Preliminary Investigations on a Pair of Giant Fibers in the Central Nervous System of Dipteran Flies

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This paper describes some of the salient features of the pair of giant fibers (GF) which link the subesophageal neuropile with the ventral ganglion via the cervical connective. The GF were first described by Power [1] in *Drosophila melanogaster* and their presence in that species is confirmed in this report. In *Calliphora erythrocephala* and *Musca domestica* we have also been able to identify fibers which because of their size and topographical location may be considered to be homologous to the GF of Power. It is hoped that the larger size of the latter species will render them more amenable to electrophysiological investigations. Special attention will be given to a description of the branching pattern of the GF within the ventral ganglion of *M. domestica*.

The branching patterns were determined primarily by iontophoretic injection of Procion Yellow dye using suction electrodes (modification of a technique of Iles and Mulloney [2]). Injection of cobalt chloride (Pitman *et al.* [3]) was also used but with less success. Tissue was fixed in a paraformaldehyde-glutaraldehyde mixture (Karnovsky [4]), dehydrated in an ethanol series and embedded in Spurr's medium (Spurr [5]).

Sections, twenty microns thick, were cut and examined using either fluorescence or Nomarski interference contrast microscopy. EM preparations were post-osmified and cut at about 600 Å. Contrast was enhanced by treating the sections with uranyl acetate and lead citrate.

Cross-sections through the cervical connectives of the three species of flies can be seen in Fig. 1 **. In *M. domestica* and *D. melanogaster* the GF are always found just beneath the dorsal surface of the connective whereas in *C. erythrocephala* they are usually more ventrally situated, one member of the pair often more so than the other. The GF of *M. domestica* have the largest absolute diameter in the connective of all the species observed. In the particular specimen of *D. melanogaster* shown in Fig. 1 one of the GF shows evidence of partial degeneration, the cause of which is unknown.

Some pertinent figures comparing the three species examined are found in the following Table:

	D. melanogaster ***	*M. domestica*	*C. erythrocephala*
Mean diameter of giant fibers [μm]	6	15	10
Mean diameter of cervical connective [μm]	35	80	110
Approximate number of fibers in the cervical connective	3600	5600	8000
Mean body weight [mg]	1	20	50

The GF enter the ventral ganglion dorsally and medially, then course ventrally and somewhat laterally. At location I (Figs 2 a, b, c ****) small collateral fibers branch medially to partially encircle members of bundles of vertically oriented fibers.

At location II in Figs 2 a and c the GF branches into two segments, one of which courses laterally, caudally and ventrally, passing directly through and ending in the mesothoracic neuromere. The terminal branching pattern of this lateral segment has only been partially elucidated.

In EM preparations, contact with numerous small diameter fibers can be observed throughout much of the length of the lateral segment. These points of contact are characterized by the presence of T-shaped ribbons as well as numerous vesicles (diameter 200 to 400 Å) within the small fibers (Fig. 2 d). The ribbons themselves have the same morphological characteristics as those previously described at other points of apparent synaptic contact in various Dipterans. The GF contacts however, often involve as many as four or five such ribbons closely aggregated on a dense osmiophilic band (300 to 400 Å thick) along the membrane of one small diameter fiber. For a more complete discussion of and references to this type of synapse see

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*** For more precise quantitative data concerning the fibers in the cervical connective of *Drosophila melanogaster* see R. Hengstenberg, Z. Naturforsch. **28 c**, 593 [1973].

** Fig. 1 see Table on page 784 a.

**** Figs 2 a–d see Table on page 784 b.

Boschek [6, 7]. For various reasons the fiber containing the ribbon has generally been considered to be the presynaptic member. This would imply that at all points of contact to the GF thus far observed the small diameter fibers are presynaptic and the GF are postsynaptic.

The medial segment at location II branches a number of times and several of these small diameter fibers cross the midline of the ganglion to enter the contralateral side. A number of contacts which are morphologically similar to those observed on the lateral segment have been found on the medial segment. Here again the GF appears to be the postsynaptic element.

Perhaps one of the most interesting assertions made by Power in his study of *D. melanogaster* is that each GF sends a lateral branch through the ipsilateral posterior dorsal mesothoracic nerve to directly innervate the large tergotrochanteral "jump" muscles of the mesothoracic legs. After following the branches of the GF and tracing the fibers of Procion Yellow injected posterior dorsal mesothoracic nerves we must conclude that this is not the case in *M. domestica*. Our results are consistent with the conclusion of Mulloney [8] based on electrophysiological evidence from *C. erythrocephala*, that at least one synapse must exist between fibers in the cervical connective and the motor input to the tergotrochanteral muscle.

At present it would appear that the GF is a large interneuron which integrates a variety of synaptic input, with its output as yet unidentified. That output as well as the course and synaptology of the GF in the subesophageal neuropile is currently under investigation.

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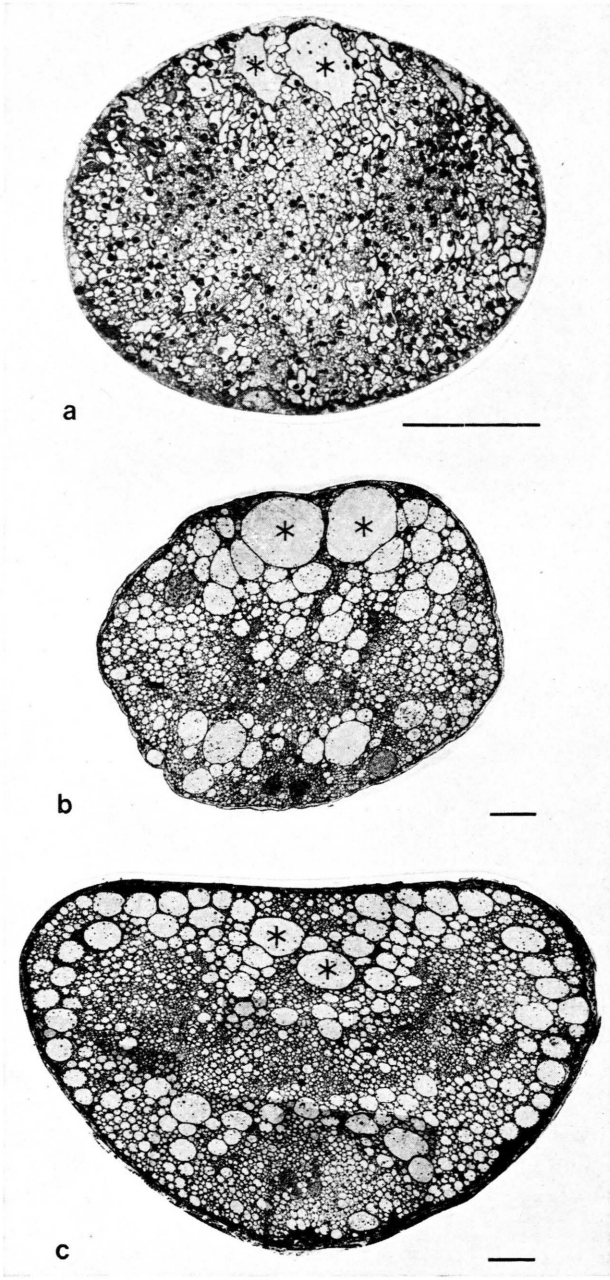

Fig. 1. Electron micrographs of cross-sections through the cervical connectives approximately midway between the subesophageal neuropile and the cervical nerve. a. *Drosophila melanogaster.* × 1800. b. *Musca domestica.* × 600. c. *Calliphora erythrocephala.* × 600. In each case the paired giant fibers are marked with stars. The scale markers indicate 10 μm.

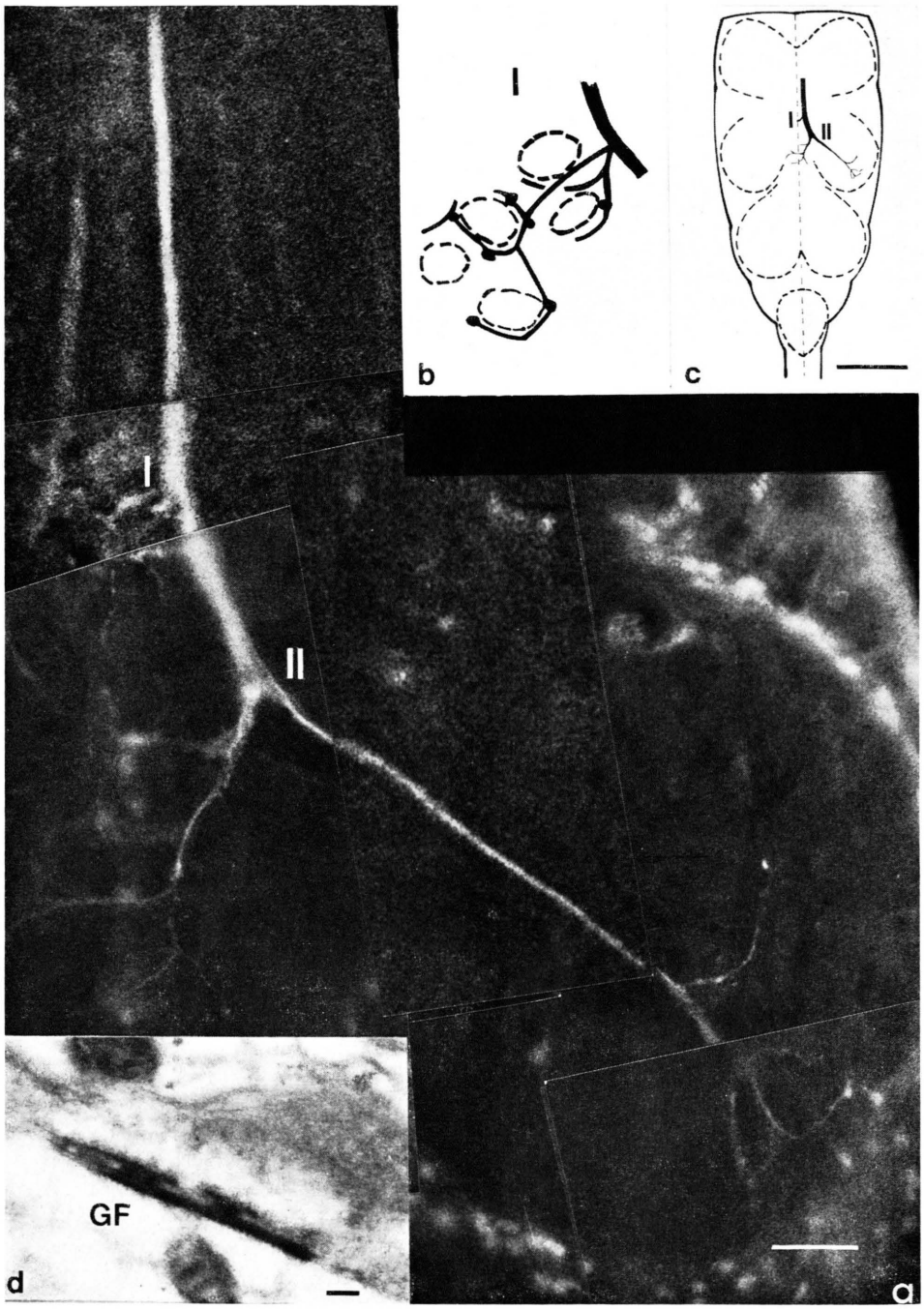

Fig. 2. a. Montage of fluorescence micrographs of a giant fiber which was injected with Procion Yellow dye. The scale marker indicates 10 μm. b. Detailed reconstruction of the small collateral branch found at location I in a and c. The broken circles indicate the position of cross-sections of vertically oriented fibers. Not to scale. c. Outline of a horizontal section through the ventral ganglion showing the position of one of the paired giant fibers. Location of the neuromeres is indicated by broken lines. Scale marker indicates 100 μm. d. Electron micrograph of T-shaped ribbons within small fibers at points of contact with the lateral segment of the giant fibers. The scale marker indicates 0.1 μm. $\times$ 45 000.