SYNAPSES BETWEEN AN IDENTIFIED GIANT INTERNEURONE AND A FILIFORM HAIR SENSORY NEURONE IN THE TERMINAL GANGLION OF FIRST INSTAR COCKROACHES (*PERIPLANETA AMERICANA* L.)

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The ultrastructure of input and output synapses of various identified insect neurones has recently been studied (for example Titmus & Hoyle, 1977; Altman, Shaw & Tyrer, 1980; Phillips, 1980; Watson & Burrows, 1981, 1982, 1983; Tolbert & Hildebrand, 1981) and connections between anatomically and physiologically defined axons have been described (King & Wyman, 1980). To date though, synapses have not been visualized in an insect central pathway in which aspects of the anatomy, physiology, pharmacology and associated behaviour have been investigated. The cercal afferent, giant interneurone synapses in the sixth abdominal (A6) ganglion of the cockroach *Periplaneta americana* are well suited for such studies. Using cobalt staining (Pitman, Tweedle & Cohen, 1972) of single neurones, seven giant interneurones (GIs 1–7) can be identified on the basis of their morphology (Harrow, Hue, Pelhate & Sattelle, 1980; Daley, Vardi, Appignani & Camhi, 1981). In adult cockroaches these interneurones receive sensory input from numerous cercal mechanoreceptor afferents (Callec, Guillet, Pichon & Boistel, 1971; Daley et al. 1981). There is considerable evidence for cholinergic monosynaptic transmission at these synapses (Callec, 1974; Sattelle, 1980) and postsynaptic acetylcholine receptors sensitive to the nicotinic cholinergic antagonist α-bungarotoxin are present on GI 2 and GI 3 (Harrow, Hue, Pelhate & Sattelle, 1979; Sattelle, David, Harrow & Hue, 1980; Harrow & Sattelle, 1983; Sattelle et al. 1983). It has been shown that the cercal afferent, giant interneurone pathways play a part in mediating the escape response of the cockroach following stimulation of the cercal mechanoreceptors (Ritzmann & Camhi, 1978; Camhi, Tom & Volman, 1978).

Cobalt-backfilling of whole connectives reveals that giant interneurones can be recognized in the first instar cockroach, but at this stage of development their sensory input is greatly simplified (Blagburn & Beadle, 1982). Each cercus bears only two functional filiform hair sensilla (Dagan & Volman, 1982) and their identifiable sensory

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neurones send large axons into the terminal ganglion where they terminate in the neuropile, overlapping the dendritic fields of interneurones 1, 2 and 3, the cell bodies of which are located contralaterally. The present study was undertaken to identify synaptic contacts between a particular interneurone and an identified cercal sensory axon by ionophoretically labelling GI 3 with horseradish peroxidase (HRP) and examining the distribution of this electron-dense marker at the fine structural level.

Experiments were performed on newly-hatched first instar nymphs of *Periplaneta americana*. A saline designed for use with the intact CNS and of the following composition (mM), was used (cf. Treherne, Buchan & Bennett, 1975): NaCl, 120; KCl, 8·0; CaCl₂, 2·0; MgCl₂, 2·0; KH₂PO₄, 1·8; KH₂PO₄, 0·2; KHCO₃, 2·5; trehalose, 10·0; Tris-HCl, 28·8; Tris (base), 3·1; pH 7·2. A preparation consisting of the cerci, cercal nerves X and XI on each side and the abdominal ganglia was dissected, mounted in a pool of saline on a glass slide and observed using a Zeiss microscope with Nomarski interference contrast optics and a ×40 water immersion lens. The cell body of GI 3 was located visually by its characteristic size and position (Blagburn & Beadle, 1982). For cobalt injection of the filiform hair sensory neurones the distal two segments of the cercus were removed and the dorsal half of the remaining segment excised. The cercus was held in place with sections of human hair mounted in petroleum jelly and examined with Nomarski optics. The cuticular cupola and neuronal cell body of the filiform hair receptor were visible. Fig. 1 illustrates these features in semidiagrammatic form. The neurone soma was subsequently injected with cobalt ions. Neurones were impaled with microelectrodes containing either 6% hexammine cobaltic chloride or, for electronmicroscopic identification of GI 3, 4% HRP with 0·3 m-KCl and 0·2 m-Tris (pH 7·4) buffer. Square, positive, current pulses of approximately 4 nA amplitude and 0·5 s duration were applied at a frequency of 0·5 Hz for 3 min. The preparation then remained in saline for 15–20 min at room temperature to allow orthograde movement of the tracer molecules. Cobalt ions were precipitated with dilute ammonium sulphide and the

Fig. 1. Semi-diagrammatic representation of the dissected first instar cercus, seen under Nomarski optics. The cercus is held in place with a human hair (h). The filiform hair (fh), inserted in a cuticular cupola (cc), is innervated by a sensory neurone whose soma (ns), visible beside the receptor lymph cavity, is approximately 20 μm across. The sensory axon joins a branch (n) of cercal nerve XI. me, microelectrode.
Preparations were then fixed in alcoholic Bouins' fixative and subsequently silver-intensified (Bacon & Altman, 1977) and mounted in neutral Canada Balsam. HRP-filled specimens were fixed in 2.5% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4) containing 0.2 M-sucrose for 60 min, washed in buffer, and the peroxidase reaction product was developed using the method described by Watson & Burrows (1981). After fixation in 1% osmium tetroxide in 0.1 M-cacodylate buffer the tissue was prepared for electron microscopy.

Cobalt injection and subsequent silver-intensification of first instar GI 3 (Fig. 2B) reveals its morphology in greater detail than the less precise method of cobalt-backfilling whole connectives (Blagburn & Beadle, 1982). Similarities in shape between the first instar and adult cell (cf. Harrow et al. 1980; Daley et al. 1981) are evident.

Cobalt injection of the cell body of the lateral filiform hair sensory neurone on the left cercus stains the most lateral of the two large axons within the A6 ganglion (Fig. 2A). This axon branches extensively in the region occupied by the dendrites of GI 3. The enlarged axon shaft can be recognized in both 1 μm and ultrathin resin sections of the ganglion (Blagburn & Beadle, 1982), without tracer injection. The filiform hair sensory axons are the largest in the first instar A6 ganglion, often reaching 7 μm in diameter, and each has a characteristic branching pattern. The lateral filiform hair sensory axon contains large numbers of mitochondria and forms output synapses on all branches that could be traced in thin sections and also forms output synapses which are concentrated on the medial side of the expanded shaft, (Fig. 2C). In places there is an almost continuous sheet of densely-packed, predominantly ovoid vesicles, 45±7 ± 0.7 nm maximum diameter 36 ± 0.7 nm minimum diameter (N = 50).

Numerous dendritic branches of GI 3 form contacts with the axon. The density of such contacts is approximately 1–2 μm⁻² on the medial side of the axon shaft. Examination of lateral filiform hair sensory axon contacts with GI 3 (Fig. 2D) shows that synaptic vesicles are clustered around bar-shaped presynaptic densities with a round or triangular cross-section (width 20–30 nm, height 30–60 nm). The average length of the bar is 150–300 nm, with a maximum of 500 nm. Some thickening of the postsynaptic membrane is evident. In Fig. 2D the process from GI 3 is one of a postsynaptic pair. This dyadic configuration is a typical feature of lateral filiform hair sensory axon output synapses.

Thus HRP-staining of GI 3 allows unequivocal resolution of its fine dendrites, many of which form synaptic contacts with the large, identifiable axon of the lateral filiform hair sensory neurone. The synaptic ultrastructure is similar to that of the bar-type
synapses described in the cockroach metathoracic ganglion (Wood, Pfenniger & Cohen, 1977) and to that of synapses formed by locust motoneurones and interneurones (Watson & Burrows, 1981, 1982, 1983). The arrangement of the synapses along one side of the expanded axon shaft in addition to distinct presynaptic endings is an unusual feature which has not been reported in the adult (cf. Farley & Milburn, 1969), and which may be related to the small number of filiform hair sensory axons in the first instar.

The present study provides ultrastructural evidence for the existence of a monosynaptic connection between an identifiable filiform hair sensory neurone and giant interneurone 3 in the terminal ganglion of the first instar cockroach Periplaneta americana. This cholinergic pathway is currently under investigation in a study of synaptogenesis.

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Identifiable cercal afferent, giant interneurone synapses


