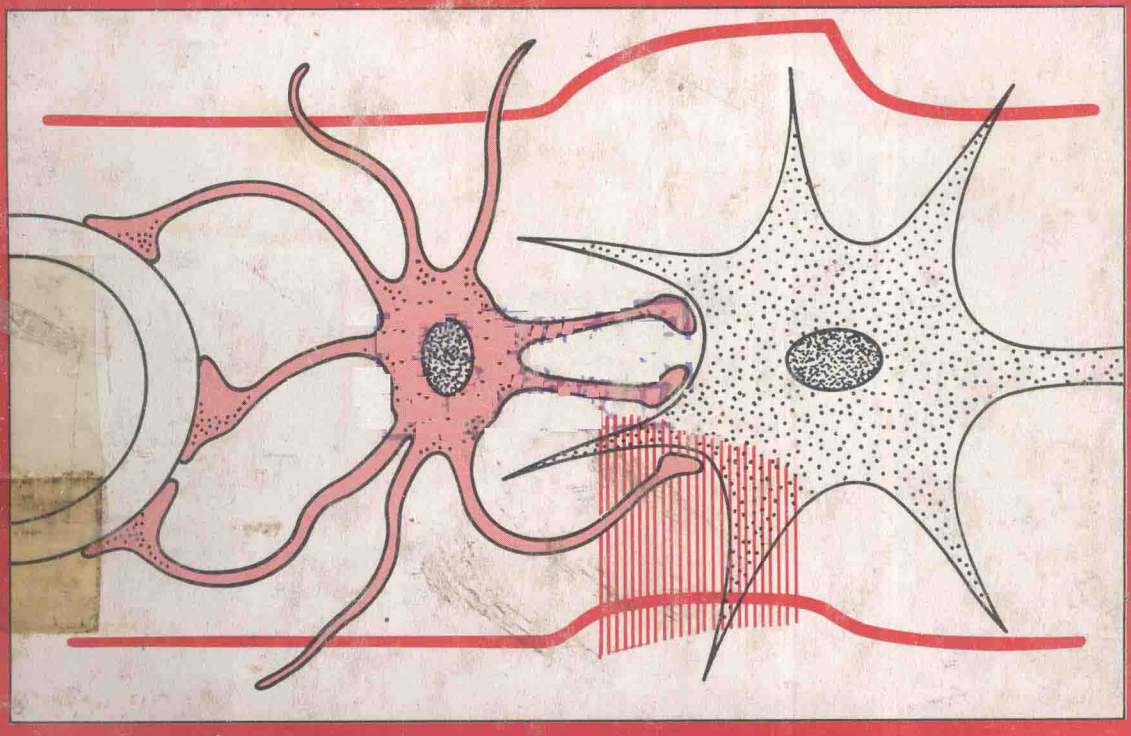
Glial-neurone interactions

J.E.Treherne



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PREFACE

This volume deals with what was until recently a neglected subject. Thus, although glial cells were discovered nearly a century and a half ago it is only during the past decade and a half that any concerted attempts have been made to understand their functional role. Even these are insignificant when compared with current research on the physiology of nerve cells which (as shown by a recent computer search of the literature) outstrips that on neuroglia by about 12 to 1. The concentration of research on the conducting elements of the nervous system is, of course, inevitable. However, it is indisputable that an adequate understanding of the functioning of those elements must incorporate knowledge of their interactions with the neuroglia that delimit the brain microenvironment and constitute half of the volume of the brain.

The papers contained in this volume are based on the third Company of Biologists Ltd. Discussion Meeting which was held in April 1981 at Titisee in the Black Forest. They cover a wide range of topics. These include various aspects of the structure of neuroglia, their role in blood–brain barrier systems, in the control of the ionic composition of the neuronal environment, the metabolic interactions between glial cells and neurones, the role of neuroglia in transmitter inactivation, in myelin synthesis and in neuronal differentiation and growth. The contributions provide authoritative accounts of recent advances in these topics and, in addition, indicate the potential and likely direction for future research.

I am grateful to the friends and colleagues who travelled to the Black Forest to create the stimulating and enjoyable meeting on which this volume is based. I am particularly indebted to Herr Dr Hasso Schroeder of Karl Thomae GmbH for once again collaborating with the Company of Biologists Ltd. in financing the Discussion Meeting and for his many kindnesses to the participants. At what other meetings are the afternoon deliberations enlivened by the arrival of a file of waiters bearing champagne? Finally, it is a pleasure to acknowledge the help of Mrs M. V. Clements for her invaluable assistance in organising the meeting.

I am indebted to Academic Press, the American Medical Association, American Physiological Society, American Society of Biological Chemists, Cambridge University Press, Chapman & Hall, Elsevier/North-Holland Biomedical Press, the Massachusetts Institute of Technology, Rockefeller University Press and John Wiley & Sons Inc. for granting permission to reproduce text illustrations.

J. E. TREHERNE

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INTRODUCTION

PERSPECTIVES ON THE CELL BIOLOGY OF GLIA

By JOHN NICHOLLS

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From the vantage point of 1981, advances in our understanding of glial cells over the last 20 years seem at once significant, provocative and high in promise. It is sobering to remember that at that time speculation about their role included a spectrum of romantic possibilities, from blood–brain barrier to memory, from constituting the total extracellular space of the brain to playing a key role in learning, from being essential for the neuronal action potential to producing impulses themselves, from ferrying vital materials into and out of neurones to having no function at all other than support. Unfortunately, the ratio of speculation to sound experimental observations was so high that, apart from continuing contributions from anatomists, to be interested in glia was almost disreputable, somewhat akin to dabbling in parapsychology or memory transfer.

A major change in emphasis was the approach of Stephen Kuffler and David Potter which dealt with the cell biology of glia. The starting point for their experiments in about 1961 was the frustration of trying to teach medical students neurobiology without mentioning the cells that made up the bulk of the brain. After an extensive search for a suitable preparation they chose a simple invertebrate, the leech, to ask questions such as: how did the membranes of glial cells compare to those of neurones, did they have resting potentials and give impulses, what ions were their membranes permeable to, what ions did they contain, were they required for neurones to give impulses, and what electrical and ionic interactions did take place between the two types of cell? These and similar down-to-earth experiments set the stage for Sidney Goldring's studies of glial cells in the mammalian brain, where they turned out to be strikingly similar in their membrane properties to those in the lowly leech. In its own way the paper by Kuffler and Potter in 1964 therefore served as watershed, changing dramatically the nature of the work on glia.

The papers at this symposium cover a far broader scope, showing progress in novel, unexpected directions, but again with emphasis on glial and Schwann cells as cells, with characteristic structures, junctions, relationships and interactions with neurones, in the normal nervous system, during development and in disease.

One key problem today concerns the morphology of membrane specializations and the distinctions between the various types of glial cells and Schwann cells. Freeze fracture has now revealed membrane particles distinctive for astrocytic glial cells located at specific sites in relation to blood vessels (p. 35). Immunological (p. 215) and enzymological techniques also offer high promise for establishing how oligodendrocytes, astrocytes and Schwann cells and invertebrate satellite cells can be distinguished chemically. It will be of considerable interest to understand the functional

roles played by the various marker proteins (p. 167) as well as the fundamental interrelationships between the various types of glial cells. In spite of much additional information about the various junctions that may allow for coupling and for barrier functions (p. 7), it is somewhat surprising that we do not yet know just what part is played by electrical coupling between glial cells or even whether oligodendrocytes are coupled to astrocytes. And, apart from paranodal regions in myelinated nerve, what specialized structures or junctions (if any) occur between neurones and glia?

Since the 1960s, major progress has been made in our knowledge of the fluid environment of the CNS (p. 129) and the contribution of glial cells. Their membranes have now been shown to pump potassium (p. 49) and in insects to play a crucial role in determining the ionic environment of the neurones (p. 61). This homeostatic function is reinforced by glial blood–brain systems in the insect nervous system (pp. 7, 61) and (as described at the meeting by Joan Abbott) in animals such as the cuttlefish (*Sepia*) it is the glia rather than endothelial cells which constitute the barrier that limits exchange of large molecules (such as peroxidase) between the blood and extracellular fluid. A major incentive for studying glial properties has been the interest in potassium accumulation caused by physiological stimuli. As Kelly and Van Essen first showed, an oligodendrocyte situated in a column of the cat visual cortex can 'respond' selectively to a bar of light shone on to one particular part of the visual field with one particular orientation. Thus, it recognizes one specific visual stimulus. But what then? In certain instances there is evidence that the potassium acts as a trigger for metabolic effects (pp. 49, 75) and it has also been shown that potassium accumulation can affect synaptic transmission (p. 93) or such phenomena as spreading depression (p. 111). Yet even today there is still no conclusive quantitative answer to the question of how important the role of glia is in redistributing potassium at various sites within the brain that contain different populations of glial cells and neurones with different geometry.

Closely related are the findings of Kelly and Currie, David Brown and others on transmitters. Non-neuronal cells clearly need to be considered in relation to transmitter uptake in which they could play a quantitatively important part (p. 181). In the case of the squid giant axon acetylcholine has been implicated by Villegas in a series of events as a factor released by Schwann cells (p. 135). as at degenerated neuromuscular junctions of the frog. There, Miledi and his colleagues demonstrated that release of acetylcholine from Schwann cells gives rise to miniature endplate potentials in the denervated muscle.

In this same system of squid giant axon and Schwann cells the transfer of specific proteins including actin has been clearly demonstrated (p. 153). Indeed, such protein transfer can also occur between various types of neurones, as for example in the mammalian visual system after radioactive amino acids have been injected into the eye. A tantalizing question is whether sufficient quantities are transferred for those proteins to play a role in normal neuronal function.

It is perhaps in relation to development that among the most provocative new vistas have been opened. Starting with Mains and Patterson's work, it is now known that non-neuronal cells, including glia, can in culture release factors that profoundly influence the type of transmitter to be synthesized by a neurone (p. 195). Specific

factors within the brain regulate the division of Schwann cells and astrocytes (p. 215); and in *Aplysia* during development glial cells may play a role in regulating division by neurones (p. 205).

The extensive series of experiments by Aguayo (p. 231) and his colleagues bear on two other important properties of glial cells and Schwann cells. One, not represented at this meeting, is the importance of myelin in speeding conduction; the other concerns the role of Schwann cells and glial cells in regeneration. That glial cells in the developing mammalian brain can act as guides for neurones to grow to their destination has been shown by Rakic and his colleagues. The use of nerve transplants and grafts by Aguayo now indicates that regeneration of the adult CNS may be far more practicable and extensive than had previously been thought. Conduits can be formed by Schwann cells that allow damaged central neurones to grow for long distances. It will be of considerable interest to know whether such neurones form synaptic connexions and, if so, whether they can be used to restore function. Other work by Aguayo and by Brockes and their colleagues bears on the key problem of the signals required for myelination to occur (pp. 215, 231).

From these considerations, one is struck by the new avenues that have been explored, the hard evidence now available about aspects of glial and Schwann cell properties and the testable new speculations that have evolved. At the same time there is the challenge of certain major unanswered questions. We are still quite ignorant about glial cells in demyelinating diseases such as multiple sclerosis; it is still not yet known whether the primary lesion is in the neurones or the oligodendrocytes. What does seem appealing is that highly complex problems may now become more approachable with the advent of structural, immunological and biochemical techniques, together with the extensive basic information about the cell biology of glial cells and Schwann cells.

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INVERTEBRATE NEUROGLIA–JUNCTIONAL STRUCTURE AND DEVELOPMENT

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SUMMARY

The morphological characteristics of the neuroglial cells of invertebrates are reviewed, including the ultrastructural and enzyme cytochemical features of their cell bodies and attenuated cytoplasmic processes, the various ways they ensheath the nerve cells, including the loosely myelinated condition, their modifications due to intraganglionic localization and their interactions with other glial cells in the form of homocellular junctions. The spectrum of heterocellular axo-glial associations that occur in invertebrates is considered with particular reference to the different kinds of intramembranous organization they exhibit as revealed by freeze–fracture. Recent studies on glial cell development in a range of arthropods, during embryonic and pupal stages, reveal the importance of glial cell tight junctions in forming the tracer-excluding blood–brain barrier. These occluding junctions are now shown to be, in some cases, vertebrate-like in their complexity. The stages in their assembly, which may be concurrent with those of gap junction formation, reveal a number of differences from vertebrate glia. During metamorphosis, glial cells dissociate and the dynamics of the concomitant interglial junctional disruption and their intramembranous particle dispersal without apparent internalization, as well as their subsequent reassembly, are examined. The stimuli triggering these glial events and the physiological significance of the various glial modifications are considered.

I. INTRODUCTION

The glial cells in the invertebrates are a varied assemblage, and, unlike those of vertebrates, have thus far defied attempts to categorize them in any rigorous way; there is little that is consistent about their distribution and morphology in different organisms to enable neurobiologists to make definitive classifications. On the other hand, some differences in cytological features and topographical arrangements between different types of neuroglial cell are discernible, and glia have for example, been subdivided into plasmatic, fibrous, perineurial and Schwann-like categories (Radojcic & Pentreath, 1979), although this classification, as with any others put forward, suffers from a number of limitations. Reviews on glial cells in the tissues of invertebrates include specialized reports (Nicaise, 1973) and more generalized accounts (Clayton, 1932; Roots, 1978; Radojcic & Pentreath, 1979; Varon & Somjen, 1979; Treherne, 1980; Lane & Treherne, 1980).

Neuroglia occur in the higher invertebtates, such as Annelids, Arthropods, Molluscs and Aschelmintha, possibly are present in the Platyhelmintha, and seem to be absent

in such phyla as the Porifera and Coelenterata (Roots, 1978; Radojcic & Pentreath, 1979). The glial cells that are found in invertebrate ganglia may be loosely defined as those cells in the nervous system which are non-neural and which ensheath the neurones, ramifying between them. Inevitably, other non-nervous elements are also present, such as those of the blood or connective tissue, and mesenchymal cells or granulocytes (for example, Baskin, 1971*a*) may be difficult to distinguish from true glial cells. In some cases, particularly in early embryonic tissues, any distinction between glial and nerve cells is impossible. Later on in development, the glia become, in comparison with the nerve cells, more irregular in outline and much more attenuated (Fig. 1), and may be rather more electron opaque in thin-sections (Fig. 2). Their initial embryonic origin is not well understood although in annelids and arthropods they could be ectodermal (Roots, 1978); in the latter it is thought that they may arise from the same neuroblast cells that give rise to the nerve cells themselves (Edwards, 1980), although they might also originate from different epidermal cells (Bate, personal communication). No really convincing evidence is as yet forthcoming for either possibility.

The functions of the glial cells in invertebrates appear to be manifold. Clearly, since they encompass the neurones, they, or their intercellular adhesions, provide a degree of mechanical support and are undoutedly also protective; for example, it has been suggested that they dampen the spread of compressional forces (Baskin, 1971*b*). Where they occasionally form extensive myelin-like sheaths they may insulate and presumably speed up impulse conduction by a saltatory mechanism (Heuser & Doggenweiler, 1966; Günther, 1976). In some cases, notably in arthropods, they may form permeability barriers (Lane & Treherne, 1972*a*; Treherne, 1974; Lane, Skaer & Swales, 1977*a*; Lane, Swales & Abbott, 1977*b*; Lane & Skaer, 1980; Lane, 1981*c*). They have always been considered to have a trophic role (Holmgren, 1900; Smith, 1967) with regard to the nerve cells, providing metabolic reserves (Fahrenbach, 1976; Wolfe & Nichols, 1967), and transferring metabolites such as proteins and transmitter enzymes to the axoplasm, although the precise mechanism of this interaction is as yet poorly understood (Gainer, 1978). They may be active in the destruction of nerve cells (Bittner & Mann, 1976; Griffiths, 1979) which could be important during development where cell death of neurones occurs naturally. They may also regulate the ionic composition of the fluid bathing the nerve cells (Treherne & Pichon, 1972), restrict the extracellular space which may serve as a cation reservoir, may sequester and/or release neurotransmitters or transmitter enzymes (Orkand & Kravitz, 1971; Salpeter & Faeder, 1971; Evans, 1974; Houk & Beck, 1977), or indeed, in some cases, may synthesize them. They may also have a tactic role in guiding migrating neurones Lopresti, Macagno & Levinthal, 1973; Lane, 1979*a*; see also Edwards, 1980). The

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Fig. 1. Thin-section of axons (A) surrounded by attenuated glial processes (G) between which lies extracellular space (E) containing collagen fibrils. Crayfish (*Procambarus clarkii*) central nervous system. Arrow indicates short trans-glial channel. × 33 550.

Fig. 2. Enhanced electron density of the glial cells (G) ensheathing axons (A) and nerve cell bodies (NCB). Locust (*Schistocerca gregaria*) nervous system. × 35 000.

Fig. 3. Nerve cell bodies (NCB) and axon (A) encompassed by attenuated glial cell (G) processes which emanate from the glial cell body (GCB). E, extracellular space. Snail (*Helix aspersa*) ganglion. × 15 600.

morphological basis for these various kinds of activities and interactions are not all well established. In this chapter it is hoped to assess what is known about the diverse structures of neuroglia in the invertebrates and the avenues of approach used in the elucidation of their characteristic features.

The techniques available for the study of the structural/functional correlates in these enormously important, yet enigmatic, cells, include electrophysiological approaches and related injection procedures whereby electrodes implanted into the glial cells may fill the cells with fluorescent dyes or with tracers which render them electron opaque in thin sections (fig. 3 in Treherne & Pichon, 1981); the electrically-characterized cells are then identifiable morphologically. Conventional fixation with thin-sectioning and freeze-fracturing has yielded much of the basic information regarding glial distribution, topography, fine structure and membrane modifications. Cytochemical techniques, including immunocytochemistry, have characterized certain aspects of their enzymatic make-up and autoradiographic studies are beginning to elucidate the parameters of glial uptake, storage and glial–neuronal exchanges. Recent developments in the production of cell-type-specific antigenic markers for glial cells may facilitate their classification as well as the study of their features during differentiation.

II. CYTOLOGICAL FEATURES

(A) *Glial cell body*

The cell body of invertebrate glial cells is normally relatively small; from its nuclear region emanate many attenuated cytoplasmic processes (Fig. 3) which may extend for very considerable distances into the surrounding nervous tissue. The glial cell body proper may possess relatively little cytoplasm (Fig. 3), but usually contains endoplasmic reticulum, rough or smooth, free ribosomes, mitochondria, the occasional Golgi complex, microtubules and or filaments. In some cases storage products such as glycogen or lipid may also be found as well as glia grana or lysosomes. The latter may be numerous (Lane, 1968*a*) and of quite a considerable size (Fig. 4) containing lamellae and granules; in such cases they are often referred to as 'gliosomes' (Scharrer, 1939; Pipa, Nishioka & Bern, 1962). Cytochemical studies show, however, that they possess acid phosphatase as well as some thiamine pyrophosphatase (TPPase) and, ATPase (Lane, 1966, 1968*a*), so they do actually seem to be a form of lysosome (Lane,

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Fig. 4. Large gliosome (lysosome) in a glial cell which is lying within the substance of the neuropile. Spider (*Aranaeus*) ganglion. × 12900.

Fig. 5. Axons (A) surrounded by glial cells (G) containing massive, ordered arrays of microtubules as is typical of arthropods. Blood-sucking bug (*Rhodnius prolixus*) nerve. × 81900.

Fig. 6. Glial cells, associated by desmosomes (D) and gap junctions (GJ), exhibiting extensive bundles of intracellular filaments (F), seen cut longitudinally and in cross-section, as is typical of molluscs and annelids. Snail ganglion. × 80000.

Fig. 7. Axons (A) surrounded by glial cells (G) after fixation with colloidal lanthanum. The tracer has leaked past the limited perineurial junctions, has infiltrated the extracellular space and is being endocytosed by the glial cells as indicated by the dense omega profiles (arrows) and vesicles (thick arrows). Spider (*Aranaeus*) peripheral nerve. The insert shows a nerve cell with adjacent glial cell containing omega profile in a locust ganglion. × 57000; Insert × 58000.

1968*b*). On the other hand, the glial granules in certain molluscs are thought to be specialized for the storage of inorganic cations (Nicaise, 1973).

Gliosomes have been shown to increase in number during CNS differentiation and then to decrease after the completion of ganglionic development, in the oligochaete, *Tubifex* (Djaczenko & Cimmino, 1976); this was taken to mean that the gliosomes were important in trophic interactions between glia and neurones during differentiation. They have also been found to increase in number with age or with enhanced phagocytic activity, presumably since they are accumulated rather than extruded (Lane, unpublished data).

(B) *Attenuated glial cell processes*

The narrow glial projections spiral around axons or nerve cell bodies, and interdigitate with other glial processes, ramifying within the tissues of the nervous system extensively. These processes may be excessively thin (Fig. 3); where they are wider, cytoplasmic organelles are found and, depending on the nutritional state of the organism, glycogen and lipid.

The most striking glial components are the cytoskeletal elements and these seem to be either microtubules or filaments. The first abound in the glia of arthropods (Fig. 5) (Smith, 1968; Lane, 1974) where the latter are rarely to be seen. The reverse is true in molluscs and annelids, where bundles of filaments extend through the glial processes (Fig. 6) (see Nicaise, 1973); here microtubules are rare or non-existent. The physiological significance of this is not clear, although it has been suggested that the microtubules may be required for adequate transport of materials along glial processes given that the arthropods are active organisms with a poor vascular supply to the nervous system (Roots, 1978). This situation contrasts with that in certain vertebrate macroglia, where there is a differential distribution depending on maturity; in astrocytes, for example, filaments abound in differentiated cells but are rare in undifferentiated glia where microtubules are plentiful (Allt, 1980).

The plasma membranes of the glial cell system have the potential to form junctional complexes (see section IV) and of being modified in other ways. They are capable of endocytosing tracer material such as lanthanum (Fig. 7) or ferritin (Reinecke, 1976) by vesicular uptake, rather as horseradish peroxidase is pinocytosed into caveolae by vertebrate Schwann cells (Mugnaini *et al.* 1977); exocytosis also seems possible (Lasek, Gainer & Barker, 1977). Freeze-fracture evidence of such cytotic activity in neuroglial membranes is also available (Fig. 14, insert). In certain situations, well documented only in the crustacea, the glial membrane forms permanent endocytotic vesicles which form trans-glial channels, or a tubular lattice system (Fig. 8) (Holtzman, Freeman & Kashner, 1970; Lane & Abbott, 1975; Nordlander, Masnyi & Singer, 1975; Shivers, 1976; Shivers & Brightman, 1976) which are patent to the entry of tracers (Holtzmann *et al.* 1970; Shivers, 1976; Lane *et al.* 1977*b*). This produces a potential short-circuit route for ions and molecules in their attainment of the axonal surface. The channels also contain cholinesterase and so may be involved in neurotransmitter degradation (Holtzman *et al.* 1970).

Glial cells have been shown to contain certain putative neurotransmitters. For example, in arthropods, acetylcholine and dopamine are preferentially localized in glial processes (Houk & Beck, 1977), while glutamate (Faeder & Salpeter, 1970;

Evans, 1974) and γ-amino butyric acid (GABA) (Orkand & Kravitz, 1971) are also more concentrated in glia than neuronal cells. This may be due to their selective uptake by glial cells in situations where there is no mechanism for extracellular neurotransmitter inactivation or enzymatic degradation.

Glia plasmalemma in the invertebrates, as in vertebrates (see for example Wood *et al.* 1977*a*), are characterized by cytochemically-demonstrable ATPase (Fig. 9) (Lane, 1968*a*; Houk & Beck, 1975) as well as by alkaline phosphatase (Fernandez, 1966) and also diphosphatases. Energy-requiring regulatory mechanisms may therefore be localized in glia, such as the function of their processes as a 'K^+ sink' (Kuffler & Potter, 1964). In addition, non-specific esterase (Wigglesworth, 1958) and eserine-sensitive cholinesterase have been reported in glial cell folds around nerve perikarya in insect ganglia (Smith & Treherne, 1965) as well as in the glia of molluscs and earthworms (Nicaise, 1973). Moreover, acetylcholine receptors are found in glial membranes of squid nerve fibre (Rawlins & Villegas, 1978) so that the glial cells must be involved in some way in nervous activity.

Perhaps surprisingly, ATPase as well as thiamine pyro(di)phosphatase is also to be found in the endoplasmic reticulum (ER) of some insect glial cells (Lane, 1968*a*). The similarities in enzymatic activity of ER and plasma membrane suggests a functional interrelationship, perhaps relating to transport of substances synthesized in the glia via the extracellular spaces to the neurones (Lane, 1968*a*). Supporting this contention, acid phosphatase-rich smooth ER in glia may show continuity with extracellular sites between glia and axons in fly eyes (Griffiths, 1979); it is thought that the enzyme is exported into axons, particularly those that have been injured. These results argue for a 'destructive' role for glia as has also been proposed in crustaceans (Bittner & Mann, 1976). Moreover, recent enzyme studies on the nervous system of the larval blowfly, *Calliphora*, reveal, in preliminary cytochemical investigations, that adenyl cyclase activity is localized in the plasma membranes of the innermost perineurial cells, that is, the 'bracelet' cells (Lane & Swales, unpublished data). These are the cell borders between which are situated the gap and tight junctions that become disassembled at the onset of metamorphosis (Lane & Swales, 1978*b*, 1980) as the glial cells become separated prior to pupal reorganization. Membrane-bound adenyl cyclase splits ATP to cyclic AMP (cAMP), which is an active phosphorylating intermediate well-known to act as a secondary messenger in many cellular control systems, mediating the activity of a variety of peptide hormones (Bitensky & Gorman, 1973;

Fig. 8. Glial processes (G) with hemi-desmosomes (arrows) abutting onto the collagen-filled extracellular space (E) as is typical of crustaceans. The ad-axonal glia exhibit trans-glial channels (curved arrows and in *insert*), crossing from extracellular space to axonal (A) surface. Crayfish ganglion. × 25000; Insert, × 38000.

Fig. 9. Glial processes (G) surrounding nerve cell bodies (NCB) and exhibiting cytochemically-demonstrable ATPase in their plasmalemma as well as some vesicles (arrows), possibly endocytotic. Grasshopper (*Melanoplus differentialis*) ganglion. × 15500. From Lane (1968*a*).

Fig. 10. Glial cell (G) processes exhibiting hemi-desmosomes (HD) where they abut onto extracellular space (E) which contains a moderately electron opaque matrix, as is typical of insects. An interglial gap junction (GJ) exhibiting the reduced intercellular cleft occurs between the membranes of the glial cells ensheathing the axon (A). Note the projections from some of the glial microtubules. Cockroach (*Periplaneta americana*) abdominal nerve cord. × 102000.

Fig. 11. Thin glial (G) processes separated by electron lucent extracellular spaces possessing only slight fibrous striations. Blood-sucking bug (*Rhodnius prolixus*) ganglion. × 18600.

Berridge, 1979). It therefore seems possible, as suggested earlier (Lane & Swales, 1980), that the late larval hormones of holometabolous insects can induce the glial cell changes and related junctional modifications which occur at the commencement of pupation. If these hormones are steroids, which do not require a second internal signal, they may activate another protein hormone(s) that can then act on the glial cells via an intermediate such as cAMP. Hormonally-induced changes in glial gap junctions have been reported previously for other systems such as those of amphibians (Decker, 1976). Moreover, when isolated glial fractions from the CNS of *Manduca sexta* are treated with serotonin, an increase in cAMP levels is obtained, suggesting the activation of a glial-associated adenyl cyclase (Taylor, Dyer & Newburgh, 1976).

Glial processes are usually separated from the nerve cells or each other by a cleft of only 10–20 nm (Fig. 1) and so they restrict the immediate cellular environment. However, in certain regions of some ganglia the extracellular space may be dilated into extensive spaces or sinuses (Fig. 11) which sometimes contain a fibrous matrix. This matrix may display collagen-like fibrils (Figs. 1 and 8) as in the crustacea, or the spaces may possess deposits of an electron opaque ground substance (Fig. 10) which has been characterized histochemically in insects as hyaluronic acid (Ashhurst & Costin, 1971). In molluscs, and many other systems, the glial cells themselves are thought to elaborate the dense extracellular material (Johnston & Roots, 1972; Prior & Lipton, 1977). This extracellular matrix may be important in furnishing a pool or reservoir for substances, possibly cations, and hence may be of considerable physiological importance (Abbott & Treherne, 1977; Abbott, Pichon & Lane, 1977; Abbott, 1979).

III. TOPOGRAPHICAL AND CELLULAR DISTRIBUTION

(A) *Trophospongial processes*

The attenuated nature of the neuroglial cells in invertebrate ganglia is such that the glial folds are often almost no more than the width of their two membranes. Around the perikarya of large nerve cell bodies, glial processes often project into the peripheral cytoplasm (Fig. 12), which otherwise is not readily accessible to diffusion from the

Fig. 12. Nerve cell body (NCB) surrounded by attenuated glial cell (G) processes, some of which have invaginated into the perikaryon as trophospongia (GT). Note the sub-surface cisternae (arrows) of endoplasmic reticulum, smooth-surfaced where facing the neurolemma, lying near the glial cells. E, extracellular space. Snail ganglion. ×28500. Insert shows tannic-acid treated glial cells (G) and axons (A) where different glial cells are distinguishable by virtue of their differing electron opacity. Locust ventral nerve cord. ×17300.

Fig. 13. Glial nucleus (N) and 'loose' myelinated glial folds (G) encompassing axons (A); the glia are associated with one another by multiple desmosomal-like radial attachment zones (arrows). Prawn (*Leander serratus*) ventral nerve cord. ×7800. Insert shows giant axon (A) with which the innermost glial layer is joined by a heterocellular desmosome (thick arrow), the other glial folds (G) being held together by homocellular desmosomes (D). Earthworm (*Lumbricus terrestris*) ventral nerve cord. ×36000. From Roots & Lane (1981*a*, *b*).

Fig. 14. Freeze-fracture replica to show the 'loose' myelin, typical of some oligochaetes and crustacea, wherein multiple stacks of glial membranes (G) exhibit very little cytoplasm (C) between one another. Occasionally the intracellular filaments are seen in cross fracture (arrows). Earthworm ventral nerve cord, giant fibre. ×39000. Insert shows *en face* fracture of a glial membrane displaying many endo- or exocytotic pits. Prawn interganglionic connective. ×45000. From Roots & Lane (1981*a*, *b*).

surrounding glial cells. These structures were originally called trophospongia (Holmgren, 1900) and are also called 'canals of Holmgren', the former name implying, of course, that they are trophic with respect to the nerve cell bodies. This contention is supported by the presence of subsurface cisternae of endoplasmic reticulum which are found lying in close apposition to the trophospongial invaginations (Fig. 12). These have been thought to be the possible sites of specific metabolic exchange (Smith, 1967, 1968) as there is some evidence that directional transfer of glucose from glial to nerve cells may occur during glycogen synthesis in both insects (Wigglesworth, 1960) and the leech (Wolfe & Nicholls, 1967). It is also possible that, as proposed for vertebrate neuronal subsurface cisterns (Henkart, Landis & Reese, 1976), they may couple some intracellular activity to the electrical activity of the plasma membrane.

(B) *Concentric glial wrappings, mesaxons and loose myelin*

The glial cells in invertebrates may send one or more spiral folds around the neurone they are encompassing (Fig. 12, insert); the term tunicated is sometimes applied to this arrangement (Smith & Treherne, 1963). In other cases they form 'mesaxon' folds, which are multiple spiral ensheathments around giant axons; in most cases, however, they do not form myelin as do the vertebrate Schwann cells. The ensheathments of the invertebrate axons tend to be uncompacted so that some glial cytoplasm remains in the spiral wrappings (Fig. 12, insert).

There are a few exceptions to this generalization where a loose or pseudo-myelinated condition occurs; this involves the glial processes wrapping around axons in spiral configurations which may in some cases be so attenuated as to appear to have little or no residual cytoplasm (Fig. 13). This is to be found in the glial wrappings around the giant fibres of the earthworm (Fig. 14) (Hama, 1959; Coggeshall, 1965; Levi, Cowden & Collins, 1966; Günther, 1976; Roots & Lane, 1981*a*) and in the wrappings around most of the axons in the optic nerves and ventral nerve cord of the prawn and shrimp (Fig. 13) (Holmes, 1942; Kusano, 1966; Heuser & Doggenweiler, 1966; Hama, 1966; Doggenweiler & Heuser, 1967; Roots & Lane, 1981*b*) as well as the crab, *Cancer irroratus* (McAlear, Milburn & Chapman, 1958). The number of glial layers or lamellae is variable, from a few to several hundred, and frequently the adjacent glial membranes are associated with one another by contacts which in the

Fig. 15. Desheathed preparation with outer glial layer removed prior to 60 min incubation in 10 mM ionic lanthanum. The CNS is thereby rendered accessible to the dense tracer which has penetrated the matrix of the extracellular spaces of the glial lacunar system lying between glia, nerve cells and tracheoles (T). Cockroach ganglion. ×4000.

Fig. 16. Perineurial cells in the synthetic state actively producing and extruding collagen fibrils (at arrows). Blowfly (*Calliphora*) ganglion. ×31000. Insert shows a septate junction after incubation in 10 mM ionic lanthanum during which the tracer has penetrated its length. Locust (*Schistocerca gregaria*) ganglion. ×94000.

Fig. 17. Colloidal lanthanum impregnation into spaces between glial cells (G) in which occur gap junctions (GJ) characterized by loosely clustered particles, each of which contains a stained central channel. Garden spider ganglion. ×117000.

Fig. 18. Freeze-fracture replica of an arthropod glial–glial gap junction with Eface (EF) particles and Pface (PF) pits. Note that the particles and pits may be closely clustered (as in I) or very loosely aggregated (as at II) within one and the same junction. Locust ganglion. ×85000.