

## Unusual Presynaptic Inclusions in the CNS of the Marine Polychaete, *Nereis virens*\*

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**Summary.** Unusual electron-dense inclusions occur in the nerve terminals of the *Nereis* CNS. These structures are closely associated with synaptic vesicles and contain what appear to be incomplete vesicles within them suggesting that they may be an organelle involved in synaptic vesicle production.

**Key words:** Inclusion — Organelle — Synaptic vesicles — Synapse.

### Introduction

A wide variety of pre- and postsynaptic structural specializations have been described in neurons (see Pappas and Purpura, 1972, for examples), but with the exception of synaptic vesicles their roles remain largely speculative. It is now believed that at the vertebrate neuromuscular junction newly produced synaptic vesicles are associated with electron-dense material which forms a shell or coat over the vesicle, this vesicle coat eventually being recycled at the site of vesicle production (Heuser and Reese, 1973).

We have found that synapses in the central nervous system (CNS) of the marine polychaete, *Nereis virens*, have extensive amounts of highly localized electron-dense granular material associated with their synaptic vesicles. It appears that these structures may be involved in the production of synaptic vesicles.

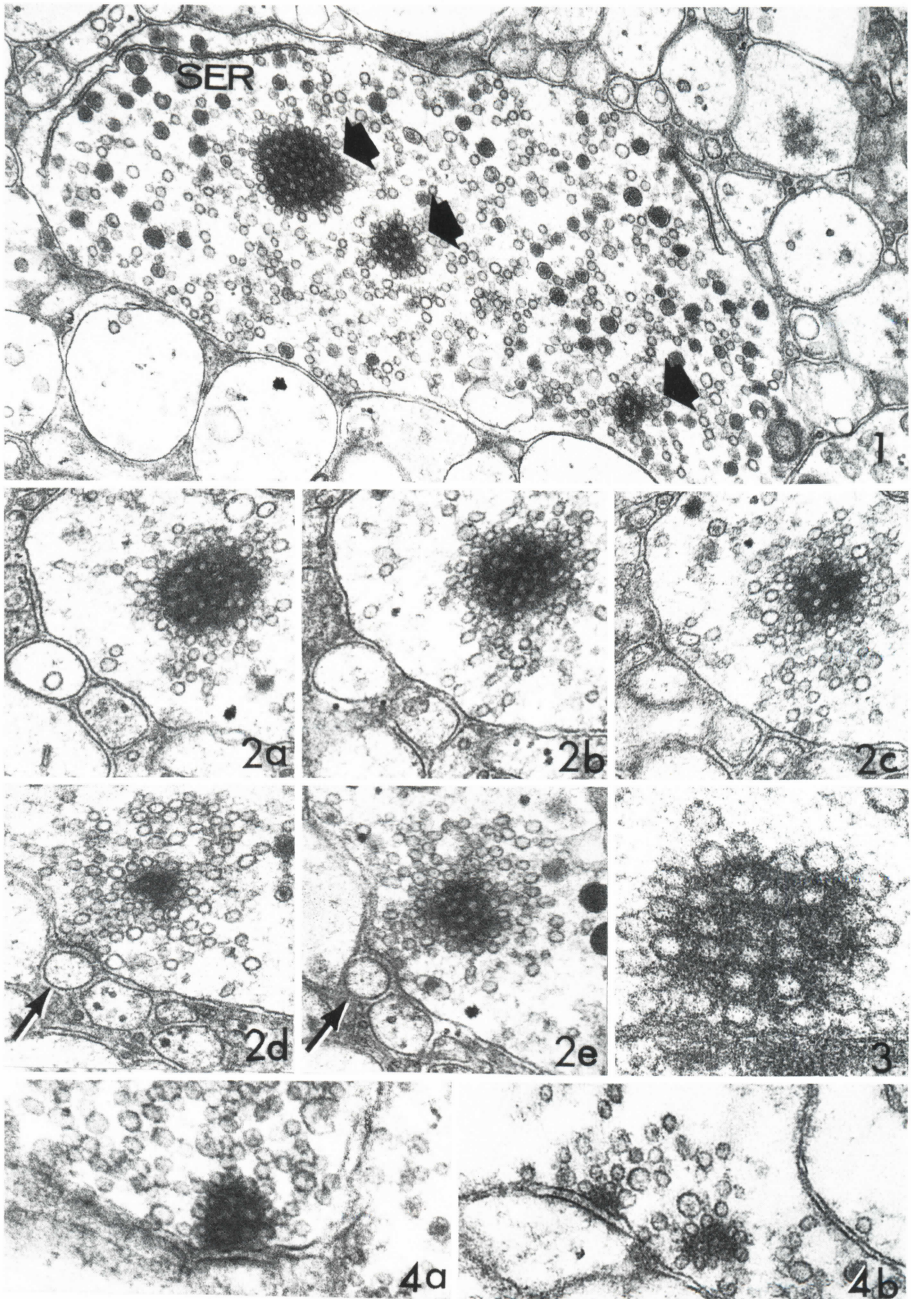
Observations were made primarily on the supraesophageal ganglion (brain) of *Nereis* which is organized into several cell and fiber masses consisting of the central stomodeal mass, the corpora pedunculata, and the fiber tracts arising from the four eyes (Bullock and Horridge, 1965). The segmental ganglia of the ventral nerve cord were also examined for the presence of the unusual presynaptic inclusions found in the brain.

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**Fig. 1.** Synaptic terminal in *Nereis* supraesophageal ganglion containing smooth endoplasmic reticulum (SER), dense core vesicles, synaptic vesicles scattered and in association with three electron-dense inclusions (arrows).  $\times 30,000$

**Fig. 2a-e.** Series partially through one dense inclusion. Note vesicles streaming out to synaptic site in d and e (arrows). Supraesophageal ganglion.  $\times 40,000$

**Fig. 3.** Electron-dense inclusion in supraesophageal ganglion. Vesicles within inclusion less distinct than on its border.  $\times 90,000$

**Fig. 4a and b.** Synapses from segmental ganglion. **a** Dense inclusion adjacent to presynaptic membrane. **b** Edge of dense inclusion adjacent to presynaptic membrane giving impression of "conventional" synapse. Note second dense inclusion within cytoplasm.  $\times 50,000$



## Material and Methods

Adult specimens of *N. virens* (15–30 cm) were obtained from the Woods Hole Marine Supply House and maintained in sea water aquaria until used. The region of the prostomium containing the supraesophageal ganglion was placed in fixative consisting of 2% formaldehyde and 2% glutaraldehyde in 0.2 M sodium cacodylate (pH 7.4). The central nerve cord was exposed and fixative applied in situ, after which sections of nerve cord were excised and placed in fresh fixative. Our best results were obtained by storing the tissue in fixative for 10 days at 4° C. The tissue was post-fixed for 1 h in 2% OsO<sub>4</sub> in veronal acetate buffer (pH 7.4). Araldite sections were stained with 1% aqueous uranyl acetate (20 min) followed by lead citrate (10 min) and examined in a Siemens la electron microscope.

## Results

Presynaptic terminals within the *Nereis* CNS contain a variety of organelles including endoplasmic reticulum, mitochondria, large (660–1375 Å) dense core vesicles, and smaller synaptic vesicles scattered throughout the cytoplasm as well as in association with electron-dense inclusions which are more conspicuous than any usual organelles (Fig. 1). These unusual dense inclusions are composed of a granular matrix and are not membrane bounded; profiles of vesicle membranes can, however, be seen within the inclusion (Figs. 1–3). There is a gradation of vesicle diameter from those in the interior of the inclusion (160–330 Å) to those free in the cytoplasm (440–700 Å) (Figs. 1–3). In the supraesophageal ganglion the dense inclusions are usually located near the center of terminal profiles (Fig. 1) with synaptic vesicles streaming into the cytoplasm toward the point of synaptic contact (Fig. 2). Several of these inclusions may occur in one terminal (Fig. 1) and one inclusion may contribute vesicles to several synapses. The dense inclusions themselves appear to be slightly elongated structures inasmuch as the serial reconstruction of one terminal showed its inclusions to have long axes of at least 6000 Å and short axes ranging from 700 to 4300 Å. The actual synapse can be identified by a regular widening of the extracellular space to a cleft of 300 Å as well as the presence of dense material both on the pre- and postsynaptic membranes and in the cleft (Figs. 2d, e, 4).

Examination of the segmental ganglia shows similar electron-dense inclusions occurring in the synaptic terminals, but unlike those in the brain they are often adjacent to the presynaptic membrane and appear to be continuous with the presynaptic densification (Fig. 4a). At other synapses, however, the dense inclusion is located away from the synaptic site as described in the brain (Fig. 4b).

## Discussion

Synaptic contacts in the *Nereis* CNS appear structurally similar to chemical synapses described in a variety of nervous tissues (Pappas and Purpura, 1972). The presynaptic terminals, however, contain large electron-dense inclusions which are closely associated with synaptic vesicles. What is the function of these unusual inclusions? Immature or partially formed vesicles are seen within the electron-dense matrix which appear smaller than the synaptic vesicles in the adjacent cytoplasm and which show a gradation of membrane definition from the center to periphery of the inclusion (Fig. 3). These observations coupled with the appearance of vesicles streaming out from the dense region to the

point of synaptic contact suggest that this may be an organelle for organization and assembly of synaptic vesicles. We have not found "coated" vesicles in any of our material; these coats have been associated with newly assembled vesicles (Pearse, 1975, 1976) including synaptic vesicles in vertebrate photoreceptors (Gray and Pease, 1971) and at the neuromuscular junction (Heuser and Reese, 1973). The dense matrix found in the *Nereis* synaptic terminals may thus be analogous to the dense proteinaceous material (Pearse 1975, 1976) forming the shells of coated vesicles in other animals.

Regardless of the plane of section these electron-dense organelles are ultrastructurally different from other presynaptic electron-dense organelles such as the ribbons or bars found at vertebrate and invertebrate photoreceptor synapses (Dowling and Boycott, 1966; Gray and Pease, 1971; Dowling and Chappell, 1972; Lasansky, 1973; Holmberg and Öhman, 1976) and synapses of the vestibular system (Smith and Rasmussen, 1965), inasmuch as the dense organelles found in *Nereis* have profiles of vesicles within the dense matrix, while the synaptic ribbons and bars have vesicles occurring solely as a surrounding halo. The electron-dense organelles of *Nereis* also differ from synaptic ribbons in that they are most often located away from the point of synaptic contact with only vesicles approaching the presynaptic site.

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