The structure of the unpaired ventral nerves in the blowfly larva

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With 2 plates (figs. 2 and 3)

Summary

The anatomy and histology of the unpaired ventral nerves are described from light- and electron-microscopical studies. Seven unpaired nerves arise from the median dorsal surface of the ventral ganglionic nerve-mass, and terminate in abdominal segments 2 to 8 respectively. Each unpaired nerve bifurcates, sending a lateral nerve to both sides of its respective segment. Subsequently the lateral nerve gives rise to 2 nerve-branches. The first branch runs to a multipolar neurone that is associated with the tracheal system, and the second branch passes beneath the ventral longitudinal muscles and terminates on a ventral transverse muscle. Structurally, the unpaired nerve consists of axons ensheathed by Schwann-cells, the cytoplasmic processes of 2 giant Schwann-cells forming the entire neurilemma sheath of the nerve and its branches. A basement membrane, or connective-tissue sheath, about 2 μ thick surrounds the Schwann-cells. Four nerve-fibres are found in the unpaired nerve; 2 of these bifurcate, sending a fibre into both lateral nerves, but the other 2 fibres pass without dividing, into the left and right lateral nerves respectively, making a total of 3 fibres in both lateral nerves. One fibre from the lateral nerve enters the nerve-branch leading to the multipolar neurone associated with the tracheal system, and eventually joins its cell-body. This fibre is the axon of the neurone. The remaining 2 fibres in the lateral nerve pass into the nerve-branch that runs to the ventral transverse muscle. A comparison is made between the unpaired nerves of the blowfly larva and those in other insects.

Introduction

In a recent paper on the sensory nervous system of the blowfly larva (Osborne, 1963), certain multipolar neurones were described which have connexions with the unpaired ventral nerves. At that time there was no reason to suppose that the unpaired nerves were any different, histologically, from the segmental nerves. It came as a surprise, therefore, when Whitten (1963) concluded that the unpaired nerves, which she refers to as the 'dorsal nerves', are not true nerves composed of axons with their attendant Schwann-cells, but giant cells which she alleges have a neurosecretory function at the onset of pupation. Consequently it was decided to investigate the structure of the unpaired nerves with light- and electron-microscopical techniques. The results of these studies are described in the present paper.

Material and methods

The unpaired nerves from 3rd instar larvae of Phormia terrae-novae were used for this work. Some were stained supravitally with methylene blue by [Quart. J. micr. Sci., Vol. 105, pt. 3, pp. 325-329, 1964.]
means of a previously described technique (Osborne and Finlayson, 1962), while others were fixed in 0.6% K_MnO_4 (Luft, 1956) for electron-microscopical investigation. Fixation was carried out at 0° C for 1/2 to 1 1/2 h, and the nerves were subsequently embedded in epon (Luft, 1961). Sections were cut on the Huxley ultramicrotome, mounted on copper grids covered with a carbon-stabilized formvar film, and examined in the Akashi 'Tronscope' TRS 50 E1.

Results

Seven unpaired nerves arise from the median dorsal surface of the ventral nerve-mass, one nerve with its associated branches terminating in each of the abdominal segments 2 to 8 (Whitten, 1963). The nerves bifurcate at progressively greater distances from the central nervous system, so that each nerve sends a lateral nerve-branch to both sides of the segment that it innervates. The lateral nerve runs alongside its corresponding segmental nerve until it reaches the ventral longitudinal muscles, where it divides into 2 branches (fig. 1, A). One branch follows approximately the course of the intersegmental fold, and runs to the multipolar neurone that is attached to the

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**Fig. 1.** A, diagram to show the lateral nerve and the terminations of its branches in the left side of the 6th abdominal segment of a Phormia larva. ax, axon of multipolar neurone; ln, lateral nerve; mn, multipolar neurone; stc, stigmatic cord; tim, transverse intersegmental muscle; uvn, unpaired ventral nerve; vln, ventral longitudinal muscles; vtm, ventral transverse muscle.

B is a diagram showing the course of the nerve-fibres at the point of bifurcation of the unpaired nerve.
posterior edge of the transverse intersegmental muscle, and the other branch passes below the ventral longitudinal muscles to terminate on a ventral transverse muscle (fig. 1, A).

Although the unpaired nerves proved difficult to stain with methylene blue, successful preparations clearly show that nerve-fibres are present (fig. 2, C, E, F). Four nerve-fibres are found in the unpaired nerve, and at the point where it divides, 2 of the fibres bifurcate and send a fibre into both lateral nerves. However, the remaining 2 fibres do not bifurcate, and these run into the left and right lateral nerves respectively (figs. 1, B; 2, F). Consequently, there are 3 nerve-fibres in each lateral nerve, one of which enters the nerve-branch leading to the neurone and ultimately fuses with the cell-body (fig. 1, A), while the other 2 fibres in the lateral nerve pass into the nerve-branch that terminates on the ventral transverse muscle.

Each neurone has 3 main processes, 2 dendrites and the axon (figs. 1, A; 2, B). One of the dendrites runs along the edge of the transverse intersegmental muscle and eventually innervates a strand of connective-tissue, the so-called stigmatic cord, which anchors the main lateral tracheal trunk to the body wall (fig. 1, A). The 2nd dendrite runs into a motor nerve that is associated with the ventral musculature. In an earlier description (Osborne, 1963), the neurone was referred to as cell mm, and it was suggested that its axon might be the fibre which enters the motor nerve, but in the light of the present work there is little doubt that the axon is the fibre that runs into the lateral branch of the unpaired nerve.

Methylene blue, in some preparations, stained the connective-tissue fibres in the nerve-sheath (fig. 2, C, E). Similar fibres are also found in the segmental nerves (fig. 2, D).

Material examined in the electron-microscope proved conclusively that the unpaired nerves contain axons (figs. 2, A; 3). The numbers of axons counted confirmed the observations made with methylene blue, namely that there are 4 fibres in the unpaired nerve and 3 in each lateral nerve. The axons average about 1 µ in diameter and contain mitochondria and neurofibrils (figs. 2, A; 3). Two giant Schwann-cells, whose nuclei are situated at or near to the point of bifurcation of the unpaired nerve, form the neurilemma sheath along the entire length of the nerve and its branches. The invaginated plasma membrane of the Schwann-cell does not form complicated wrappings and spirals round the axons as in the ordinary peripheral nerves of insects (Edwards and others, 1958a, b; Hess, 1958; Edwards, 1959, 1960). Immediately surrounding the Schwann-cell is a layer of basement membrane material, or connective tissue, about 2 µ in thickness. Electron-dense areas in the connective-tissue sheath (fig. 3) are the connective-tissue fibres that stain so readily with methylene blue.

**Discussion**

It is evident that the unpaired nerves of the blowfly larva are 'true nerves' comprising axons ensheathed by Schwann-cells. This is in complete
disagreement with Whitten's (1963) suggestion that they are peripherally disposed giant neurosecretory cells. The 'giant cells' which Whitten refers to are in fact Schwann-cells. Therefore, as Schwann-cells are not neurosecretory in function, there remains the question of the interconnecting 'secretory channels' which she describes and figures. These look so like the connective-tissue fibres which are abundant in the nerve-sheath that the most likely explanation is that Whitten has mistaken connective-tissue fibres for secretory channels. Of course it is possible that at certain times in the life history of the blowfly the axons in the unpaired nerve and its branches function as neurosecretory pathways. Johnson (1962), for instance, by means of specific neurosecretory stains, has shown in aphids that neurosecretory material passes along the axons in the sympathetic nerves that run from the corpora cardiaca to the hind-gut. Whitten (1963) did not use these staining techniques in her work. Therefore it still remains to be established whether the unpaired nerves in the blowfly larva subserv a neurosecretory function.

The unpaired nerves of the blowfly larva do not innervate spiracular closer muscles as do the unpaired nerves of other insects (Beckel, 1956; Case, 1957; Hoyle, 1959). However, the nerves in the blowfly larva are so similar topographically to the unpaired nerves described in other insects that there seems to be little reason for not regarding them as homologous structures. Moreover, Escherich (1902) has shown that unpaired nerves are present in the embryo of the muscid fly, *Lucilia caesar*, and further that they develop in exactly the same way as do the unpaired nerves of other insects (Snodgrass, 1935).

Case (1957) has reported that there are 2 motor axons in the unpaired nerve of the cockroach, and that these bifurcate at the point of division of the unpaired nerve to provide a common double motor innervation for the 2 spiracular closer muscles in each segment. Therefore, by homology, it is probable that the 2 axons which bifurcate at the division of the unpaired nerve of the

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**Fig. 2 (plate).**

A, electron-micrograph showing the cytoplasmic inclusions of an axon (*ax*) from the unpaired nerve. They are mitochondria (*m*) and neurofibrils (*nf*). *Sc*, Schwann-cell.

B, methylene blue preparation of the neurone (*mn*) that is attached to the posterior edge of the transverse intersegmental muscle. The axon (*ax*) enters the lateral nerve; one dendrite (*di*) runs along the edge of the muscle and innervates the stigmatic cord (see fig. 1, A), and the other dendrite (*d2*) eventually enters a motor nerve. The preparation is from left side of 6th abdominal segment.

C is a photomicrograph of part of the lateral nerve. One axon (*ax*) has stained and also the connective-tissue fibres (*cf*). Methylene blue.

D, methylene blue preparation of a portion of a segmental nerve. Note the connective-tissue fibres (*cf*) in the nerve sheath.

E, point of division of lateral nerve (*ln*) into 2 branches. One branch (*bmn*) runs to the multipolar neurone, whose axon (*ax*) is seen to enter this nerve. The other branch (*brm*) is destined to terminate on the ventral transverse muscle. Note the connective-tissue fibres (*cf*). Methylene blue.

F, methylene blue preparation of the unpaired nerve (*uvn*) in the region where it bifurcates to form the lateral nerves (*ln*). There are 4 nerve-fibres in the unpaired nerve, but in this preparation only 3 fibres have stained. Note that only one of the fibres bifurcates sending a fibre into both lateral nerves. The other 2 fibres pass into the left and right lateral nerves respectively.
Fig. 2

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FIG. 3

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blowfly larva are motor axons, and that they furnish the common motor supply for the 2 ventral transverse muscles. The other nerve-fibres in the unpaired nerve which do not bifurcate are most likely the axons of the sensory neurones.

Electrical stimulation of the unpaired nerves results in closure of the spiracles (Case, 1957; Hoyle, 1959). Further, it has been shown (Beckel and Schneiderman, 1957; Hoyle, 1960) that CO₂ acts directly on the spiracular closer muscle, causing it to relax even if the unpaired nerve is simultaneously stimulated. Thus opening of the spiracle under high CO₂ tensions does not necessarily involve a reflex mechanism initiated by the excitation of CO₂-sensitive receptors, as has been proposed by Case (1956, 1957) and Beckel and Schneiderman (1957). Histological investigations on the unpaired nerves and spiracular closer muscles (Beckel and Schneiderman, 1957; Case, 1957) have failed to reveal peripheral neurones which may be sensitive to CO₂. Zawarzin (1924) described sensory fibres in the unpaired nerves of the dragonfly larva, but did not trace them to peripheral neurones. It was thus of interest to find, in the blowfly larva, a sensory neurone with at least one of its dendrites associated with the tracheal system, and its axon running into the unpaired

erve.

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References

— 1958b. Ibid., 4, 251.
— 1960. Ibid., 4, 63.

Fig. 3 (plate). Electron-micrograph of a transverse section of the unpaired nerve. It contains 4 axons (ax) surrounded by the Schwann-cell (Sc). Immediately outside the Schwann-cell sheath is the basement membrane, or connective-tissue sheath, in which are embedded the connective-tissue fibrils (cf). The inset shows these fibrils at higher magnification. m, mitochondria; nf, neurofibrils.