ULTRASTRUCTURAL EVIDENCE OF POLARIZED SYNAPSES IN THE NERVE NET OF *HYDRA*

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INTRODUCTION

Synapses provide pathways of communication between cells throughout the animal kingdom wherever a nervous system is present. The most primitive or elementary nervous system that can be readily recognized is the nerve net of coelenterates. The first synapses described at the ultrastructural level in nerve net animals were symmetrical synapses in marginal ganglia of jellyfish (Horridge and Mackay, 1962). Later, two types of synapses were reported (Jha and Mackie, 1967) in a hydromedusan, *Sarsia*: (*a*) symmetrical synapses with vesicles on both sides of a specialized membrane and (*b*) asymmetrical synapses with vesicles on only one side of the synaptic junction. Asymmetrical or morphologically polarized synapses have now been demonstrated ultrastructurally in a sea pen (Buisson and Franc, 1969; Buisson, 1970), a primitive hydromedusan (Westfall, 1970 *a*), a sea anemone (Westfall, 1970 *b*), and various other species of Cnidaria (Westfall et al., 1970).

The ultrastructural confirmation of neurons in *Hydra* by Lentz and Barrnett (1965) excited a number of investigators to the possibility of demonstrating synaptic contacts in this elementary nervous system. To date, there have been no reports of morphologically specialized contacts between cells of *Hydra* that might indicate the precise region of chemical mediation of an impulse (see Lentz, 1968). In the present study ultrastructural evidence is provided for the presence of polarized interneuronal synapses and neuromuscular and neuronematocyte junctions in two species of *Hydra*.

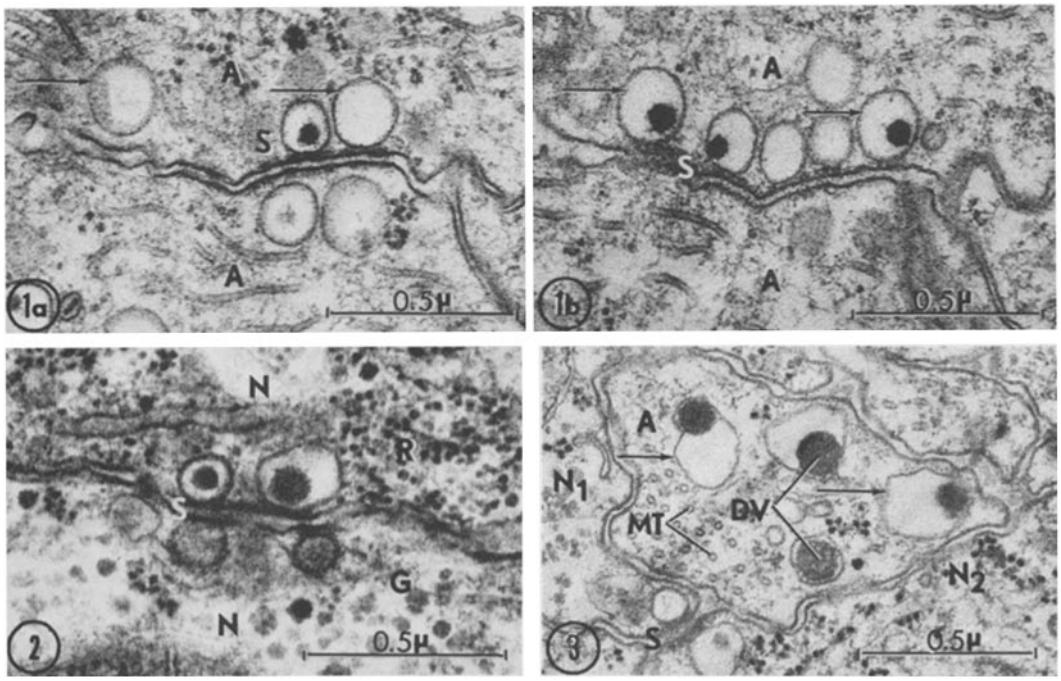
MATERIALS AND METHODS

Specimens of *Hydra littoralis* and *Hydra pseudoligactis* were obtained from Dr. Helen D. Park, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md. Entire animals were fixed for 2 min by adding 5 ml of fixative to a specimen relaxed in 1 ml of culture medium. They were then transferred to fresh fixative: 4% glutaraldehyde in either 0.1 M *s*-collidine at pH 7.3 or 0.05 M cacodylate buffer at pH 7.2. The specimens were rinsed in cacodylate buffer before the tentacles were extirpated, and post-fixed in 1% osmium tetroxide in 0.08 M cacodylate buffer at pH 7.2. All specimens were dehydrated in a graded series of ethanol and propylene oxide, then embedded in Epon (Luft, 1961). Sections 0.5 μ thick were cut and stained with equal parts of 2% toluidine blue and 2% sodium borate for light microscopy. Thin sections for electron microscopy were cut on a Porter-Blum MT-1 or MT-2 ultramicrotome with a diamond knife in a Westfall-Healy section mounter. The sections were stained with uranyl acetate followed by lead citrate and were examined in either an RCA EMU-3G or an HU-11 electron microscope.

OBSERVATIONS

When the epidermal nerve net of tentacles of *Hydra* was examined with the electron microscope, interneuronal, neuromuscular, and neuronematocyte synapses were seen—with these characteristics in common: several dense-cored or clear vesicles (1200–2000 A in diameter) associated with two parallel, thickened, electron-opaque, closely-apposed plasma membranes separated by a cleft approximately 150 A wide.

INTERNEURONAL SYNAPSES appeared either morphologically polarized (asymmetrical), as in Figs. 1 *a* and 1 *b*, or nonpolarized (symmetrical), as in Figs. 2 and 3. Asymmetrical synapses occurred between neurites, which resembled vertebrate axons by possessing fascicles of long parallel microtubules, aggregations of vesicles (Figs. 1 *a*, 1 *b*, and 3), and mitochondria. Symmetrical synapses were found between neuronal perikarya (Fig. 2). The membrane densities that indicated the precise site of contact between neurites were composed of an electron-opaque coat of fine filaments on both cytoplasmic surfaces and a filamentous material within the synaptic cleft (Figs. 1 *a*, 1 *b*). The synaptic vesicles (1400–2000 A in diameter) contained dense cores (600–800 A in diameter) that did not always appear in the plane of section (compare Figs. 1 *a* and 1 *b*). Serial sections showed that the vesicles were in contact with the filamentous coat on the presynaptic membrane. Symmetrical synapses occurred between the perikarya of adjacent neurons which could be dis-



FIGURES 1 *a* and 1 *b* Serial sections of synapse (*S*) between two neurites (*A*) in a tentacle of *Hydra pseudoligactis*. Two vesicles are attached to the fibrous coat on the presynaptic membrane in 1 *a* and five vesicles in 1 *b*. Arrows indicate dense-cored vesicles that appear clear in 1 *a*. × 48,000.

FIGURE 2 Symmetrical synapse (*S*) between two neurons (*N*) in a tentacle of *Hydra littoralis*. The synaptic vesicles in the cell rich in ribosomes (*R*) have dense cores, whereas the synaptic vesicles in the cell rich in glycogen (*G*) have no cores in this section. × 52,000.

FIGURE 3 Cross-section of neurite (*A*) containing microtubules (*MT*) and dense-cored vesicles (*DV*), which often have an asymmetrically swollen membrane (arrows). Note symmetrical synapse (*S*) between glycogen-rich neuron (N_1) and ribosome-rich neuron (N_2) in *Hydra littoralis*. × 45,000.

tinguished by the large concentration of ribosomes in one and by glycogen particles in the other (Fig. 2). Dense cores (Figs. 2, 3) were present or absent in the synaptic vesicles (1100–1900 A in diameter). The neurite in Fig. 3 has a granule (1200 A in diameter) that nearly fills its limiting membrane and that resembles the neurosecretory granules described in *Hydra* by other electron microscopists (Lentz and Barrnett, 1965; Lentz, 1968; Davis et al., 1968). Many vesicles had an irregular swelling of the vesicular membrane (arrows, Fig. 3) which made accurate measurements difficult and indicated that, perhaps, many "clear vesicles" resulted from sectioning swollen regions of dense-cored vesicles.

EN PASSANT SYNAPSES along a neurite, in contradistinction to nerve terminal synapses, appeared to characterize the tentacular nerve plexus of *Hydra*. Such synapses occurred between neurites (Fig. 1 *a*) or between a neurite and the basal regions of epitheliomuscular cells. In Fig. 4, three en passant neuromuscular synapses are seen within 4 μ along a single neurite. A gap junction, composed of two closely apposed trilaminar unit membranes, is present between two epitheliomuscular cells.

NEUROMUSCULAR SYNAPSES (Figs. 4, 5) resembled asymmetrical interneuronal synapses. The glycogen-rich neuronal process in Fig. 5 has synaptic contacts both with an epitheliomuscular cell and with another neuronal process. The synaptic vesicles are approximately 1200 A in diameter and have cores approximately 700 A in diameter. The neuromuscular synapse has a prominent filamentous coat, extending on both the presynaptic and postsynaptic surfaces, but lacks an intracleft basal lamina characteristic of vertebrate neuromuscular junctions. Serial sections revealed seven dense-

cored vesicles in contact with the presynaptic coat, but no specific features appeared to characterize the postsynaptic region. The neuromuscular synapse in Fig. 5 lies near a gap junction between the myofilament-containing basal regions of two epitheliomuscular cells. Other gap junctions were seen in association with desmosomes (not shown) connecting the muscular bases of epitheliomuscular cells; they resembled the intercalated discs of Purkinje cells in canine cardiac muscle (Martinez-Palomo et al., 1970).

NEURONEMATOCYTE SYNAPSES were found between neurites and the bases of mature nematocytes (Fig. 6) and also between the perikarya of neurons and developing cnidoblasts (Fig. 7). Neuronematocyte synapses had the same features as neuromuscular synapses, except that the synaptic vesicles (1400–1900 A in diameter) appeared to be agranular.

A unique feature of the tentacular nerve plexus was the dual innervation of a cnidoblast and an epitheliomuscular cell by a single neuron (Fig. 7). Nowhere else in the animal kingdom is there ultrastructural evidence of a single neuron having synaptic contacts with two different types of effector cells.

DISCUSSION

Electron microscopy has revealed, for the first time, morphological evidence of polarized synapses between neurites of *Hydra* and has demonstrated the presence of specialized neuromuscular and neuronematocyte junctions. The synaptic contacts of nerve net animals and higher forms have basic similarities such as: (*a*) the presence of vesicles in association with a pair of membrane densities between two cells, (*b*) the occurrence of mitochondria near the vesicles and (*c*) a 150–200 A wide synaptic cleft containing intracleft filaments. Comparison between synapses of *Hydra* and those of vertebrates indicates several subtle differences: (*a*) the presence of only a few vesicles at synaptic contacts, (*b*) the large size of the synaptic vesicles (1200–2000 A in diameter), and (*c*) the occurrence of en passant synapses along neurites.

The synaptic vesicles of *Hydra* usually have dense cores. Dense-cored vesicles were first observed ultrastructurally in neurons of *Hydra* by Lentz and Barrnett (1965) who classified them as neurosecretory granules because of their large size (approximately 1000 A) and their resembling elementary neurosecretory granules of higher animals. Neurons with many membrane-bounded granules were termed neurosecretory cells because they lacked synaptic contacts with other neurons or effector cells (Lentz and Barrnett, 1965; Lentz, 1968; Davis et al., 1968). Because *Hydra* have morphologically specialized contacts that indicate precise foci to transmit an impulse from one cell to another, it is possible that the vesicles at these sites contain a neurotransmitter substance.

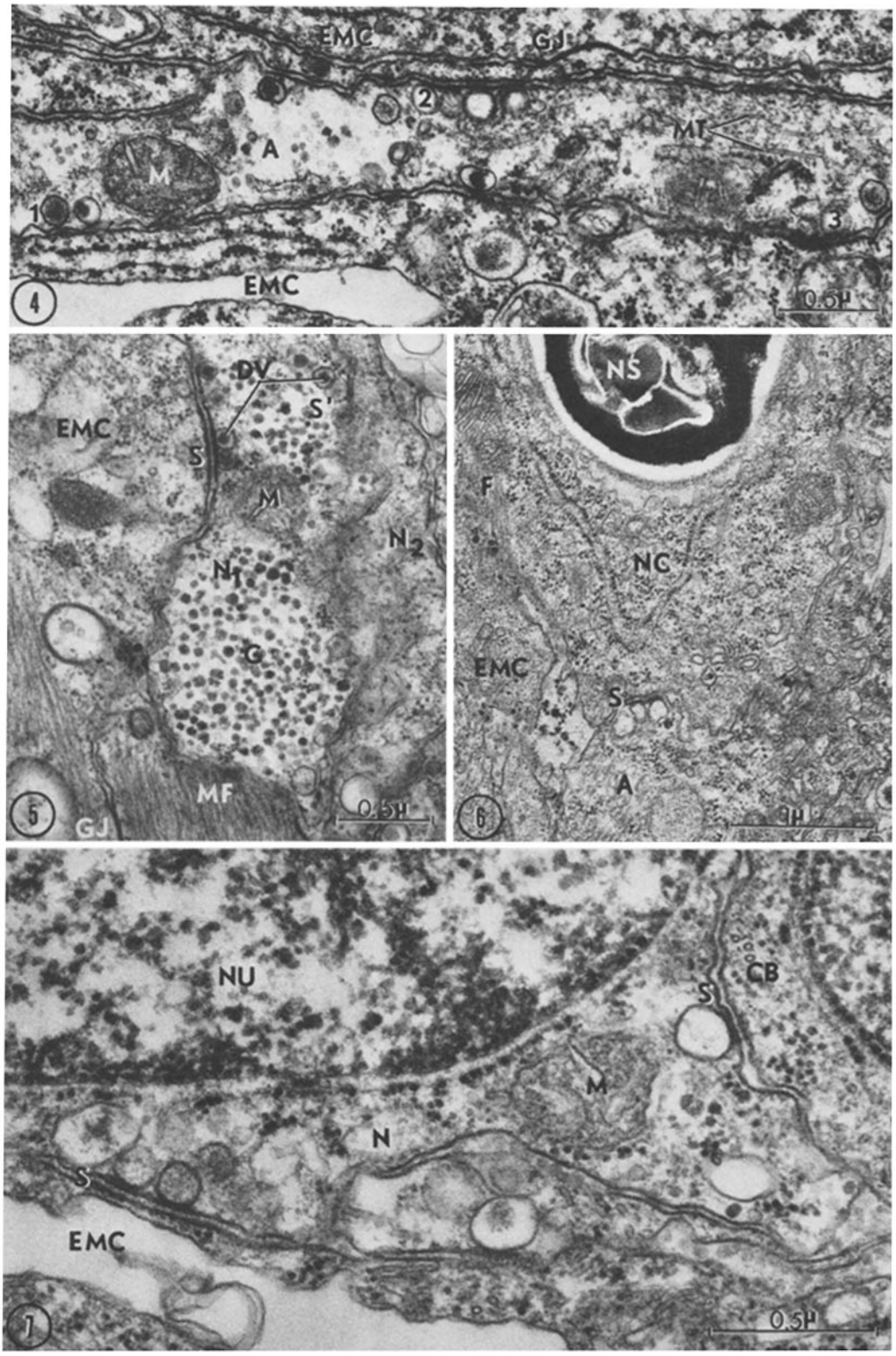
Biogenic amines have been correlated with the presence of dense-cored vesicles in various adrenergic nerve terminals of higher animals (Wolfe et al., 1962; De Robertis, 1964; Bloom and Barrnett, 1966) and have been demonstrated at a light microscope level in *Hydra* (Wood and Lentz, 1964). This suggests that dense-cored vesicles at specialized synaptic junctions in *Hydra* may contain an adrenergic neurotransmitter substance.

FIGURE 4 Longitudinal section of neurite (*A*) with three en passant synapses (*1*, *2*, *3*) to different regions of one or more epitheliomuscular cells (*EMC*) of *Hydra littoralis*. Serial sections showed that the vesicles at site *1* were also associated with membrane densities. Note gap junction (*GJ*) between epitheliomuscular cells. *M*, mitochondrion; *MT*, microtubules. × 30,000.

FIGURE 5 Neuromuscular synapse (*S*) between process of glycogen-rich neuron (N_1) and base of epitheliomuscular cell (*EMC*) in *Hydra littoralis*. Note synapse (*S'*) between neurons (N_1, N_2) and gap junction (*GJ*) between epitheliomuscular cells. *DV*, dense-cored vesicles; *G*, glycogen; *M*, mitochondrion; *MF*, myofilaments. × 28,500.

FIGURE 6 Synapse (*S*) between neurite (*A*) and nematocyte (*NC*) in a pocket of an epitheliomuscular cell (*EMC*) in *Hydra littoralis*. Note nematocyst (*NS*) and bundle of filaments (*F*) within nematocyte. × 22,000.

FIGURE 7 Part of perikaryon of neuron (*N*) with synapses (*S*) to both an epitheliomuscular cell (*EMC*) and a cnidoblast (*CB*) in *Hydra littoralis*. *M*, mitochondrion; *NU*, nucleus. × 50,000.


EMC
GJ
2
A
MT
M
1
3
EMC
4
0.5μ
DV
S'
EMC
S
M
N2
N1
G
MF
5
GJ
0.5μ
NS
F
NC
EMC
S
A
6
1μ
NU
CB
S
M
N
S
EMC
7
0.5μ

Our laboratory is currently testing, by electron microscope radioautography, for various monoamines at these sites. The nature of synapses of *Hydra* will need to be resolved by further investigations at the biochemical and electrophysiological levels.

That the synaptic vesicles at neuronematocyte junctions are clear, may indicate another type of neurotransmitter substance like acetylcholine. Acetylcholinesterase has been demonstrated at the light microscope level in neuronal endings on cnidoblasts of *Hydra* (Lentz and Barrnett, 1961). Moreover, it has been shown that acetylcholine augments the discharge of nematocysts (Lentz and Barrnett, 1962). Such observations suggest that the neuronematocyte junctions may be cholinergic.

A unique feature of the nerve net of *Hydra* is the presence in one neuron of synaptic junctions with two different effector cells: an epitheliomuscular cell and a nematocyte. This unusual situation may result from the arrangement of a battery of nematocyst-containing cells in a single epitheliomuscular cell. Slautterback (1967) noted long desmosomal connections between nematocytes and slender modified bases of epitheliomuscular cells. He did not find ultrastructural evidence of any specialized neuronematocyte synaptic junctions, and thus, postulated that the firing of an entire battery of nematocysts might be controlled by a single epitheliomuscular cell by way of the specialized desmosomal connections.

We have observed evidence of neuronematocyte synapses with each of three types of nematocysts in *Hydra*: stenoteles, desmonemes, and isorhizas, which suggests that the nervous system is the probable coordinator of nematocyst discharge in *Hydra*. However, the dual innervation of an epitheliomuscular cell and a nematocyte by one neuron may coordinate muscular contraction and simultaneous discharge of one or more types of nematocysts.

The polarized interneuronal and neuroeffector synapses described in this paper are morphological indications of synapses that may transmit in only one direction. Changes in electrical potential recorded in response to light and mechanical and electrical stimuli indicate excitable cells in *Hydra* similar to those of the central nervous system of higher animals (Passano and McCullough, 1962, 1964; Rushforth et al., 1963). *Hydra* thus possesses all the building blocks necessary to form elementary neuronal pathways with a precise, rapid, polarized system of conduction similar to that of higher animals.

SUMMARY

Transmission electron microscopy revealed specialized synaptic junctions formed by an aggregation of vesicles in association with two thickened, parallel, electron-opaque plasma membranes in tentacles of *Hydra littoralis* and *Hydra pseudoligactis*. The synaptic vesicles (1200–2000 A in diameter) generally contain dense cores from 600 to 1200 A in diameter and may be associated with one or both cytoplasmic surfaces of the synaptic membranes. The synaptic junction, up to 0.8 μ long, is composed of two parallel plasma membranes, both thickened by a coat of fine filaments on their cytoplasmic surfaces. The synaptic cleft is approximately 150 A wide and has a dense filamentous material in it. The epidermal nerve net of *Hydra* has asymmetrical and symmetrical interneuronal synapses and neuromuscular and neuronematocyte junctions.

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