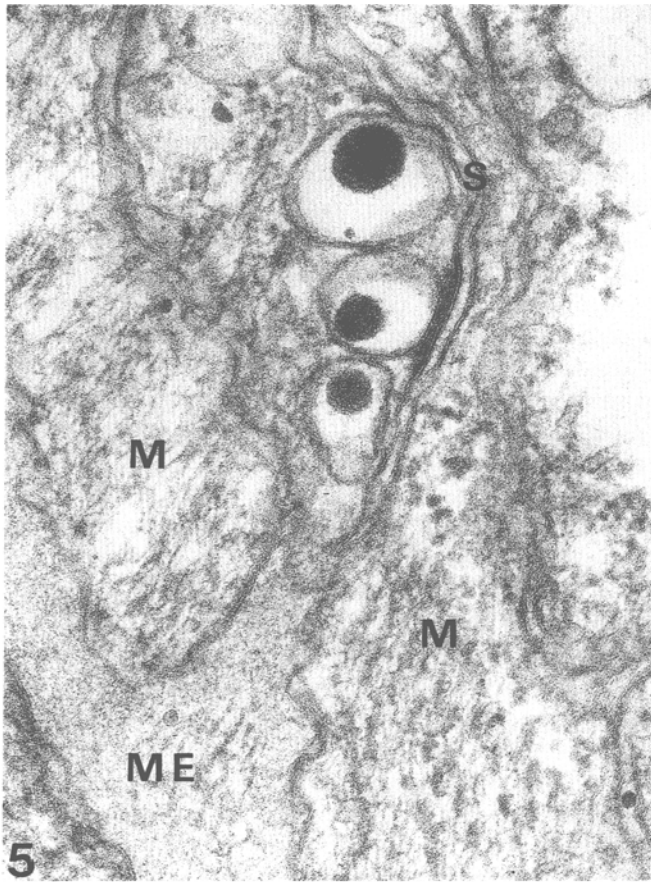
The first neuronematocyte synapse, which involves a type of cell unique to the Cnidaria, was demonstrated ultrastructurally in the hydromedusan *Gonionemus vertens* (Westfall, 1970a). This observation lent support to the hypothesis that nematocyst discharge is under control of the nervous system. In addition, the synapse was part of a serial neuro-neuro-nematocyte sequence, suggesting that presynaptic inhibition might already be present in this example of a first-evolved nervous system. The question of neural control of nematocyst discharge remains a controversial issue today, partly because of the recent work on sea anemones, which indicates that nematocyst discharge is controlled by prey-produced N-acetylated

**Fig. 1.** A two-way synapse (S) with mixed vesicles between two neurites (N) in the epidermal nerve plexus of a tentacle from the jellyfish *Chrysaora quinquecirrha*.  $\times 52\,600$ .

**Fig. 2.** A one-way synapse (S) with dense-cored vesicles between two neurites (N) in the epidermal nerve plexus of a tentacle from the fresh water hydrozoan *Hydra littoralis*. (From Westfall *et al.*, 1971).  $\times 53\,500$ .

**Fig. 3.** A polarized interneuronal synapse (S) with clear vesicles in the epidermal nerve plexus of a tentacle from the sea anemone *Calliactis parasitica*.  $\times 64\,300$ .

**Fig. 4.** Opaque vesicles at an interneuronal synapse (S) with Antho-RFamide immunoreactivity (5 nm gold particles, arrowhead) in a tentacle from the sea anemone *Anthopleura elegantissima*.  $\times 111\,800$ .



sugars. These sugars cause a slight elongation of tunable hair bundles that respond to the frequency of pulses received by swimming food prey (Watson & Hessinger, 1991, 1992). It should be noted that electrophysiological experiments are difficult to perform on these jelly-like animals with tiny nerve cells and unmyelinated axons.

In an ultrastructural survey of 20 different cnidarians, we observed the largest synaptic loci in the jellyfish *Chrysaora* (Fig. 1). Two way synapses were up to 1.7  $\mu\text{m}$  in length, and in thin sections they displayed 26 or more, clear or dense-cored vesicles averaging 102 nm in diameter. These vesicles are mixed and the dense-cored vesicles appear somewhat smaller and more regular than the clear vesicles. Because clear vesicles occur on both sides of the synaptic cleft, the synapse does not appear to have any direction, suggesting that exocytotic release of transmitter substance can occur from stimuli originating from either direction. A thin electron-dense coat is present on both cytoplasmic surfaces and appears to attach some of the vesicles to the paired synaptic membranes. Within the cleft is an irregular series of fine cross filaments running the length of the electron-dense coats with attached vesicles. Palay (1958) coined the term *synaptic complex* for synaptic vesicles associated with the paired dense membranes of vertebrate synapses. The term also seems appropriate for this array of vesicle-associated, paired, electron-dense membranes with cross filaments in the 25 nm-wide cleft, even though vesicles occur on both sides.

Polarized synapses with a short row of three or four, 140 nm diameter, dense-cored vesicles are seen commonly in the fresh water hydrozoan *Hydra* (Fig. 2). Although a cluster of granular vesicles with a distinct but often irregular halo between the granule and membrane may be present in both sensory and ganglion cells, the actual synaptic vesicles are aligned in a row and attached individually to a presynaptic paramembraneous density. A narrower postsynaptic dense coat parallels the presynaptic density for a length of 0.7  $\mu\text{m}$ . The synaptic cleft is a uniform 23 nm wide gap having periodic densities along its length, which parallels that of the thin paramembraneous coats. Courteaux (1961) coined the term *active zone* for the assembly of synaptic vesicles and paired dense membranes in the vertebrate CNS. Such a term seems

appropriate for the *en passant* synaptic loci observed in the nerve plexus of *Hydra*.

Clear vesicles, often arranged in tiers, are seen more frequently at sea anemone synapses (Fig. 3). The epidermal nerve plexus of tentacles of the sea anemone *Metridium senile*, contains three types of vesicles: clear (electron-lucent) vesicles 100–150 nm in diameter, dense-cored vesicles 140–150 nm in diameter, and small opaque vesicles 70–90 nm in diameter. These vesicles are present at paired, electron-dense membranes separated by a 15–20 nm cleft that usually is traversed by a series of cross filaments (Westfall, 1970b). Thus, they appear to be true synaptic loci and presumably play a role in neurotransmission or neuromodulation of impulses. However, according to Van Marle (1989), no discrete chemical synapses occur in sea anemones, although he found three types of 'synaptic' vesicles in neurites of the epidermal (ectodermal) plexus of the sea anemone tentacle. The largest of these were 150 nm electron-lucent vesicles, but others were 130 nm dense-core granules and 110 nm opaque granules. Van Marle never observed dense core and opaque granules together in the same neurite. We have observed small opaque vesicles (63 nm in diameter) immunoreactive to the sea anemone neuropeptide Antho-RFamide (Antho-Arg-Phe-NH<sub>2</sub>) at two-way synapses in the sea anemones *Calliactis* and *Anthopleura* (Fig. 4). The 9 nm wide cleft in *Anthopleura* is traversed by periodic filaments for a length of 0.25  $\mu\text{m}$ . Cubomedusae have polarized synapses with clear 80 nm vesicles in the sensory cells of the ocelli (Yamasu & Yoshida, 1976).

#### Neuroeffector synapses

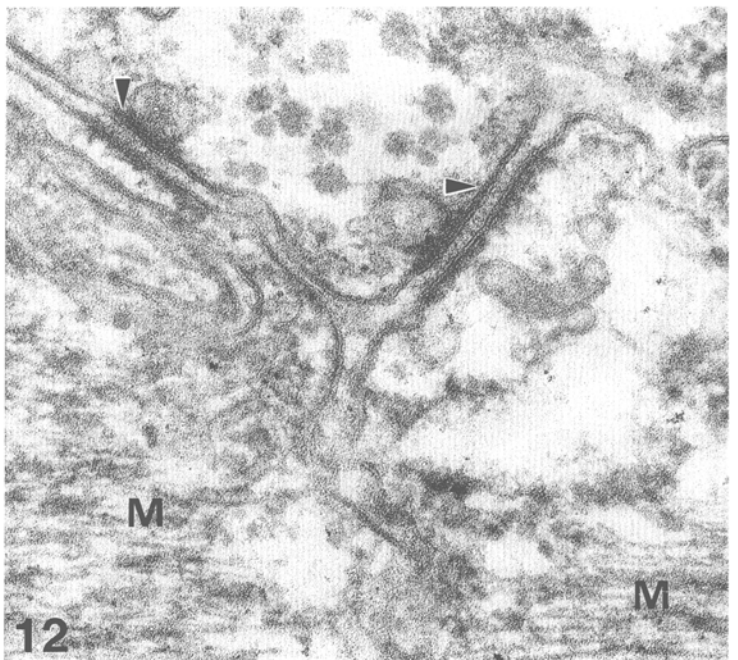
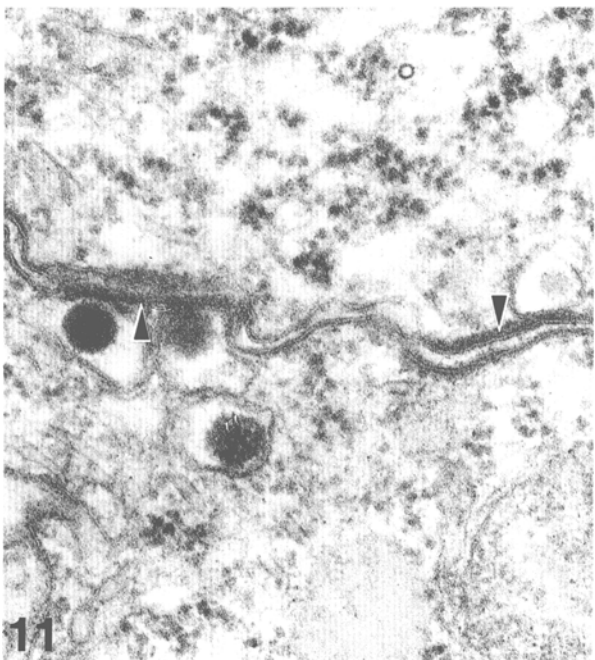
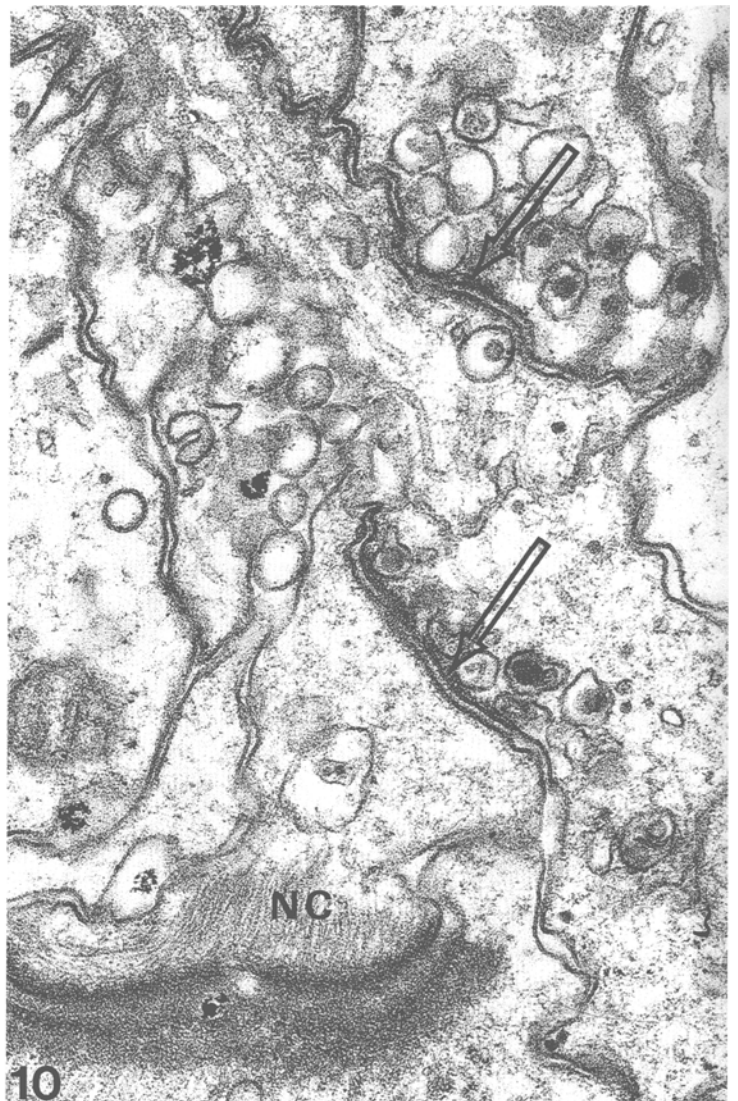
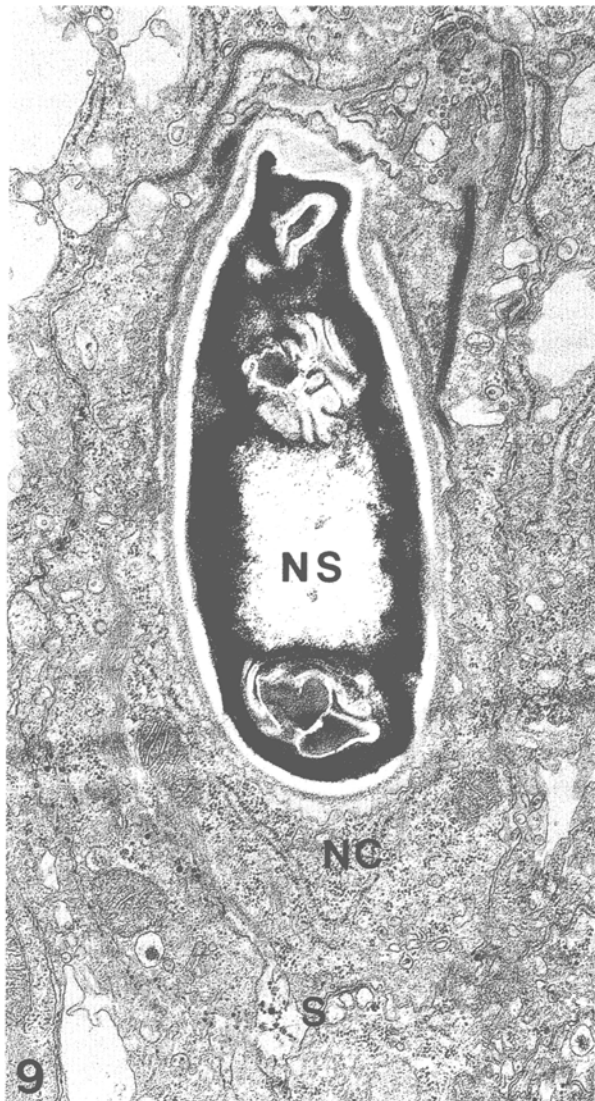
Neuromuscular synapses resemble interneuronal synapses, with the exception of certain species in which postsynaptic specializations are present. In *Hydra*, the neuromuscular synapses may show only three dense-cored vesicles at a junction (Fig. 5), whereas in sea anemones, tiers of clear vesicles may be present (Fig. 6). In *Hydra*, the vesicles are 125 nm in diameter like the interneuronal synapses and are attached to a presynaptic density at a 13 nm wide cleft. In ideal profiles, usually requiring serial sections, periodic cross filaments can be seen in the synaptic cleft. Often large mitochondria are present between the synaptic loci and the bundle of myofilaments. In

**Fig. 5.** Dense-cored vesicles at a neuromuscular synapse (S) in the tentacle epidermis of *Pelmatohydra pseudoligactis*. M: muscle; ME: mesoglea.  $\times 82\,100$ .

**Fig. 6.** Neuromuscular synapse (S) with mainly clear vesicles in the tentacle epidermis of the sea anemone *Metridium senile*. M: muscle. (From Westfall, 1970b).  $\times 53\,500$ .

**Fig. 7.** Neuromuscular synapse (S) with series of postsynaptic densities (arrowhead) opposite each presynaptic vesicle in the tentacle epidermis of the hydromedusan *Gonionemus vertens*. M: muscle. (From Westfall, 1973b).  $\times 53\,500$ .

**Fig. 8.** Neuromuscular synapse (S) with postsynaptic cisterna (C) opposite a row of presynaptic vesicles in the jellyfish *Chrysaora quinquecirrha*. M: muscle.  $\times 53\,500$ .



**Table 1.** Characteristics of synapses and associated vesicles in 36 cnidarian species studied.

<i>Synapse type</i>	<i>Vesicle type</i>	<i>Vesicle size (nm)</i>	<i>Number of cnidarian species</i>
Interneuronal (two-way)	dense-cored	70–252	11
	clear	64–151	12
	opaque	35–44	2
Interneuronal (one-way)	dense-cored	70–208	19
	clear	76–170	15
	opaque	70–90	2
Neuromuscular	dense-cored	50–190	11
	clear	44–192	13
	opaque	40–89	2
Neuronematocyte	dense-cored	120–192	4
	clear	68–190	6

the hydromedusan *Gonionemus*, postsynaptic densities occur opposite each dense-cored or clear vesicle attached to the presynaptic membrane (Fig. 7). The scyphomedusans *Chrysaora* and *Haliclystus*, have a subsurface cisterna (Fig. 8), which parallels the row of presynaptic vesicles at the neuromuscular junction (Westfall, 1973b).

Neuronematocyte synapses (Fig. 9) are unique to the Cnidaria. A controversy has long existed over whether or not nematocyst discharge is under control of the nervous system. The hypothesis of neural control of nematocyst discharge was strengthened by the discovery of neuronematocyte synapses first in the hydromedusan *Gonionemus* and later in *Hydra* (Westfall, 1970a, 1973a). The synaptic vesicles appear clear in the tentacle synapses and dense-cored in the hypostomal neuronematocyte synapses of *Hydra* (Westfall, 1973; Westfall & Kinnamon, 1984). They range in size from 90–150 nm. The synaptic loci are 0.3–0.5  $\mu\text{m}$  in length with a 13 nm wide cleft that has cross filaments. In *Gonionemus*, sequential neuro-neuronematocyte synapses (Fig. 10) occur, suggesting presynaptic inhibition (Westfall, 1970a).

#### Synaptic patterns

Polarized (unidirectional) synapses have been demonstrated ultrastructurally in 16 species of the class

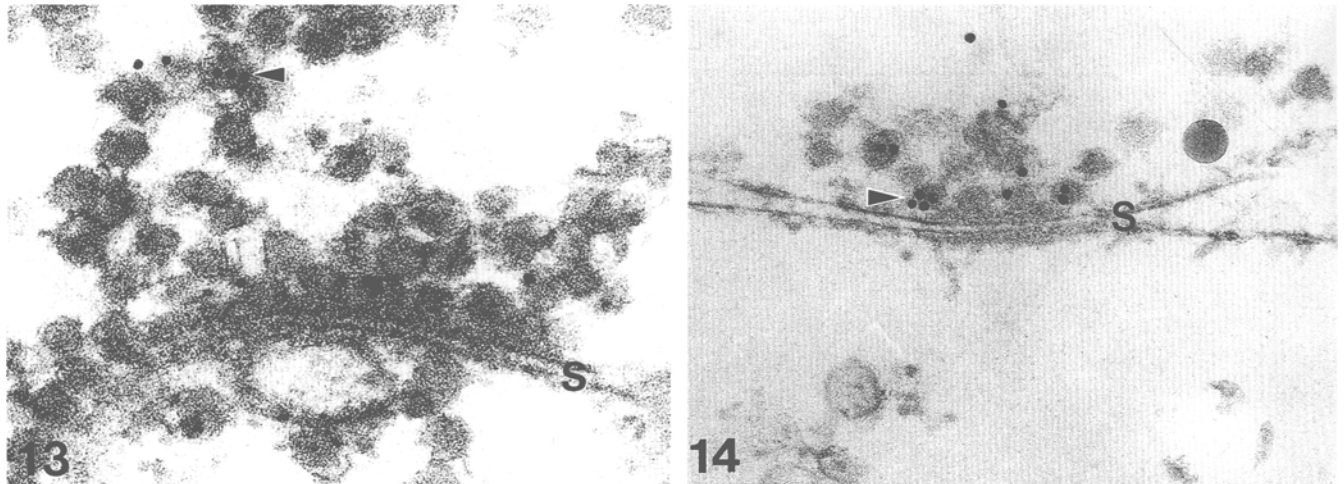
Hydrozoa, four species of the class Schyphozoa, two of the class Cubozoa, and 14 of the class Anthozoa. Thus, 36 different coelenterates have been examined to date, and all have directed chemical synapses that suggest the presence of polarized neuronal pathways (Table 1). Peteya (1973) made a comprehensive ultrastructural study of synapses in the burrowing anemone *Ceriantheopsis americanus* and found that 97% of the interneuronal synapses were polarized. Two-way (bidirectional) synapses (Fig. 15, top) have been described in two hydrozoans, two scyphozoans, and three anthozoans. Perhaps the small number of species with bidirectional synapses indicates that the first nervous systems were polarized early in evolution. However, it could also be due to the paucity of EM studies on coelenterate synapses. Reciprocal synapses are common in the burrowing anemone, where they form a continuous series of synaptic-loci (Fig. 15, bottom) without a widening of the synaptic cleft, leading Peteya (1973) to call them multipolarized, thus distinguishing them from the typical *en passant* synapses. In hydromedusan photoreceptors, reciprocal synapses provide feedback between the second-order neurons and the photoreceptor cell (Toh *et al.*, 1979). They appear to be rare in the Hydrozoa, where they have been reported only in *Hydra* (Figs 11 & 15; Westfall & Kinnamon, 1984). Reciprocal synapses also

**Fig. 9.** Neuronematocyte synapse (S) with short row of clear vesicles in tentacle epidermis of *Hydra littoralis*. NC: nematocyte, NS: nematocyst. (From Westfall *et al.*, 1971).  $\times 17\,000$ .

**Fig. 10.** Serial neuro-neuronematocyte synapse (arrows) in the tentacle epidermis of the hydromedusan *Gonionemus vertens*. NC: nematocyte. (From Westfall, 1970a).  $\times 53\,500$ .

**Fig. 11.** Reciprocal synapses (arrowheads) between ganglion cells in the hypostome of *Hydra littoralis*. (From Westfall & Kinnamon, 1984).  $\times 81\,100$ .

**Fig. 12.** One neuron innervating two different myonemes (arrowheads) in the oral region of *Hydra littoralis*. M: muscle.  $\times 99\,800$ .



**Fig. 13.** Opaque vesicles at polarized interneuronal synapse (S) with Antho-RWamide immunoreactivity (15 nm gold particles, arrowhead) in the tentacle nerve plexus of the sea anemone *Anthopleura elegantissima*.  $\times 111\,800$ .

**Fig. 14.** Opaque vesicles immunoreactive to Antho-RWamide (Arg-Trp-NH<sub>2</sub>) antisera (15 nm gold particles, arrowhead) are present at a neuromuscular synapse (S) in the sphincter of the sea anemone *Calliactis parasitica*.  $\times 111\,800$ .

have been observed in Ctenophores, along with both polarized and nonpolarized synapses (Hernandez-Nicaise, 1991). Sequential neuro-neuroeffector cell synapses have been reported in two hydrozoan and one anthozoan species (Fig. 15, bottom). They may allow for presynaptic inhibition of muscular contraction or nematocyst discharge.

Tracing neuronal synapses to their final pathway innervating an effector cell has been difficult because of the small diameter and complex intertwining of the unmyelinated neurites in the nerve plexus. Neuromuscular synapses (Fig. 15, middle) have been observed in nine Hydrozoa, four Scyphozoa, one Cubozoa, and six Anthozoa. Most neuromuscular synapses occur *en passant*, but some terminal-like junctions have synaptic loci innervating two or more myonemes (Fig. 12). Neuronematocyte synapses (Fig. 15, bottom) have been reported in only three hydrozoans and three anthozoans. In addition, neuroglandular synapses have been recently observed in the sea anemone *Calliactis* (unpublished observations), but the direct innervation of a glandular cell by a sensory cell has been observed in a Ctenophore (Hernandez-Nicaise, 1991). Ctenophores also have neuromesenchymal cell synapses (Hernandez-Nicaise, 1968). All of these ultrastructural findings in various coelenterates add up to a substantial volume of literature supporting a complex and organized nervous system operating in animals with the first-recognizable nervous systems.

#### Neurotransmitters

To date, 18 novel neuropeptides have been isolated and sequenced from coelenterates, most of them from sea anemones (Grimmelikhuijzen *et al.*, 1992, and personal communication). Such peptides as

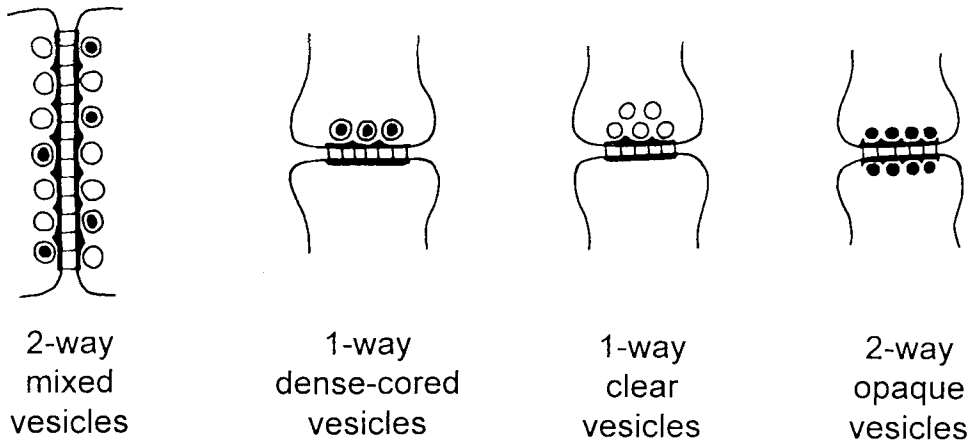
Antho-RFamide and Antho-RWamides I and II cause increased frequency and amplitude of muscle contractions in the epidermis and gastrodermis of sea anemones (McFarlane *et al.*, 1987; McFarlane & Grimmelikhuijzen, 1991). Taurine depolarizes motor nerve net neurons in the jellyfish *Cyanea*, in which immunoreactivity to a taurine-like molecule has been demonstrated (Carlberg *et al.*, 1995), suggesting that this sulfonated amino acid may be an excitatory neurotransmitter. Immunogold electron microscopy has allowed us to localize peptidergic immunoreactivity in dense-cored vesicles of *Hydra* (Koizumi *et al.*, 1989); in large dense vesicles of the hydromedusan *Aglantha* (Singla & Mackie, 1991); both in neuronal vesicles and at two-way synapses in the sea anemone *Anthopleura* (Westfall & Grimmelikhuijzen, 1993); and at neuromuscular synapses in the sea anemone *Calliactis* (Westfall *et al.*, 1995).

In anthozoans, small opaque vesicles containing the sea anemone neuropeptide Antho-RFamide are present at two-way (nonpolarized) synapses and at one-way (polarized) synapses (Westfall & Grimmelikhuijzen, 1993; Fig. 4). Similar opaque vesicles immunoreactive to Antho-RWamides I and II are present at interneuronal synapses (Fig. 13) in tentacles of the sea anemone *Anthopleura* and at neuromuscular synapses on the oral sphincter muscle of the sea anemone *Calliactis parasitica* (Fig. 14). These vesicles are comparable in size to the small clear and dense-cored vesicles of vertebrate synapses. Larger vesicles with dense cores are found at paired synaptic membranes and in aggregates in nerve cell processes. They range in size from those overlapping dopamine-containing and 5-HT-containing vesicles of vertebrates to larger types resembling elementary neurosecretory granules.

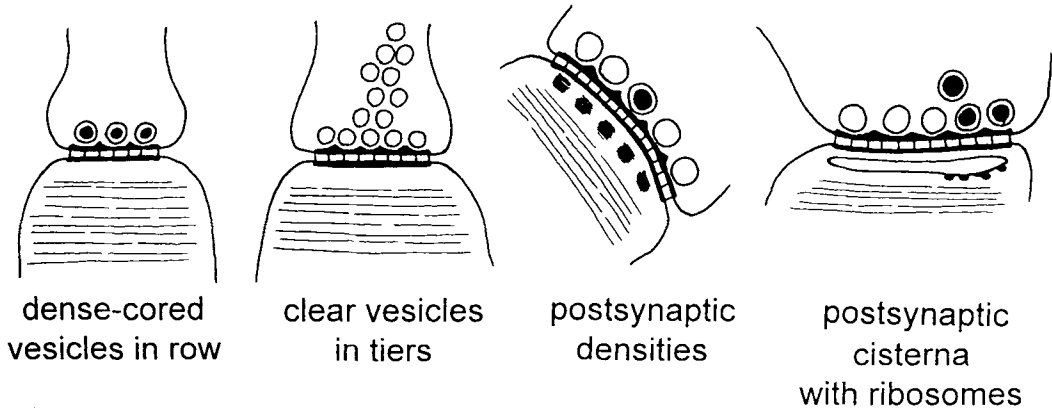
Finally, there are large neuronal granules that do not



### Interneuronal Synapses



### Neuromuscular Synapses



### Unusual Synapses

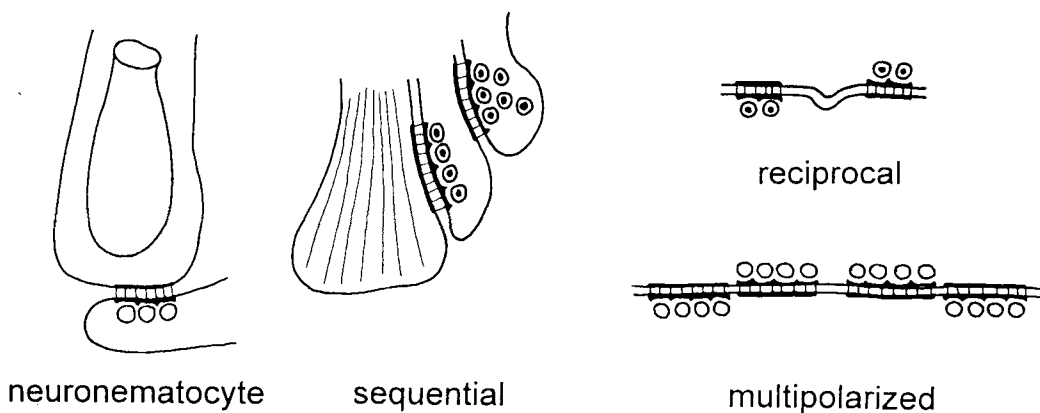


Fig. 15. Diagrammatic drawings of synaptic patterns observed in the phylum Cnidaria.

appear at synaptic membranes and may be more secretory in nature. Some synapses and neurons contain clear vesicles with a faint core, suggesting that they once contained a dense material that either has been released or somewhat diluted.

Almost nothing is known about the protein at

coelenterate synapses. Immunolabelling with synaptophysin 1, a synaptic membrane protein, demonstrated its presence at sea anemone synapses with small clear vesicles, but not at those with large dense-cored vesicles (DellaCorte *et al.*, 1994). This is an area open for research. However, finding synapses in

first-evolved nervous systems will not be easy, because they are sparse and difficult to locate in the electron microscope.

### Discussion

Transmission EM has revealed that many cnidaria have synaptic loci with characteristics similar to those of higher animals. Synapses in modern representatives of early-evolved animals have paired electron-dense membranes separated by a 13–25 nm gap containing intracleft filaments and with vesicles on one or both sides of the synaptic cleft. The synaptic vesicles fall into three basic categories: dense-cored, clear, and opaque. Some clear vesicles may represent granular vesicles that have lost their cores, perhaps by exocytosis during neurotransmission. Peptidergic neurotransmitters probably evolved early, perhaps in response to the predominance of nonstriated muscle in this largely sessile group of animals. However, some interneuronal and neuroeffector synapses have electron-lucent vesicles that appear to lack granular cores and may carry a different type of transmitter substance.

#### Neurotransmitters

In addition to the novel anthozoan neuropeptides such as Antho-RFamide and Antho-RWamides I and II isolated by C. J. P. Grimmelikhuijzen and his associates (see Grimmelikhuijzen *et al.*, 1992), some classical neurotransmitter substances have been demonstrated. Evidence has been found for dopamine, adenaline, noradrenaline, and serotonin (5-HT) in the anthozoan *Renilla* (Anctil *et al.*, 1984; DeWaele *et al.*, 1987; Umbriaco *et al.*, 1990) and for DOPA in neuronal vesicles of the sea anemone *Metridium* (Carlberg, 1983). Also, dopamine is reported to be an inhibitory neuroactive substance in the hydro-medusan *Polyorchis* (Chung & Spencer, 1991). Therefore, antisera to these substances should be used to investigate their existence either alone or with novel neuropeptides in selected granular vesicles of sea anemone neurons.

#### Synaptic vesicles

The main difference between synapses of early-evolved animals and those of higher animals is in the number, size, and types of synaptic vesicles. The vesicles are fewer in number and larger in size than those of vertebrate synapses. Controversy remains as to how to classify coelenterate synaptic vesicles that vary in size and density.

Synapses in sea anemones were reported first in 1970. Three types of synaptic vesicles were observed: clear (100–150 nm); dense-cored (140–150 nm); and small lightly granular (70–90 nm) vesicles (Westfall, 1970b). Later Van Marle (1977) reported three types

of vesicles in sea anemone nerve cells, which he defined as electron-lucent (150 nm), dense-core granules (130 nm), and opaque granules (110 nm). I suggest that we use the terms *dense-cored vesicles* for vesicles with a halo between the dark granule and the limiting membrane and *opaque vesicles* for the small, lightly granular ones.

#### Evolution

Finally, we are left with the question: how did first synapses arise? No living ancestral relative of coelenterates exists to provide clues to the forerunners of the vesicle-associated synaptic loci found in all four classes of the Phylum Cnidaria. Sponges lack chemical synapses and flatworms have rostral ganglia to go with their bilateral form. Coelenterates are radiate or biradial organisms with a nervous system that generally is defined as diffuse, although medusae contain ganglia and nerve rings. Some medusae even have well-developed photoreceptors and statocysts for sensing their environment. However, coelenterates have no brainlike equivalent, in spite of the fact that certain hydrozoans have orchestrated feeding patterns (Koizumi *et al.*, 1983); medusae have rapid escape swimming responses (Roberts & Mackie, 1980); and the muscular tentacles of some sea anemones are strong enough to allow somersaulting onto gastropod shells (Ross & Sutton, 1961).

Initially, early cnidarians may have depended on actions of individual cells, similar to the epitheliomuscular cells of *Hydra* and the muscular digestive cells of sea anemones, for movement and feeding. To obtain food, the tentacles and mouth became fortified with stinging capsules for paralyzing prey. These nematocysts (capsules with an internal eversible thread) were believed to be independent effectors, because the trigger hair (a cnidocil or modified cilium) at the apex of the cell (nematocyte) could be stimulated to fire its capsule by a combined chemical-mechanical stimulus (Pantin, 1942). The advent of electron microscopy, which allowed the first observations of synaptic loci at functional contacts between neurons, provided a means to search for morphological evidence of neuron-nematocyte synapses and led to the still somewhat controversial belief that the firing of the capsules is under control of the nervous system. When an animal is satiated, it no longer fires its capsules into the prey (Smith *et al.*, 1974). Where then is the neural control centre?

Early on, it was postulated that a sensory cell with a cilium in contact with the watery environment of the animal might be connected directly to the muscular base of an epitheliomuscular cell and thus provide a two-cell system for an input-output behaviour such as contraction of the tentacles. Using electron microscopy, such a two-cell synaptic pathway was found in *Hydra*, (Westfall & Kinnamon, 1978). Parker (1919) was

the first to suggest a hypothetical two-cell system, to which an intermediate adjuster cell (the ganglion cell) was added later as a modulator of the stimulus and response. Such modulatory cells are represented by the bipolar and multipolar neurons that make up the bulk of the through-conducting nerve net of coelenterates. Finally, ganglia, or clusters of neurons, were observed at the base of the tentacles of *Hydra*, which we believed could act as endogenous pacemakers (Kinnamon and Westfall, 1981). Thus, we can postulate a progression of complexity from single cells behaving independently to paired interactions and finally to modulated behaviour via interneurons, which also function as motor neurons for the final common pathway.

Once the epithelial cells of a multicellular system have evolved into excitable and conducting cells with secretory capabilities, they may become primitive neurons (Lentz, 1968). Such primitive neurons having the multifunctional features of sensory cells, interneurons, neurosecretory cells, and motor neurons combined into one cell have been observed in *Hydra* (Westfall, 1973a). We might speculate that similar multifunctional cells acted as ancestral neurons to cells that later became specialized for specific functions,

such as sensory, motor, neuromodulatory, and neurosecretory. Such specialized nerve cells with independent functions are present in the flatworms, in which rostral brains already are evolved. The position of the phylum Cnidaria and its sister phylum Ctenophora somewhere between the multicellular sponges and bilateral triploblastic flatworms continues to be an enigma. Perhaps studies on ribosomal RNAs that establish patterns of gene expression will provide the necessary information on the development and interrelationships of the first-evolved nervous systems to give us clues about their origin. One such study suggests that the Bilateria emerged before the Radiata (Hori & Satow, 1991), indicating that coelenterate synapses may not be representative of the first-evolved nervous systems and may instead reflect a dead-end evolution.

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