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A Note on the Structure of Synapses in the Ventral Nerve Cord of the Onychophoran *Peripatoides leuckarti*

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Summary. The fine structure of synapses, their distribution and arrangement in the ventral nerve cord of *Peripatoides leuckarti* (phylum Onychophora) is described. The asymmetric synaptic junctions show a well developed synaptic cleft (300 Å) and pre- and subsynaptic electron dense apposition. They frequently show an array of presynaptic projections and a subsynaptic cisterna of endoplasmic reticulum. The onychophoran synapses differ from those of annelids and insects.

Key words: Synapses – Ventral nerve cord – Onychophora.

The Onychophora have been of great interest due to the fact that they possess anatomical features of both the annelids and arthropods, and are regarded as a phylogenetic link between the two phyla. Phylogenetic considerations have been extended to the nervous system of Onychophora on the basis of light microscopical studies (Holmgren, 1916; Hanström, 1935; Feodorov, 1926; 1929; see Horridge, 1965). Evidence for giant fibres in the ventral nerve cord was originally presented by Sänger (1870). Their presence in *Peripatoides leuckarti* was recently confirmed by morphological and electrophysiological investigations (Schürmann and Sandeman, 1976). A few studies have dealt with the ultrastructure of onychophoran sense organs (Eakin and Westfall, 1965; Eakin and Brandenburger, 1966; Storch and Ruhberg, 1977); however, knowledge of the fine structure of the central system is still lacking.

The present study describes the structure of synapses and synaptic configurations of nerve fibres in the ventral nerve cord of the Australian onychophoran, *Peripatoides leuckarti,* and compares this structure to that of annelid and insect synapses.

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Materials and Methods

Two colour morphes of *Peripatoides leuckarti* were collected near Canberra (A.C.T.) and Armidale (New South Wales) in Australia. Intact animals of both sexes and various lengths (1–7 cm) were anaesthetised with carbon dioxide or immobilised by cooling, pinned down in order to prevent bending from muscular contraction and opened dorsally by a longitudinal cut. After removing the glands, intestine and reproductive system, the animals were immersed in the fixative. Fixation fluids of low ionic strength gave satisfactory results and a solution of 1% glutardialdehyde – 1% formaldehyde buffered with sodium cacodylate (0.05 M, pH 7.4) yielded the best preservation of nervous tissue. After 2–6 h the animals, with nervous system undissected, were transferred into a 1% osmic acid solution (with the same buffer). For special staining of paramembranous synaptic substances the ethanolic phosphotungstic acid (EPTA) impregnation of Bloom and Aghajanian (1968) was applied without modification. Subsequent processing followed conventional techniques for electron microscopy. Transverse and longitudinal thin sections through the ventral nerve cord were double stained with uranyl acetate and lead citrate. Sections were examined with an electron microscope at magnifications up to 80,000×. Light microscopy was carried out with paraffin sections stained with Heidenhain's haematoxilin, and with 1 μm thick plastic sections stained with toluidine blue.

Results

Distribution of Synapses. Synapses in the ventral nerve cord of *Peripatoides leuckarti* are restricted to the neuropil and do not occur in the ventro-medial perikaryal layer. They were not observed in the leg and tegmental nerves or in the fine tract-like commissures (nine per segment) which link the widely separated nerve cords on either side. The neuropil is continuous along the neurosomites (Feodorov, 1926) and has no connective-like parts free of synapses ("Markstrang", s. Hanström, 1928). It consists predominantly of a fine meshwork of fine naked fibres which are filled with synaptic and other organelles. The fibres make abundant synaptic contacts with each other. A glial enveloping of fibres is not prominent but occurs around larger fibres, e.g. partially around the giant fibres (Schürmann and Sandeman, 1976). The majority of nerve fibres contains and is sometimes densely packed with different classes of vesicles. The spherical, translucent synaptic vesicles have a diameter of 350–450 Å (Fig. 2a). Different types of dense core vesicles with various degrees of electron opacity show diameters up to 1200 Å (Fig. 1a, b, d).

Structure of Synapses. Synaptic junctions normally show the structural polarity typical for chemical synapses which allows a distinction of the pre- and postsynaptic fibre element (Figs. 1, 2). At the presynaptic side, electron dense substances cover parts of the presynaptic membrane. In some sections these osmiophilic appositions appear as a regular array of protrusions projecting up to 400 Å into the fibre matrix (Fig. 1c, e, f). These pillar-like projections line the presynaptic membrane at intervals up to 400 Å. As serial sections and tangential views of the presynaptic apparatus are lacking, the three-dimensional pattern of dense projections remains unclear. Clear or dense core vesicles are accumulated around the presynaptic structures and membrane appositions (Figs. 1, 2). There is no evidence that they cannot contact the presynaptic membrane. A fusion of vesicles with the membrane at synaptic sites was not observed.

The synaptic cleft measures up to 300 Å in width, whereas the normal intercellular space is about 100 Å. The synaptic cleft is filled with electron dense

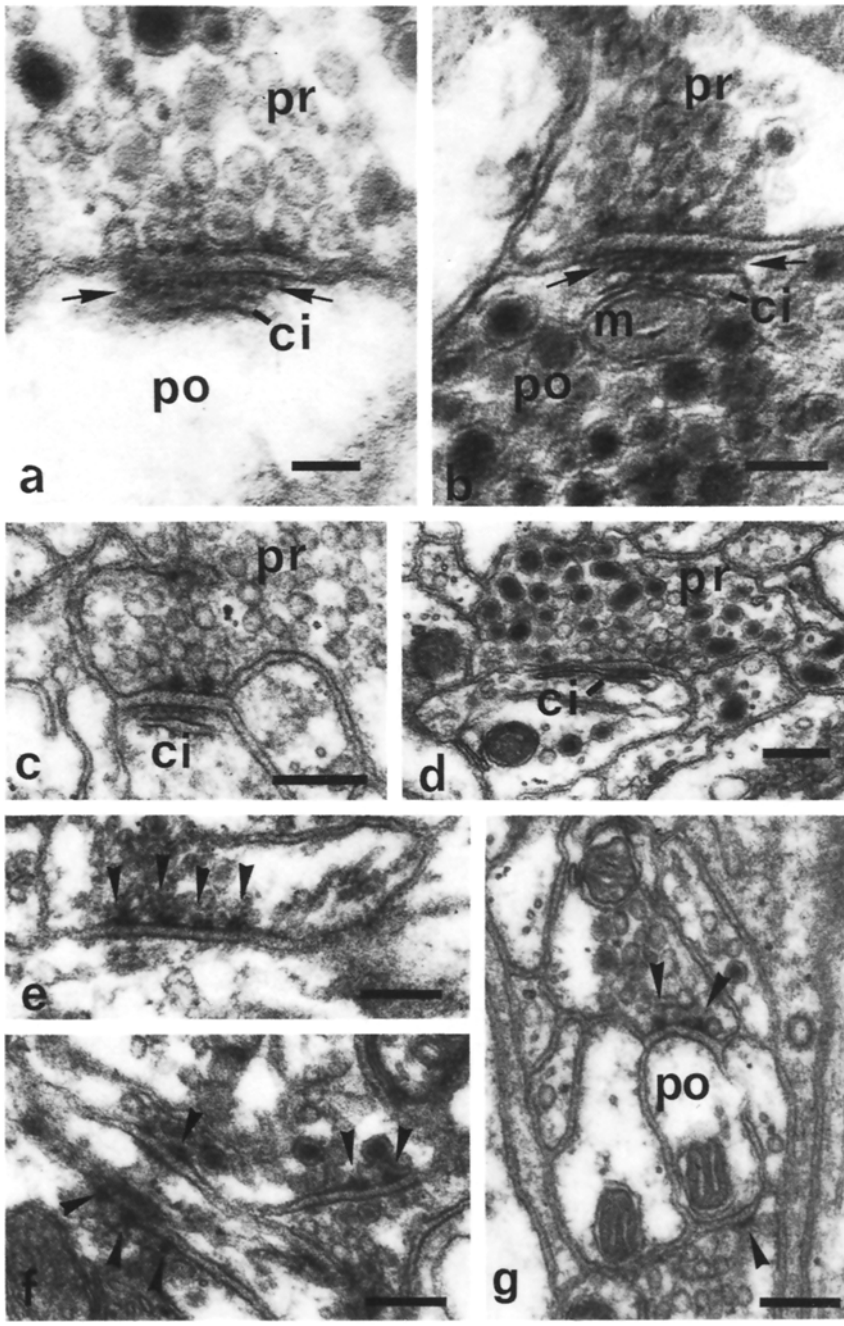

Fig. 1 a–g. Different types of synapses. Presynaptic fibre (*pr*); postsynaptic fibre (*po*); subsynaptic cisterna of the endoplasmic reticulum (*ci*); mitochondrion (*m*); subsynaptic appositions (*arrows*); presynaptic projections (*arrowheads*). **a** Dense core vesicles with faint osmiophilic content at the presynaptic membrane, dense midline in the synaptic cleft; scale 0.1 µm. **b** Dense core vesicles and smaller translucent vesicles accumulated at the presynaptic side; postsynaptically a population of large dense core vesicles; scale 0.15 µm. **c** Clear presynaptic vesicles; note denser intracleft midline; scale 0.2 µm. **d** Clear vesicles and small dense core vesicles near the presynaptic membrane; a group of large dense core vesicles more distant from the membrane; denser intracleft midline; scale 0.2 µm. **e** Synapse with presynaptic projections; subsynaptic appositions poorly developed; scale 0.2 µm. **f** Synapses with presynaptic projections; scale 0.2 µm. **g** Two presynaptic fibres contact a postsynaptic element; subsynaptic cisterna missing; scale 0.2 µm

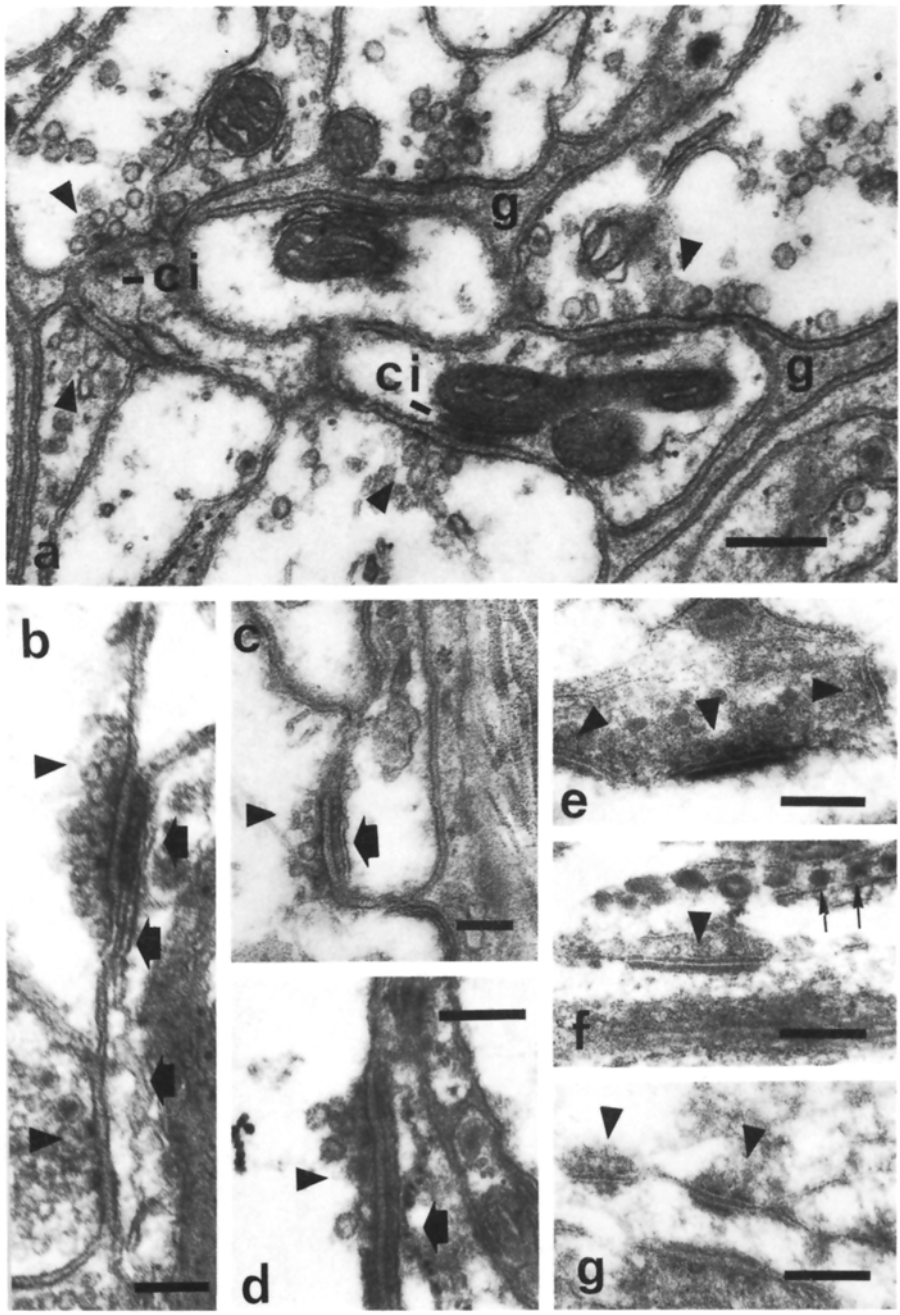

Fig. 2a. Synapses with clear presynaptic vesicles (*arrowheads*). Swollen cisterna of endoplasmic reticulum (*ci*) at left which contacts two synaptic sites; glial protrusions (*g*) between nerve fibre elements; dense intracleft midlines at the right; scale 0.2 µm. **b** A cisterna of endoplasmic reticulum (*arrows*) extends from the subsynaptic site to other parts of the axoplasm; triangles mark synaptic sites in two presynaptic fibres; scale 0.2 µm. **c–d** "En passant" synapses in larger fibres; giant fibre. **c** Synapse of a giant fibre (*arrowhead*: presynaptic element); subsynaptic cisterna (*arrow*); scale 0.2 µm. **d** Subsynaptic cisterna with ribosomes (*arrow*); scale 0.2 µm. **e–g** EPTA-impregnation of synapses leaves the membranes unstained; synaptic intra- and intercellular substances appear electron dense. Note the broad subsynaptic appositions and a faint clear intracleft midline corresponding to the electron dense cleft line in aldehyde-osmium fixed material. Scales 0.2 µm Presynaptic bouton (*arrowheads*) in **b**; dense core vesicles in **b** and **c** (*arrows*); clear presynaptic vesicles in **d**

substance, but does not appear as electron opaque as the intracellular synaptic paramembranous material. A denser midline can be detected in most synaptic clefts (Figs. 1a, d; 2a). Subsynaptic deposits of high electron density are normally well developed. They line the membrane along the cleft and project up to 300 Å into the postsynaptic fibre matrix. Very frequently, the subsynaptic densities border a flattened cisterna of smooth endoplasmic reticulum arranged in parallel to the subsynaptic membrane (Figs. 1a–d, 2a–d) and often close to a mitochondrion. The membranes of the subsynaptic cisternae often exhibit an increased electron density compared with other unit membranes of the endoplasmic reticulum. The extension and full distribution of this postsynaptic ER system is unknown, but from favourable sections it is obvious that it may extend to other than subsynaptic areas of the postsynaptic fibre element (Fig. 2b); in addition, different subsynaptic cisternae are continuous with each other (Figs. 1f, 2a). The subsynaptic reticulum is occasionally covered with ribosomes (Fig. 2d).

With the EPTA-impregnation, pre- and subsynaptic paramembranous substances as well as the intracleft material are visualised more clearly and completely (especially the contents of the synaptic cleft) (Fig. 2e–g) in comparison to aldehyde-osmic acid treated material. A clear midline is found in the synaptic cleft, corresponding to the denser midline of synaptic clefts of aldehyde-osmic acid fixed synapses. A proteinaceous nature for the intra- and intercellular synaptic membrane appositions can be assumed from the EPTA- impregnation (Bloom, 1970; Pfenninger, 1973). The contents of the dense core vesicles react positively to the EPTA-stain (Fig. 3e, f). That different types of synaptic junctions are present in

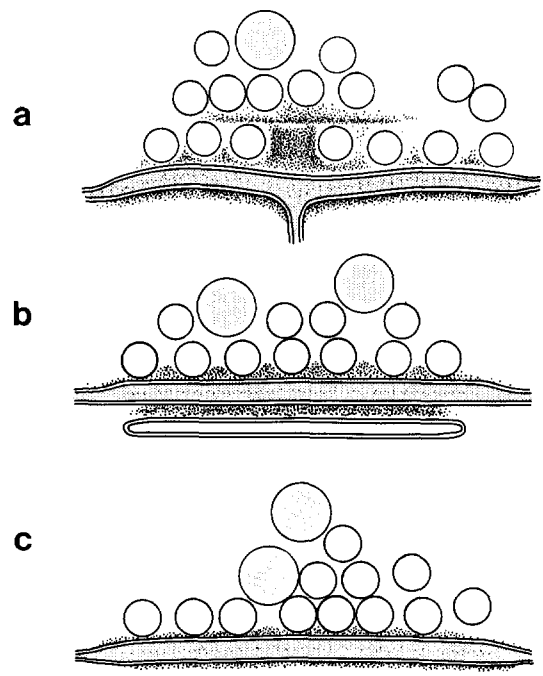

Fig. 3a–c.
Schematic drawing of an insect **a**, onychophoran **b** and annelid **c** synapse

the central nervous system of *Peripatoides* is mainly suggested by differences in their vesicle populations and the extent of pre- and subsynaptic appositions.

Arrangement of Synapses. Synapses are localised "en passant" (Fig. 2c, d) and in nerve fibre terminals. It was occasionally observed that two given fibres synapse with each other at more than one synaptic site. Two postsynaptic fibres are sometimes connected with one presynaptic element at the same presynaptic site (dyad of Lamparter et al., 1969). A presynaptic element (bouton) can be connected with a group of surrounding postsynaptic fibres (divergent type of junction), and a number of tiny presynaptic elements can converge on a postsynaptic element which appears dendritic (Fig. 1g). Serial synapses are found as well. Complex configurations of synapses are not ensheathed with glial processes and are therefore not separated from neighbouring nerve fibres.

Discussion

The synapses in the ventral nerve cord of the onychophoran *Peripatoides leuckarti* show the same principal ultrastructural features as the chemical synapses in the central nervous systems of vertebrates (Gray and Guillery, 1966; Pfenninger, 1973) and invertebrates (e.g. Arthropoda: Smith 1967; Trujillo-Cenóz, 1969; Krasne and Stirling, 1972; Strausfeld, 1976; Annelida: Coggeshall and Fawcett, 1964; Dhainaut-Courtois and Warembourg, 1969; Günther and Schürmann, 1973; Muller and Mc Mahan, 1976; Mollusca: Jones, 1968; Plathelminthes: Best and Noel, 1969; Coelenterata: Westfall et al., 1971). Structural polarity in asymmetric synapses is established by the clustering of presynaptic vesicles at the presynaptic projections and at the membrane and by the assymetry of the pre- and subsynaptic membrane appositions.

A comparison of onychophoran synapses with synapses of the closest related phyla, the annelids and arthropods, shows the onychophoran synapses to differ from both in fine structural details. Compared with annelid synapses (see Günther and Schürmann, 1973), the pre- and subsynaptic electron opaque appositions are much more prominent and always present in onychophoran synapses. Paramembranous synaptic substances are very often poorly developed in the earthworm synapses and therefore often overlooked (Myhrberg, 1972). Many insect synapses exhibit a well ordered array of presynaptic densities forming distinct presynaptic figures projecting more than 500 Å into the presynaptic fibre (see Trujillo-Cenóz, 1969; Schürmann, 1971; Burckhardt and Braitenberg, 1976), whereas presynaptic figures in onychophoran synapses appear smaller. The extent of subsynaptic appositions is similar in both insect and onychophoran synapses. Fig. 5 presents a schematic comparison of annelid, insect and onychophoran synapses.

The frequent presence of a flattened cisterna of endoplasmic reticulum parallel to the subsynaptic densities and membrane is so striking that it is considered as a part of the subsynaptic apparatus on the basis of its position, form and enhanced electron density. A similar peculiarity in postsynaptic fibres is known for some identified types of neurons in the optic lobes of flies (Trujillo-Cenóz, 1965; Boschek,

1971) and was termed "postsynaptic bag" (Burckhardt and Braitenberg, 1976). Stacks of flattened cisternae of smooth endoplasmic reticulum represent a typical postsynaptic feature of the dendritic spines (spine apparatus) in vertebrates (Gray, 1959).

The sporadic occurrence of subsynaptic cisternae with ribosomes is uncommon for invertebrate synapses. Membrane-attached ribosomes appear in dendritic fibre profiles of giant neurons in the earthworm (Günther and Schürmann, 1973), but were not observed as part of the synaptic site. The restriction of synapses to the neuropile in the ventral nerve cord of Onychophora is consistent with the observation that neuronal perikarya in invertebrates are in general devoid of somatic synapses although exceptions do occur (Schürmann and Günther, 1973).

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