Degeneration and Regeneration in the Insect Central Nervous System. II

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Received June 16, 1969

Summary. 1. When the neck connective of Sphodromantis is cut in two places posterior to the suboesophageal ganglion and anterior to the prothoracic ganglion, 100% of the axons in the isolated piece of tissue are degenerating by the second day after injury, and the connective is phagocytosed.

2. In contrast, if a neck connective of Schistocerca or Sphodromantis is cut once between the ganglia, only 2% of the axons degenerate rapidly, the remainder show non-degenerative reactive changes.

3. Approximately 2% of the reactive axons contain osmiophilic granules 30—100 m\(\mu\) in diameter which are not seen in undamaged axons. This granular material is also present in regenerating axons up to one week after injury. Neurosecretory-stains colour axons in the connective. These axons have a distribution and frequency corresponding to those found in electronmicrographs to contain the granular material.

4. It is suggested that the granular secretion is produced in response to injury in some axons which are connected to their perikaryon. The material may act as an inhibitor to phagocytosis and to axon degeneration, and possibly also stimulate axon regeneration.

5. The results and hypothesis are discussed in relation to previous work.

Key-Words: Insect: Nerve — Secretion — Regeneration — Degeneration.

Recent experimental work shows that when arthropod axons are cut, swift degeneration does not always occur in the portion separated from the perikaryon. When one neck connective of Schistocerca is cut, approximately half the axons on each side of the lesion are presumed to be severed from their cell bodies, but up to 23 days after injury, only 2% of the axons show signs of degeneration; the remainder show reactive morphological changes, and regeneration from both connective stumps occurs after a week. In the transganglionic connectives (pro/mesothoracic) there is no morphological change from normal in either the axons or glial cytoplasm (Boulton 1 in press). Rowell and Dorey (1967) cut one metathoracic/abdominal connective in Schistocerca and ten days later found no numerical loss in the axons anterior or posterior to the lesion. Melamed and Trujillo-Cenoz (1963) could find no degenerative changes during the first four days after injury in the sectioned connectives of Laplatacris which would distinguish between the distal and proximal portions of the axon. Crayfish motor axons will remain excitable for an hundred days after they have been severed from their cell bodies before degeneration takes place (Hoy, Bittner and Kennedy, 1967). Hess (1958 c) suggested that two lateral giant axons in the ventral nerve cord of Periplaneta americana are syncytial, as the axons showed no degenerative change on either side of the cut. Recent work by Farley and Milburn

* Supported by a “Study and Serve” grant from the British Government, and a grant from the Worshipful Company of Goldsmiths.
(1969) shows that all the giant axons have their origin in the last abdominal ganglion, but vary in the rate at which degeneration occurs after sectioning the nerve cord.

There are four possible explanations for the longevity of apparent enucleated axons. (a) The neuron nucleus is not essential for the short term physiological activities of the axon. (b) The axons form a syncytium with cell bodies in more than one ganglion. (c) Axon degeneration is slow, and not apparent until many days after injury. (d) Degeneration of enucleated axons is prevented by interaction with nucleated cells.

(a) Non-nucleated cells, such as mammalian erythrocytes are incapable of growth or division, and have a limited range of metabolic activities. Experimental enucleation has clearly demonstrated the longterm dependence of cells on the nucleus — the concept of neurons being indefinitely independent of a nucleus is implausible, but from recent experiments, it appears that invertebrate neurons can survive and function adequately for some time without a nucleus. (b) Although giant axons in Periplaneta may be syncytial, (Hess, 1958c; Farley and Milburn, 1969), the overall evidence from electrophysiological and anatomical work denies the existence of a syncytial arrangement in the arthropod nervous system. Landolt (1965) showed the number of nuclei in the corpora pedunculata of Formica to be one tenth to one fiftieth of the number of axons. Coggeshall (1967) has estimated that there are a total 25,000 axons and 2,000 cell bodies in Aplysia. Jacklet and Cohen (1967) identified neuron cell bodies of damaged motor axons in the metathoracic ganglion of Periplaneta americana and found one cell body per neuron. Staining of neuron cell bodies and their axons, whether by methylene blue, reduced silver Golgi impregnation, or the more recently developed technique of thiazol yellow injection, have all shown single cell bodies in each neuron.

(c) Results presented in this paper show that if all the axons in a connective are isolated from their cell bodies, by cutting the connective immediately anterior to the prothoracic ganglion, and posterior to the subesophageal ganglion, all axons degenerate by Day 2. This contrasts with the morphology of the connective cut once between adjacent ganglia — here there is a limited invasion by phagocytes which engulf injured glial cells, but only 2% of the axons degenerate. Thus, rapid axon degeneration occurs in the connective when there is no communication with any of the axon nuclei.

(d) By exclusion, it seems possible that nucleated axons influence the enucleated axons after the connective is cut. Direct parasitism by axons deprived of their nucleus is unlikely, but they could be influenced by secretion from nucleated axons. Dense osmiophilic granules, (never seen in normal connectives) are described in this paper. They appear after injury in some axons, and may represent such secretions.

Two distinct, but inseparable events occur in the isolated connective. There is complete degeneration of the axons and glial cytoplasm, and invasion of the connective by phagocytes. A secretion may have a passive function, preventing phagocytosis of the enucleated axons, or may influence the axons actively, and enable them to survive and function without a nucleus.