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MORPHOLOGY OF IDENTIFIED CERCAL AFFERENTS AND GIANT INTERNEURONES IN THE HATCHLING COCKROACH *PERIPLANETA AMERICANA*

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SUMMARY

Cobalt backfills from the thoracic connectives of the hatchling *Periplaneta americana* allowed identification of giant interneurones in the terminal abdominal ganglion, morphologically comparable to GI 1, 2 and 3 in the adult. The bipolar neurone innervating each cercal filiform wind receptor hair is ultrastructurally similar to the adult cell and possesses an individually identifiable afferent axon, four of which provide the behaviourally functional escape response system with a simplified sensory input. Both pre- and post-synaptic neurones can be identified and may provide a good preparation for the study of cholinergic synapses.

INTRODUCTION

Many aspects of orthopteran cercal afferent–giant interneurone systems have been investigated because of the ease of identification and large size of the giant interneurones and their accessibility to a range of electrophysiological and pharmacological techniques (Callec, 1974; Harrow *et al.* 1979, 1980; Murphey, Jacklet & Schuster, 1980). In the adult cockroach *Periplaneta americana* many of the hundreds of cercal sensory axons form chemical synapses, believed to be cholinergic (Shankland, Rose & Donniger, 1971; Callec, 1974; Sattelle, 1980), with the giant interneurones, which play a part in mediating the escape response to mechanical stimulation of the cercal receptor organs (Roeder, 1948; Ritzmann & Camhi, 1978; Camhi, Tom & Volman, 1978). Individual sensory neurones have been physiologically characterized (Nicklaus, 1965; Dagan & Camhi, 1979) but the large number of small cercal axons makes routine investigation of a single identified cell very difficult.

The first instar nymphal cercus of *Periplaneta americana* possesses only three segments and considerably fewer sensilla than that of the adult. There are only two filiform wind receptor hairs, whereas in the adult there are over 200 (Sihler, 1924) although the escape response of the nymph is almost as efficient (Camhi & Tom, 1978; Camhi, 1980). It seems likely that at this stage of development the cercus supplies sensory input to the giant interneurones through very few axons, making the presynaptic side of the cercal afferent–giant interneurone synapse more amenable to investigation. Preliminary light and electron microscope observations indicated that two large

axons enter the first instar terminal ganglion via each cercal nerve. The aims of this work were to determine the origins of these axons and to confirm that the hatchling giant interneurones were morphologically comparable to those described in the adult ganglion (Harrow *et al.* 1980; Daley *et al.* 1981).

MATERIALS AND METHODS

Oothecae were collected daily from female *Periplaneta americana* and incubated at 29 °C in a humid atmosphere. Under these conditions the duration of embryogenesis was 30–31 days. First-instar nymphs 1 or 2 days old were studied. Giant interneurones and cercal afferents were stained by axonal diffusion of cobaltous ions and the subsequent formation of a black sulphide precipitate (Pitman, Tweedle & Cohen, 1972). The former were filled by cutting the connectives between the thoracic ganglia then sealing the cut ends in a petroleum jelly chamber of cobalt solution. The cercal afferent axons, and the cercal sensory cells, were filled using a similar technique with different portions of the cut cercal nerves. A 1% $CoCl_2$ solution with 0·13 mg/ml bovine serum albumen was used (Strausfeld & Obermayer, 1976) and the preparation was left for 18 h at 4 °C. Tissues were dissected from the animal in tissue culture medium (Chen & Levi-Montalcini, 1969). Cobalt was precipitated with freshly made ammonium sulphide in culture medium, the specimens were rinsed and placed in alcoholic Bouins fixative for ½–1 h. Tissues were dehydrated in an alcohol series, cleared and mounted in neutral Canada Balsam, and examined with a Zeiss photomicroscope.

For transmission electron microscopy specimens were excised in tissue culture medium, fixed for ½–1 h at 4 °C (2·5% glutaraldehyde, 0·1 M cacodylate buffer, 0·2 M sucrose, pH 7·2) and after a buffer wash, post-fixed in 1% osmium tetroxide for 1 h. After dehydration and embedding in Araldite the tissues were sectioned at 1 μm and at 50–100 nm on an LKB ultramicrotome. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined in a JEM electron-microscope. Cerci used for scanning electronmicroscopy were fixed in 2·5% glutaraldehyde in buffer, dehydrated, and after critical point drying were examined in a Phillips 300S microscope.

OBSERVATIONS

Cobalt fills of giant interneurones

Studies on the adult terminal ganglion have established the constancy of giant interneurone morphology (Daley *et al.* 1981) and 14 cells can be classified according to the nomenclature of Harris & Smyth (1971) and Camhi (1976). In the present study backfilling with cobalt from the connectives between the first and second thoracic ganglia enabled staining of 8–14 cells in the sixth abdominal ganglion (Fig. 1). Six large cells consistently took up enough stain to allow visualization of their cell bodies, axons, neurites and major dendritic branches. These cells are organized into bilaterally symmetrical pairs with each cell body situated contralaterally to its axon and morphological evidence indicates that they correspond to giant interneurones (GI) 1, 2 and 3 in the adult ganglion.

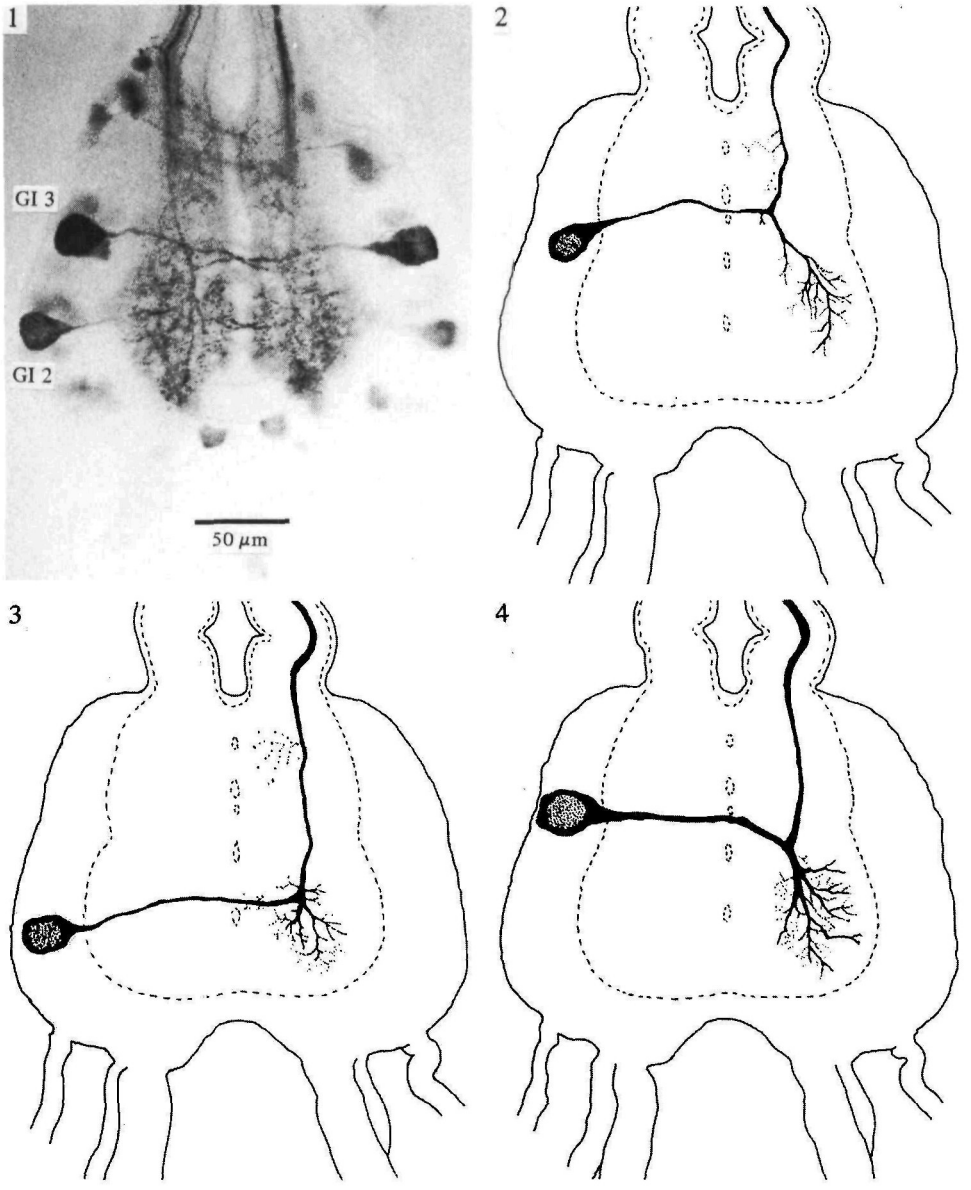
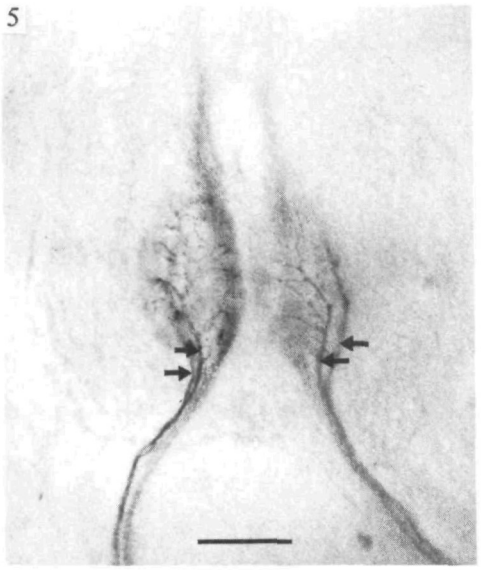
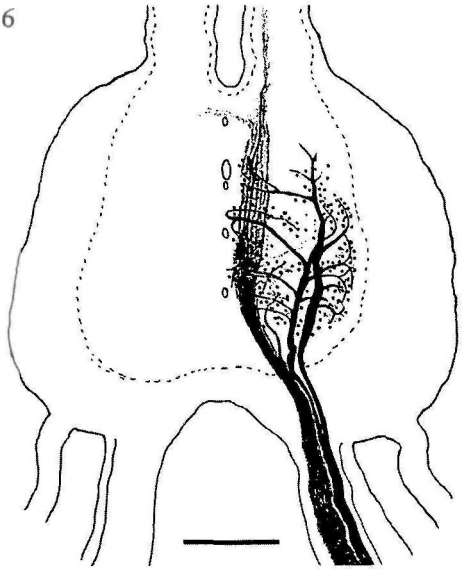


Fig. 1. Photomicrograph of a whole mount of the sixth abdominal ganglion from a 1-day-old nymph after overnight cobalt backfilling from the thoracic connectives. The neurites and dendritic arborization of the giant interneurones contain particulate cobalt sulphide. The cell bodies of GI 2 and 3 can be seen but those of GI 1 are out of the plane of focus. The cell bodies of GI 4–7 are also lightly stained.

Figs. 2–4. Camera lucida tracings of cobalt-filled, ventral giant interneurones GI 1, GI 2 and GI 3.





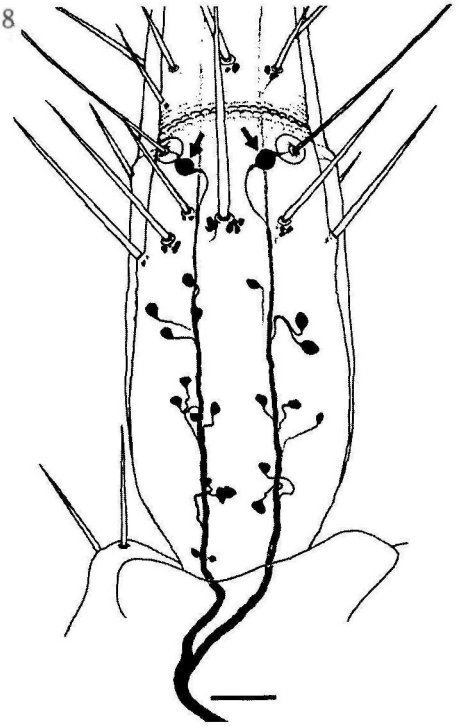


Fig. 5. Photograph of fibres filled from the cut cercal nerve 11. The two large axons from each nerve are arrowed. Scale: 50 μm.

Fig. 6. Camera lucida tracing of a specimen similar to that in Fig. 5. Scale: 50 μm.

Fig. 7. Scanning electron micrograph of a 2-day-old nymphal cercus showing the insertion of one of the two filiform hairs (arrow). The third segment of the cercus has been removed. Scale: 50 μm.

Fig. 8. Camera lucida tracing of cercus with cells filled with cobalt from nerves 10 and 11 (ventral view). The large bipolar neurones innervating the two filiform hairs are arrowed. Scale: 50 μm.

The cell bodies of the giant interneurones apparently corresponding to GI 1 and GI 3 lie close together, just anterior to nerve 8 (nomenclature of Roeder, Tozian & Weiant, 1960) and have diameters of 18 μm and 25 μm respectively (Figs. 2, 4). The smaller cell body lies just posterior and dorsal to the larger. The interneurone corresponding to the adult GI 2 has a cell body 20 μm in diameter situated 50 μm posterior and slightly dorsal to the other two cell bodies. The giant interneurone axons enter the ventral half of the connective, that of GI 3 being most ventral, and that of GI 1 lying closest to the midline. The approximate axon diameters are: GI 1, 3 μm; GI 2, 4 μm; GI 3, 6 μm. Further evidence for the equivalence of these interneurones with those in the adult was provided by the neurite shape and primary dendrite configuration. The neurites of all three neurones extend contralaterally and form a characteristic T-junction from which the axon projects anteriorly and the dendrites ramify in a well-defined synaptic glomerulus (Fig. 1). The GI 3 neurite exhibits a distinct posterior-directed curvature before it divides. GI 1 has a single primary dendrite with fine branches occupying the lateral portion of the synaptic glomerulus (Fig. 2). GI 3 dendrites occupy much of this area and arise from one or two main branches, whereas GI 2 has several primary dendrites and less extensive ramifications in the posterior part of the glomerulus (Fig. 3). The particulate precipitate (Fig. 1) did not permit unequivocal resolution of anterior branched axon collaterals or of fine dendrites arising from neurites near the midline.

Cobalt fills of afferent fibres

Cobalt fills of the cut cercal nerve 11 produced a picture of axonal projections similar to that in the adult (Daley *et al.* 1981), consisting of axons ramifying in a posterior synaptic glomerulus with longer, medial, axon tracts running anteriorly; with the major difference that the majority of the nerve 11 input is supplied by only two large axons (Fig. 5). These axons attain a maximum diameter of 7 μm, dimensions comparable to those of the first instar giant interneurone axons, and project anteriorly with numerous side branches curving dorsally around the lateral margins of the glomerulus. These terminate in globular structures approximately 2 μm in diameter, probably representing synaptic boutons (Fig. 6). The medial axon tract contains an indeterminate number of small axons (Fig. 6), some of which apparently extend to the anterior margins of the neuropile and a few still further into the interganglionic connective and fifth abdominal ganglion. No cell bodies were detected in any of the nerve 11 cobalt fills, indicating that the axons described above are not from motoneurones.

Cercal morphology and cobalt fills of cercal sensory neurones

The cercus of the first instar nymph is approximately 600 μm in length and consists of three segments, the first of which bears two filiform or thread hairs (Sihler, 1924), 350 μm long, and six bristle hairs of various sizes on the ventral surface, and five small bristle hairs dorsally (Fig. 7). The second segment bears thirteen or fourteen bristle hairs and the third, fifteen. SEM examinations shows that each filiform hair originates from a cuticular cupola (Fig. 7) similar to those on the adult cercus (Gnatzy, 1976). Cobalt diffusion from the cut ends of nerve 11 stained single bipolar neurones 20–30 μm in diameter at the base of each filiform hair, which give rise to axons that join the ipsilateral branch of the cercal nerve (Fig. 8). The cobalt precipitate also revealed

small neurones associated with the base of each bristle hair, possibly representing the positions of campaniform sensilla, and neurones in the first cercal segment that apparently do not innervate any visible cuticular structure (Fig. 8). Axons stained by these cobalt fills were too narrow to be resolved individually, preventing identification of the two large cercal afferent axons described above.

Origins of the large cercal axons

Four large axons could be seen in toluidene blue-stained 1 μm sections of Araldite-embedded terminal ganglia (Fig. 9) and their uniquely large size (up to 7 μm in diameter) and distinctive branching pattern indicated that they correspond to the large cercal axons seen in cobalt backfills of the ganglion. Ultrathin sections of the cercal nerve 11 taken every 20 μm, showed that the axons could be traced back to the base of the cercus where, at a diameter of 3 μm, they were more than twice the diameter of any other cercal afferent. The nerve then divides with one large axon in each branch (Fig. 10), the axons diminish to 0·7 μm in diameter and can be recognized only by their position in the nerve and by the glial envelope which persists along their length (Fig. 10). Each axon separates from the other afferents and, surrounded by glial and perineurial cells, can be traced to a neuronal cell body approximately 25 μm in diameter situated 250 μm from the base of the cercus (Fig. 11*a*). The cell body is surrounded by glial cells with characteristic glial evaginations or trophospongia invading the cytoplasm and gives rise to a dendrite 10 μm in length which innervates one of the filiform hairs (Fig. 11*b*). The dendrite consists of outer and inner segments divided by a constricted region containing a microtubular ciliary body, and the outer segment contains a distal tubular body and is surrounded by an electron-dense sheath (Fig. 11*b*). These features all correspond to those described by Gnatzy (1976) in the filiform hair sensillum of the adult *P. americana*.

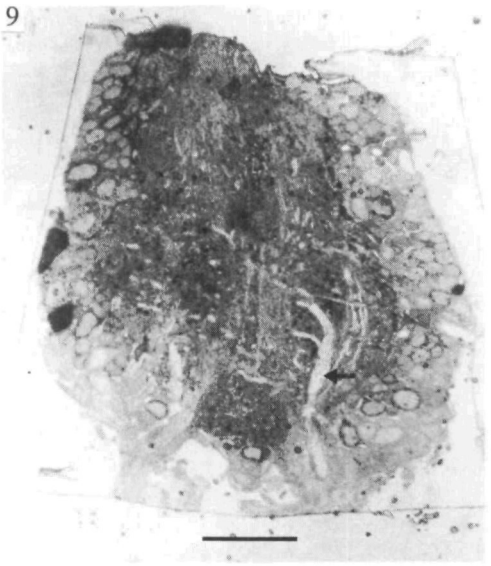
DISCUSSION

Cobalt diffusion along the axons of giant interneurones in the terminal ganglion of the first instar cockroach stains cells morphologically comparable to GI 1, 2 and 3 in the adult (Harrow *et al.* 1980; Daley *et al.* 1981) and it was inferred that these are the same neurones at an earlier developmental stage. Other neurones whose cell bodies took up the stain possibly include the dorsal giant interneurones GI 4–7. Silver intensification (Bacon & Altman, 1977) was not used because up to 14 cells contained small amounts of precipitate and intensification would have resulted in a confused picture of dendritic branching. The giant interneurones are smaller in the hatchling

Fig. 9. 1 μm horizontal section of a resin embedded ganglion showing a large axon entering via nerve 11 (arrow). Scale: 50 μm.

Fig. 10. Electron micrograph of the medial branch of nerve 11 in the cercal lumen. The large axon shown in Fig. 9 can be identified (*a*), although reduced in size, by its many mitochondria and glial enclosures. Scale 5 μm.

Fig. 11. Electron micrographs of the wind receptor cell. (*a*) The cell body (*wr*) with its nucleus (*n*), axonal process (*a*) and characteristic glial fingers, or trophospongia, invading the neuronal cytoplasm (arrow). The dendritic process (arrow) from the wind receptor cell (*wr*) containing a tubular body (*t*) and surrounded by an electron-dense sheath (*s*). The dendrite innervates the filiform hair (*f*). Scale: 5 μm.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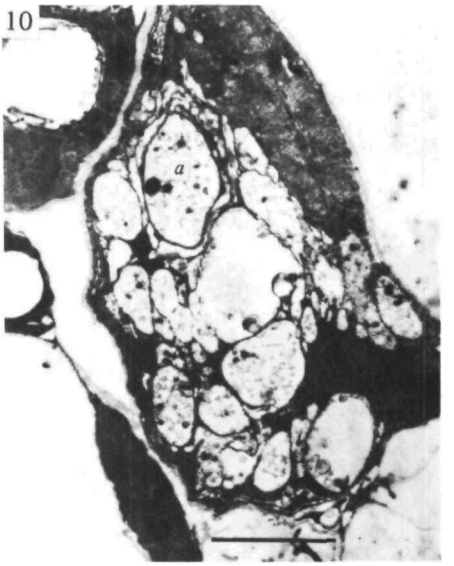
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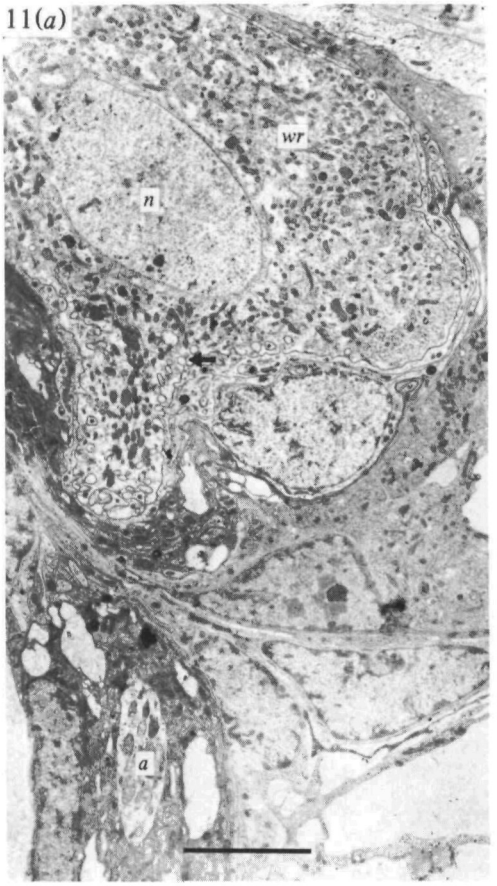
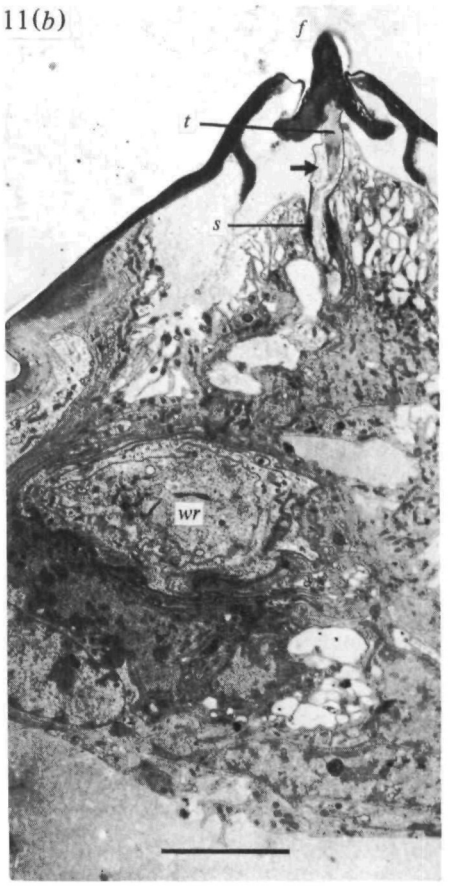

11(a)
wr
n
a


11(b)
f
t
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than in the adult (GI 3 cell body is 25 μm in diameter compared to 50 μm) but the ganglion itself is smaller, being 260 μm long compared to 670 μm. The size ratio of GI 3: ganglion is 1 : 10 in the hatchling and 1 : 12 in the adult indicating that allometric growth has taken place during postembryonic development, with a growth constant (Huxley, 1932) of 0·73.

Serial reconstruction and cobalt backfilling of the cercal nerve 11 show that the two filiform or thread hairs (Sihler, 1924) on the cercus are innervated by single bipolar neurones, ultrastructurally similar to those described in the adult (Gnatzy, 1976) which gave rise to axons that can be traced to the terminal ganglion. These axons enlarge from 0·7 μm in diameter in the cercus to 7 μm in the ganglion, then branch and apparently form synaptic boutons. It is not known why the tenfold increase in diameter occurs but it has been noted (J. M. Blagburn, unpublished electronmicroscope observations) that morphological synapses are formed by the enlarged shafts of the axons. Studies of adult cercal afferent morphology have not reported any axons of large diameter (Harrow, 1981; Daley *et al.* 1981) and it is likely that as more filiform hairs develop in subsequent instars the two original axons increase in length at the expense of a further increase in diameter. The medial tracts of small axons in the terminal ganglion probably originate from cercal sensory neurones innervating bristle hairs (trichoid sensilla) and campaniform sensilla (Harrow, 1981). Those cercal neurones that are not associated with any visible cuticular structure may innervate sensilla formed in later instars.

The escape response of the first instar cockroach is almost as efficient as that of the adult (Camhi, 1980) and although there is no direct physiological evidence it is likely that the filiform hairs and associated bipolar neurones serve the same function in the first instar as the physiologically characterized wind receptors in the adult (Dagan & Camhi, 1979). The two wind receptor axons terminate in the ipsilateral posterior synaptic glomerulus, overlapping the dendritic fields of GI 1, 2 and 3 (and possibly also GI 4–7) and the existence of the escape response suggests that functional synapses between them are present in the first instar but additional electrophysiological and microanatomical evidence is necessary to confirm this.

Information is available on the types and numbers of sensilla on the adult cercus and on the projections of afferent axons in the terminal ganglion (Harrow, 1981; Daley *et al.* 1981) but the multitude of these axons makes the study of single identified sensory neurones difficult, although Nicklaus (1965), Westin, Langberg & Camhi (1977), and Dagan & Camhi (1979) succeeded in recording from physiologically characterized receptor axons. The cell bodies and axons of giant interneurones 1, 2 and 3 and the wind receptor axons are all visible in the living hatchling ganglion when viewed with Nomarski interference optics (D. B. Sattelle, personal communication) and it seems likely that single, easily identifiable cells on both the pre- and postsynaptic sides of the wind receptor-giant interneurone synapse may be amenable to investigation by conventional electrophysiological techniques.

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