COCKROACH GIANT INTERNEURONES STAINED BY COBALT-BACKFILLING OF DISSECTED AXONS

BY I. D. HARROW,* B. HUE,† M. PELHATE‡ AND D. B. SATTELLE‡

*Dept. of Zoology, Cambridge University, Downing Street, Cambridge, CB2 3EJ., U.K.
†Laboratoire de Physiologie, Faculté de Médecine, Université d’Angers, Angers 49000, France.
‡A.R.C. Unit, Dept. of Zoology, Cambridge University, Downing Street, Cambridge CB2 3EJ., U.K.

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Detailed neuroanatomical information is an essential prerequisite for functional analysis of specific neurones in the central nervous system. In this investigation we have combined the dissection of single axons with cobalt-backfilling to reveal the structural organisation of a class of interganglionic giant interneurones in the central nervous system of the cockroach, Periplaneta americana (L). Each giant interneurone possesses a cell body, neurite and dendrites within the sixth abdominal ganglion and also has a giant axon which ascends the ventral nerve cord (Harris & Smyth, 1971). Cereal sensory neurones provide monosynaptic input to at least some giant interneurones (Callec et al., 1974). Furthermore the cereal afferent, giant interneurone pathway has proved to be a useful system for quantitative pharmacology of putative cholinergic synapses (Callec & Sattelle, 1973; Callec, 1974).

A preparation consisting of the cereal nerves, the abdominal ganglia and their interganglionic connectives was excised from an adult male cockroach and transferred to a drop of saline on a microscope slide. A single giant axon was located and dissected under a binocular microscope (× 150 magnification), as previously described (Pichon & Callec, 1970; Hue, Pelhate & Chanelet, 1978), from one of the paired connectives linking either the fourth and fifth abdominal ganglia or the fifth and sixth abdominal ganglia. The dissected preparation was removed to a perspex chamber in which the cereal nerves and sixth abdominal ganglion were located in a compartment containing physiological saline of the following composition (in mM): NaCl, 120; CaCl₂, 2·0; MgCl₂, 2·0; KCl, 8·0; KHCO₃, 2·5; KH₂PO₄, 0·2; K₂HPO₄, 1·8; tris HCl, 28·8; tris base, 3·1; trehalose, 10·0; pH 7·15; 330 m-osmol. The isolated axon was immersed in a paraffin-oil channel which separated the saline from a compartment containing 2–5 % (w/v) cobaltous chloride in which the remainder of the abdominal nerve cord was submerged.

The rate of cobalt penetration into those elements of a giant interneurone located in the sixth abdominal ganglion was influenced by temperature and applied current. For example, backfilling in preparations dissected midway between the fifth and sixth
abdominal ganglia typically required the application of a 50 nA current for 8 hr. to fill the finer dendritic branches (Fig. 1d, e). In all cases, following the cobalt backfill the tissues were transferred to fresh physiological saline. A few drops of ammonium sulphide were added and the preparation was left for 5 min to produce a black precipitate of cobaltous sulphide. The stained tissue was fixed in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.2) for at least one hour and then dehydrated in a series of alcohols. The preparation was finally cleared in methyl salicylate and mounted in Canada balsam. Block silver-intensification (modified from the procedure of Bacon and Altman, 1977, but using 5% sucrose in the developer base) was performed on some preparations to enhance detail of the dendrites. Stained interneurones were photographed with a Zeiss photomicroscope and drawn with the aid of an attached drawing tube (Zeiss).

Each stained giant interneurone was identified by serially sectioning the paraffin-embedded nerve cords and locating the position of the cobalt-filled giant axon either in the connectives or in the fifth abdominal ganglion. Harris & Smyth (1971) originally devised a simple nomenclature for giant interneurones (GI) based on the characteristic positions of four giant axons (GI 1-4) forming a ventral group in transverse sections of the nerve cord. Camhi (1976) extended this nomenclature to include the three giant axons (GI 5-7) which constitute the dorsal group (Fig. id). The present study is confined to GIs 1-3 and the results are derived from 66 interneurones (20 GI 1, 25 GI 2, 21 GI 3). A typical camera lucida representation of each giant interneurone is presented in Fig. 1(b-d).

The cell body of each giant interneurone lies contralateral to its axon on the lateral edge of the ganglion (Fig. 1). The cell bodies of GI 1 and GI 3 are positioned anterior to nerve 8 (nomenclature of Roeder, Tozian & Weiant, 1960) and lie in close proximity to each other. The cell body of GI 1 lies just dorsal to that of GI 3. The cell body of GI 2 is situated posterior to nerve 8 and occupies the most dorsal position of the giant interneurone cell bodies. A neurite projects from each cell body and crosses to the opposite side of the ganglion. Numerous fine processes branch from each neurite in the contralateral half of the ganglion and near the midline. The neurites of GI 1 and GI 2 are relatively straight whereas the neurite of GI 3 has a slight but distinct curvature (Fig. 1d, e).

GI 1 possesses two primary dendrites whereas GI 2 has many primary dendrites. In GI 3 by contrast only one primary dendrite is detected. From these primary dendrites, fine branches are seen to ramify through the neuropil. In the anterior region of the sixth abdominal ganglion the ascending axons of GI 1 and GI 2 each give rise to a branched axon collateral which projects towards the midline of the ganglion (Fig. 1b, c). No branched axon collateral has been observed in preparations of GI 3, even when intentionally over-intensified and de-intensified (Pitman, 1979). We conclude that each of the three cockroach giant interneurones (GI 1-3) investigated has unique morphological features. Cell body position, neurite shape, dendritic branching pattern and the presence or absence of an axon collateral provide anatomical criteria for identifying giant interneurones.

The single-axon backfill technique allows selective staining of individual giant interneurones and in this respect differs from previous cobalt backfill results (Mendenhall & Murphey, 1974; Fuller & Vent, 1976). Our results compare favourably
Fig. 1. Morphology of three giant interneurones of the cockroach (*Periplaneta americana*, L). (a) Camera lucida drawing of a section through the fifth abdominal ganglion showing the relative positions of giant axons. (b–d) Camera lucida representations of silver-intensified cobalt-backfilled, single, giant interneurones in the sixth abdominal ganglion: (b) GI 1, (c) GI 2, (d) GI 3. (e) Photograph of the sixth abdominal ganglion containing the same cobalt sulphide stained GI 3 prior to intensification (d).
with those obtained by microelectrode injection of cobalt (Pitman, Tweedle & Cohen, 1973; N. Vardi & J. M. Camhi, personal communication). Cobalt-backfilling of dissected axons has recently been used to stain four other cockroach giant interneurones which have smaller (20 µm diameter) axons (Harrow, unpublished observations). Therefore, it should be possible to use the technique on other neurones with similar axon diameters, which are found in many other animals. Neuroanatomical studies using the method described here complement directly physiological and pharmacological studies of synaptic transmission using the oil-gap, single-fibre recording technique (Callec, 1974). For example, cobalt-backfilling a giant interneurone following pharmacological experiments has enabled us to demonstrate that α-bungarotoxin (10^-8 M) completely blocks the unitary excitatory postsynaptic potentials generated by the deflection of a single cercal mechanoreceptor and recorded from GI3 (Harrow et al., 1979). Thus we are now able to ascribe physiological and pharmacological findings to anatomically identified cockroach giant interneurones.

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