0270-6474/84/0411-2689$02.00/0

The Journal of Neuroscience
Vol. 4, No. 11, pp. 2689-2697
November 1984

GLIAL REPAIR IN AN INSECT CENTRAL NERVOUS SYSTEM: EFFECTS OF SURGICAL LESIONING[1]

J. E. TREHERNE,[2] J. B. HARRISON, J. M. TREHERNE, AND N. J. LANE

Agricultural Food Reserve Council Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, CB2 3EJ United Kingdom

Received December 27, 1983; Revised April 23, 1984; Accepted May 8, 1984

Abstract

Surgical lesioning of central nervous connectives in the cockroach (*Periplaneta americana* (L.)), although causing only local glial damage, resulted in complex and prolonged cellular changes. An early response to mechanical disruption was the appearance of granule-containing cells within the damaged perineurium, among adjacent, undamaged, perineurial cells, and between glial processes deep within the connectives. These cells, which were strikingly similar to hemocytes, were clearly involved in phagocytic activity and persisted in the damaged regions for more than a month after lesioning. There was only a slow restoration of organized perineurial glia and re-establishment of the blood-brain barrier, as indicated by the exclusion of an extracellular tracer, ionic lanthanum. These observations contrast with the speedy, ordered repair of the neuroglia observed following selective glial disruption and suggest that undamaged axons and/or the extracellular matrix exert a profound influence on the mechanisms of glial repair.

It is now increasingly recognized that the supporting cells of the brain, the neuroglia, serve a number of important physiological roles. These include metabolic interactions with neurons, homeostasis of the brain microenvironment, and transmitter inactivation and synthesis (see Treherne, 1981; Sears, 1982). Recent work has also revealed a hitherto unsuspected influence of glia in determining neuronal growth (Aguayo et al., 1981). This latter role raises the intriguing, converse, question of what determines the growth of the neuroglia themselves.

Previous work has largely concentrated on vertebrates, using two basic approaches: first, lesioning and experimental manipulations *in vivo* (e.g., Adrian and Schelper, 1981; Fugita et al., 1981), and, second, the powerful combination of cultured cell lines with monoclonal antibody and biochemical techniques (cf. Brockes et al., 1981). The first approach has suggested an involvement in neural regeneration of monocyte-derived macrophage cells (cf. Billingsley et al., 1982); the second has indicated a possible functional role of diffusible glial growth factors (cf. Brockes et al., 1980; Fontana et al., 1980). However, both of these approaches have limitations. Furthermore, the identity and origin of the "reactive" cells have not yet been resolved (see Gilmore and Walls, 1981). The daunting complexity of whole brain preparations makes it difficult to make precise electrophysiological measurements of identified components, while the results obtained from culture techniques are often difficult to relate to the reality of normal brain function.

Recent research in this laboratory has used the relatively simple nervous system of an insect, the cockroach (*Periplaneta americana* L.), to study some of the basic factors involved in the control of glial repair and regeneration. This preparation has a number of advantages: notably, relative structural simplicity, its accessibility to electrophysiological study, and the possibility of extensive experimental manipulation.

This paper, the first of a series, describes the effects on the neuroglia of cutting central nervous connectives, a situation in which glial damage is accompanied by mechanical disruption of the axons and drastic effects on the extracellular matrix. This experimental procedure contrasts with that described in the accompanying paper (Smith et al., 1984) in which only the glial cells were disrupted using a glial toxin.

This investigation is necessary because, despite much available knowledge of the structure and physiology of insect nervous systems, relatively little research has been carried out on glial damage and repair in these organisms. Apart from some earlier investigations on general structural changes (Bodenstein, 1957; Boulton, 1969; Boulton and Rowell, 1969), lesion studies have been largely confined to the investigation of neuronal damage and regeneration (Pitman and Rand, 1982; Meiri et al., 1983; Roederer and Cohen, 1983a, b; Leech and Treherene, 1984).

Materials and Methods

One of the two penultimate connectives of the abdominal nerve cord of adult cockroaches, *Periplaneta americana*, was cut, and the animals were left for varying periods before removal of the nerve cords for ultrastructural examination. Both proximal and distal segments to the cut, as well as the uncut control connective, were processed at each stage studied by fixing in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, containing 6% sucrose, postosmicated in 1% OsO_4 in phosphate buffer and sucrose and then dehydrated, after *en bloc* uranyl acetate staining, to propylene oxide. Some preparations at each stage were also incubated, prior to fixation, in physiological saline to which 10 mM ionic lanthanum had been added; these served to indicate whether the blood-brain barrier, disrupted by the initial lesioning, had undergone repair. Tissues were then embedded in Araldite, and both

[1] This research was supported in part by a grant from the United States European Research Office.

[2] To whom correspondence should be addressed.

thick and thin sections were cut and examined by light and electron microscopy. Animals were killed at 3 days, 4 days, 14 days, 21 days, 24 days, 28 days, 40 days, 110 days, and 6 months after lesioning.

In each case one of the pair of penultimate connectives of the abdominal nerve cord was severed in animals which had been immobilized by submerging them in tap water for 4 min. A flap of the integument was cut, with sterilized scissors, from the ventral abdominal surface and lifted forward to expose the penultimate connectives, one of which was cut, midway between the 4th and 5th abdominal ganglia, with freshly sterilized scissors. The flap of integument was then replaced and sealed into position with dental wax melted in an electrically heated wire loop. The animals were kept at a temperature of 28°C and fed with bran and fresh water. The uncut connective in each case served as the control.

For simplicity of description the cut connective adjacent to the 5th ganglion is referred to as the proximal stump and the other as the distal stump.

Results

Control tissues

The glial system of cockroach central nervous connectives consists, as previously described (Lane and Treherne, 1980), of a superficial layer of interdigitating cells, the perineurium, and an underlying complex of neuroglial cells whose processes ensheath the variously sized axons (Fig. 1). The perineurium is overlain by the acellular neural lamella, to which hemocytes (*H* in Fig. 1) occasionally adhere. The subperineurial glia contain, predominantly, microtubules and mitochondria, but some endoplasmic reticulum is present; lysosomes are rare. The extracellular spaces delimited by the glial and axon membranes contain an electron-dense matrix which is most clearly seen in the sporadic dilated intercellular spaces between glial processes (Fig. 1).

As shown previously (Lane and Treherne, 1972; Lane et al., 1977), the extraneously applied extracellular tracer, lanthanum chloride, penetrated into the clefts between the perineurial cells but was excluded from the underlying extracellular system (Fig. 2). This exclusion and the electrophysiological evidence of restricted intercellular access of small, water-soluble cations by the perineurium (Treherne and Schofield, 1981; Schofield and Treherne, 1984) has been postulated to be the basis of an insect blood-brain barrier and to result from the presence of occluding junctional complexes at the inner ends of the perineurial clefts (Lane and Treherne, 1970, 1972).

Cut connectives

Effects during the first 4 days. In connectives transected in saline containing ionic lanthanum, there was no appearance of the tracer within the axoplasm or in the glial cytoplasm close to the lesion. However, infiltration occurred through the damaged tissues and tracer was distributed within the intercellular glial-axonal clefts and the extracellular dilatations (Fig. 3). The extracellular system thus is not sealed after cutting and is immediately accessible to charged particles as large as those of lanthanum chloride. The stumps of the cut connectives, both proximal and distal, were still leaky to extraneously applied lanthanum 4 days after transection.

A consistent feature at this time after transection was the appearance of cells containing electron-dense granules within the damaged perineurium as well as between cells in undamaged areas of this peripheral glial layer. Such cells were also found deep within the tissues of the connectives, between axons and glial folds (Fig. 4). These cells share many of the cytological features of hemocytes, principally in the presence of a population of electron-opaque granules with relatively homogeneous contents. They also contain lysosomes of various sizes (Fig. 5, *inset*). It is not justifiable to identify these cells unequivocally as hemocytic, for they could represent transformed neuroglia with a greatly increased lysosomal content. However, more typical glia were still recognizable at this stage, but with no small opaque granules and with lysosomes that were larger and more amorphous in their outline and internal composition than characteristic granules of the hemocyte-like cells. At this stage many glial cells were clearly disorganized (Fig. 5), with cytoplasm that appeared denser than normal and projections pushing into the axoplasm.

As observed by Boulton (1969) and Meiri et al. (1983), there was an increase in the number of axoplasmic mitochondria and, in some cases, in neurosecretory granules and possibly lysosomes. There was also an enlargement in the extracellular spaces and marked changes in the appearance of the matrix material which was now separated and "pulled away" from the glial membranes. In many cases, the matrix substance was also highly fragmented (Fig. 5).

Effects after 11 to 21 days. At 11 and 14 days after cutting, there was a distinct increase in the extent of the extracellular spaces which, nevertheless, contained electron-dense matrix (Fig. 6). This could represent accumulation of newly synthesized extracellular material since it was less fragmented than earlier. At this stage and, later, there were indications that fresh matrix substance was being produced (Fig. 8) to fill the dilatations. Large, amorphous glial lysosomes were frequently encountered (Fig. 9), presumably as a result of glial phagocytic activity.

After 21 to 24 days, at the proximal cut end, the neuroglia were still loosely packed and there were areas where these cells had been very disrupted (Fig. 7); matrix material was fragmented (Fig. 7) or beginning to fill up space (Fig. 8). The severed giant axons looked normal, with axoplasm now containing more characteristic numbers of mitochondria. These axons, however, were in some cases invaded around their periphery by short (finger-like) glial processes, more extensively than usual. Some atypical thickening of the neural lamella also occurred at the tip of the proximal stump with irregular collagen alignments.

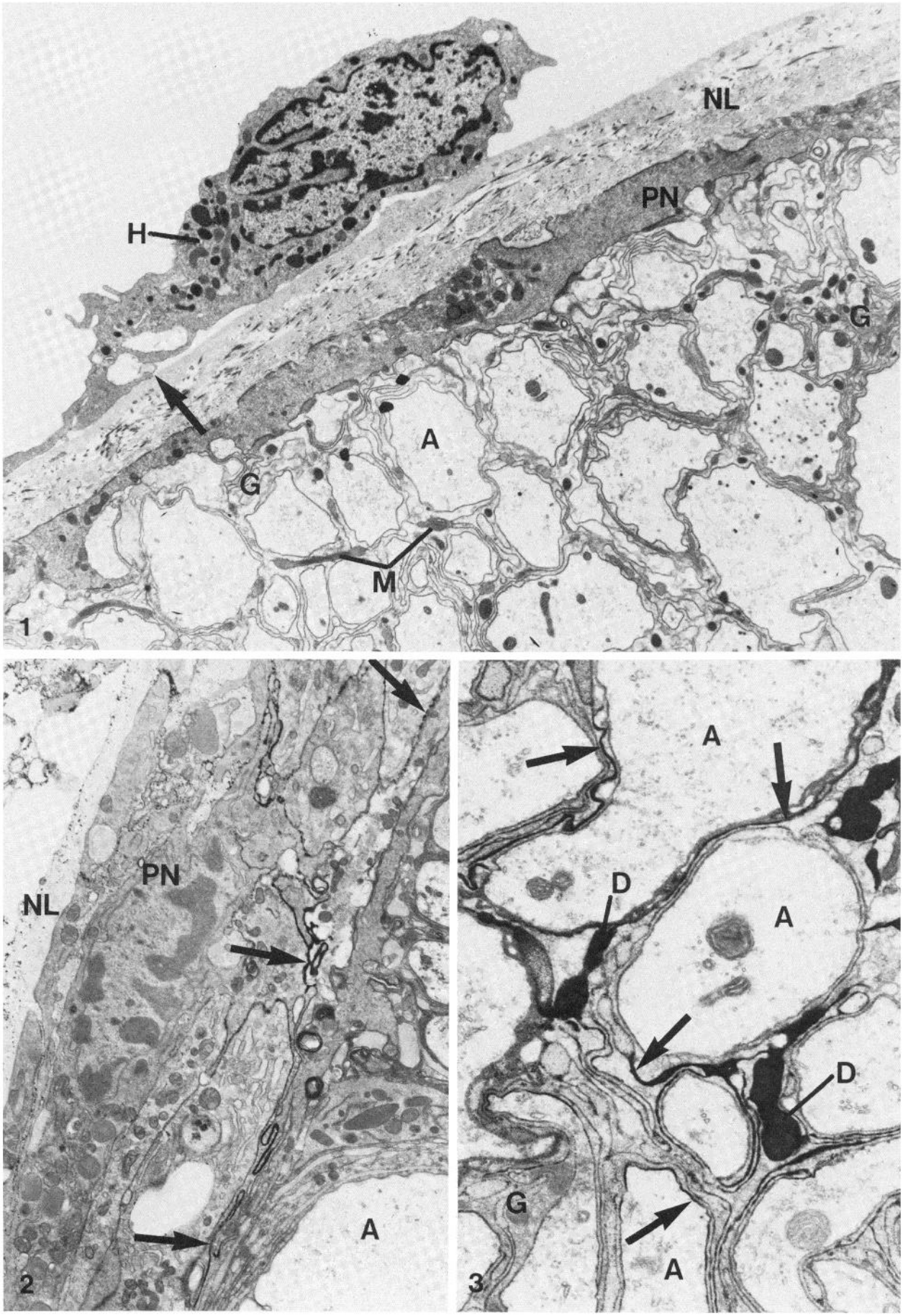
The glia and the smaller axons in the distal stump looked relatively normal at this stage, although in some preparations the giant axons had apparently collapsed. The cut stump was

[3] The abbreviations used in the figures are: *A*, axon; *D*, dilatation of the extracellular space; *ECS*, extracellular space; *F*, fibrous material; *G*, glial cell; *GJ*, gap junction; *H*, hemocyte; *L*, lysosome; *M*, matrix material within extracellular space; *MT*, microtubule; *NL*, neural lamella; *PN*, perineurium; *SJ*, septate junction; *SER*, smooth endoplasmic reticulum.

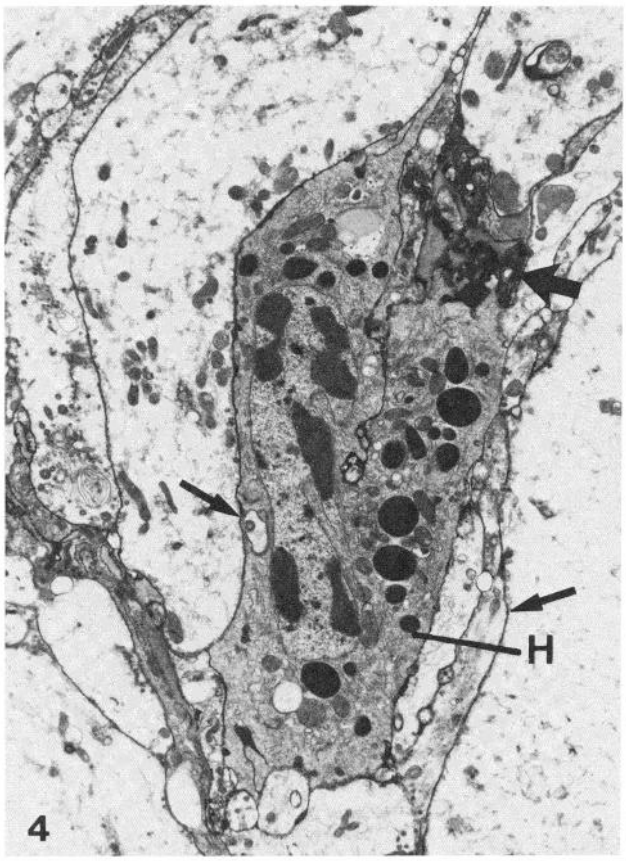
Figure 1.[3] Control preparation from an uncut control connective, 24 days after the operation in which the other of the connective pair was cut. Note that the neural lamella overlies the perineurium in the usual fashion, with underlying glial-ensheathed axons. The extracellular spaces contain dilatations filled with matrix material. On the surface of the connective is a hemocyte. These are only occasionally seen associated with the neural lamella in control connectives. No obvious morphological changes were observed in the glia or axons of control connectives following cutting of the other one. Magnification × 7,000.

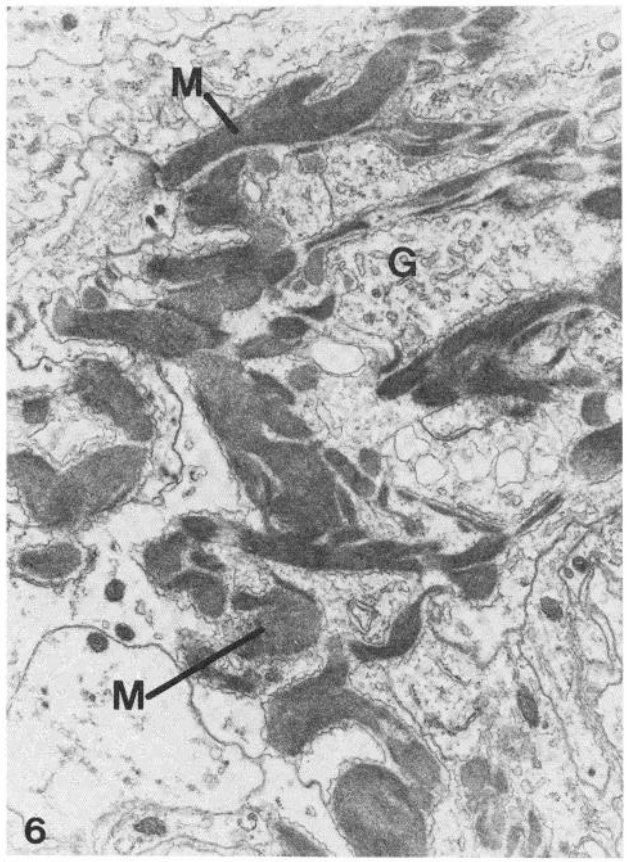
Figure 2. Control preparation, 24 days after cutting of the paired connective, after incubation for 1 hr in ionic lanthanum. Note that the dense tracer (at *arrows*) fills the perineurial clefts but does not invade the underlying nervous tissue. Magnification × 8,500.

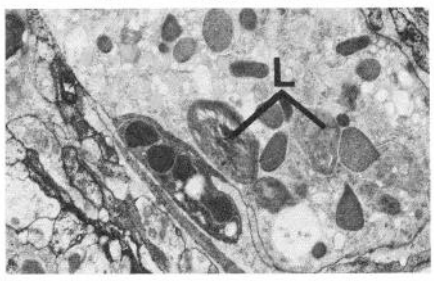
Figure 3. Cut connective, immediately incubated in ionic lanthanum for 1 hr and then fixed. The extracellular system is accessible to the exogenous tracer which can be seen (at *arrows*) filling the intercellular clefts between glia and axons, as well as binding to the matrix in the dilatations. Magnification × 24,000.



Figures 1 to 3







Figures 4 to 6

now club-shaped and the glia were loosely packed, those nearest to the tip showing clearest signs of damage (Fig. 7).

Effects after 24 to 40 days. After 24 to 40 days granule-containing cells were still present, close to the cut ends of proximal and distal stumps (Fig. 10). Some of these cells had very large lysosomes, indicative of enhanced phagocytic activity (Fig. 11). At the periphery of connectives, close to damaged regions, loose layers of cell processes had formed into what looked like reorganized perineurial glia. These processes exhibited junctional complexes, including both gap (Fig. 12) and septate junctions (Fig. 13). The neural lamella overlying these regions was relatively unstructured, containing only occasional collagen fibers (Fig. 12).

By 28 days perineurial restriction to the intercellular access of tracer molecules had not been re-established, for extraneously applied lanthanum was found to have leaked into the clefts and extracellular spaces between the axons and deeper glial processes (Fig. 14). Enlarged extracellular spaces were a prominent feature at this stage, especially the subperineurial ones which were filled with a fine, fibrous substance which could be coagulated or precipitated protein (Fig. 15).

After 40 days glial packing and extracellular spaces in the proximal stump had assumed a relatively normal appearance, and the giant axons had normal complements of mitochondria. In the distal stump, however, giant axons were no longer recognizable, and the axoplasm of the smaller ones contained atypical bodies and dense-core granules which could be neurosecretory. The surviving glial cells exhibited enhanced populations of lysosomes as if phagocytosis had occurred and, again, appeared to be involved in the production of new extracellular matrix material. The spaces between glial elements were not distended, as earlier, and septate junctions (Fig. 16) and desmosomes (not normally found between any glia other than the perineurial cells) were occasionally seen. In many cases, the glia were seen to send extensive projections into the axon peripheries, and the neural lamella surrounding the distal stump was abnormally thick.

Effects of 110 to 180 days after cutting

After 110 days the distal stump had regressed. The proximal stump, on the other hand, exhibited normal glial-axonal packing and relatively typical intercellular spaces, but with increased volume of neural lamella.

By 4 to 6 months after cutting, extraneous lanthanum did not penetrate beyond the intercellular clefts of a re-formed perineurium (Fig. 17). Very large perineurial cells were still seen in some preparations and were peculiar in possessing a fibrous component (Fig. 18) and large amounts of smooth endoplasmic reticulum (Fig. 19, *SER*); the cisternae of the latter were found mainly in close association with the subperineurial glial membranes (Fig. 19). Many microtubules were found here, lying at right angles to the underlying neuroglia (Fig. 19).

Discussion

The immediate effects of surgical lesioning on insect neuroglia are to cause local cellular damage and to disrupt the perineurial blood-brain barrier. This disruption is indicated by the extensive penetration of tracer into the extracellular system, despite the apparent sealing of the axons which do not show detectable leakage of the tracer into their axoplasm. This contrasts with the apparent accessibility of small cations indicated by the relatively rapid decline in the resting and action potentials observed in most, but not all, severed giant axons in this preparation (Meiri et al., 1981; Leech and Treherne, 1984). The exclusion of the tracer observed in our experiments suggests that the initial sealing of the axon may be a result of a selective exclusion of large, charged particles, such as those of lanthanum. The lack of penetration of the tracer into the cytoplasm of undamaged glia could result from uncoupling of the disrupted glial elements from one another, or from the inability of the tracer to pass through the low-resistance pathways provided by the gap junctions that occur between adjacent glial cells (Lane et al., 1977; Lane, 1981).

An early response to mechanical disruption is the appearance of granule-containing cells both within the damaged perineurium, among adjacent, undamaged, perineurial cells, and between glial processes deeper within the interganglionic connectives. As already emphasized, these cells bear a striking resemblance to hemocytes which were also seen adhering to, and penetrating, the neural lamella of connectives in which the neuroglia had been selectively destroyed with a glial toxin (Smith et al., 1984). Despite many obvious structural similarities with hemocytes it is not possible, at this stage, to eliminate the possibility that these cells are transformed neuroglia. The granule-containing cells are clearly involved in phagocytic activity and may be the insect equivalent of vertebrate macrophages. They often showed pseudopodia in the manner consistent within the engulfment of substances by phagocytosis, as well as possessing lysosomes that appeared to derive from phagocytic vacuoles. Phagocytosis by the granule-containing cells persisted, for such cells were found to be present, exhibiting both granules and lysosomes in the vicinity of the cut surface 1 month after lesioning.

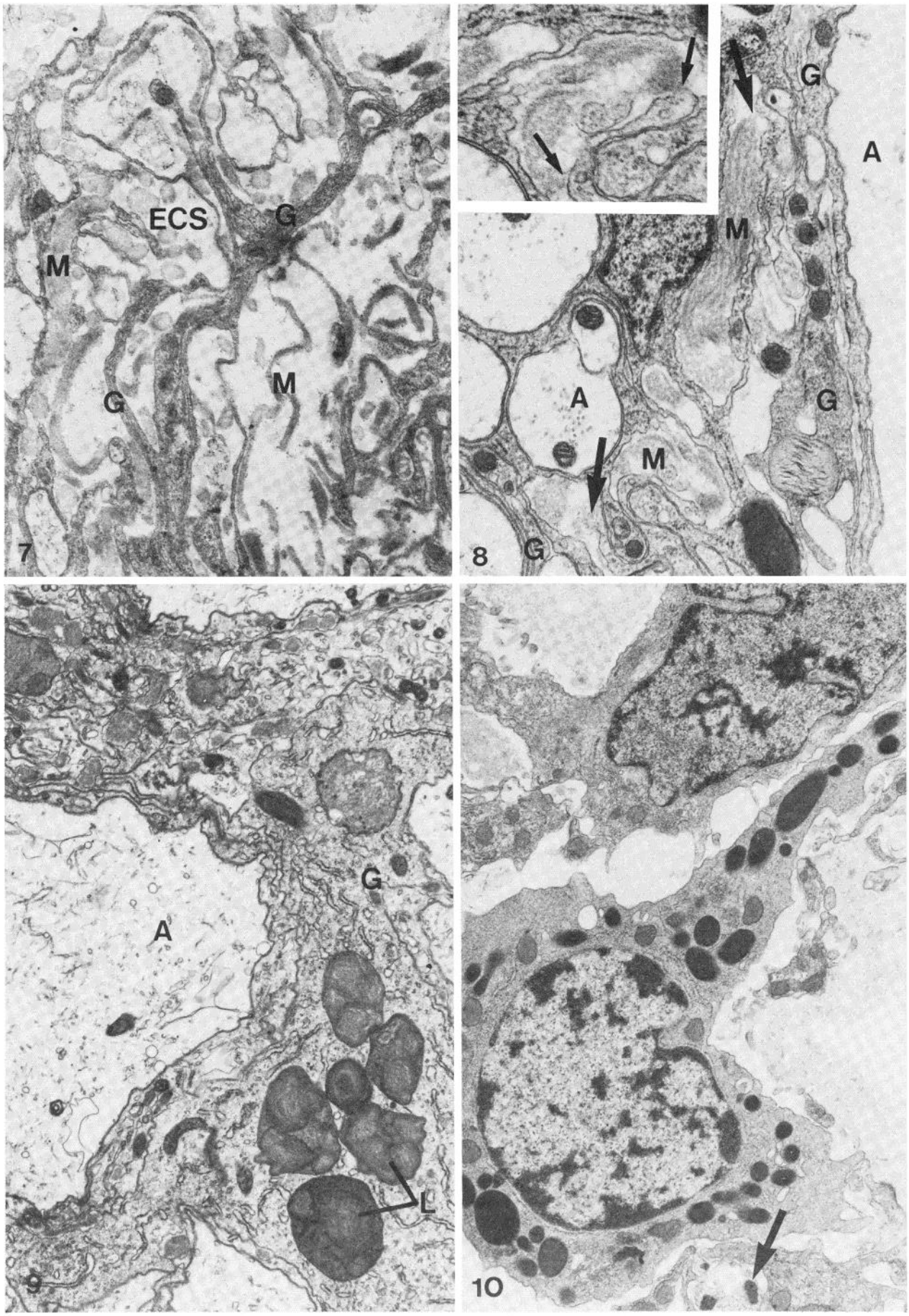
Another notable feature of the nervous tissue responses to lesioning in the cockroach was the increase in extracellular spaces, especially subperineurial ones. These spaces tended to be filled with amorphous matrix material which could represent precipitated protein from circulating body fluids which had leaked in, or material synthesized by, the glial cells in response to the injury. The more internal spaces contained the characteristic dense material normally present in interglial clefts, but after lesioning, it was much more voluminous; this could be due to enhanced synthetic activity on the part of the disturbed glial cells.

Our observations indicate that the blood-brain barrier in insects is only slowly re-established following surgical damage. After 28 days extraneously applied ionic lanthanum still penetrated deep into the extracellular system beyond the perineurium and was found in intercellular clefts between axon and glial membranes. This contrasts with the situation in which only the glia were selectively disrupted by the glial toxin ethidium bromide (Smith et al., 1984); in such preparations, which were initially leaky to lanthanum ions, the tracer was excluded from the general extracellular system only 4 days after treatment, despite the extensive glial disruption caused by the ethidium. The rapid re-establishment of the blood-brain barrier

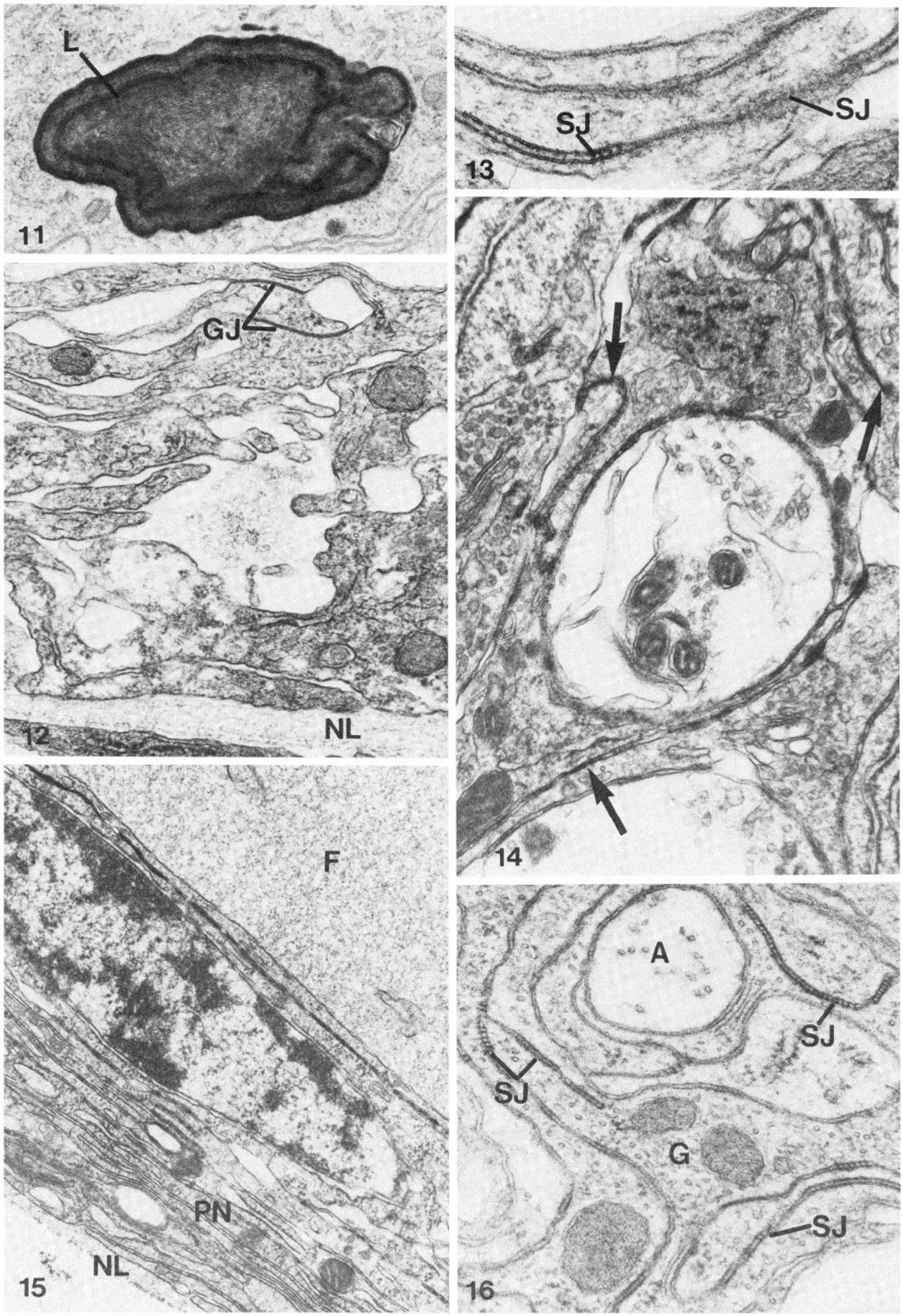
Figure 4. Cut connective left for 3 days and then incubated in lanthanum. Note that the tracer still has unrestricted access to the intercellular clefts (*small arrows*). A hemocyte-like cell is present, lying between axons in a typically glial position. This cell is phagocytosing material (*large arrow*) and contains the characteristic dense hemocyte-like granules. Magnification × 6,750.

Figure 5. Cut connectives, left for 4 days and then fixed, show the highly disrupted glial system with much extracellular space and fragmented matrix material. Magnification × 10,750. *Inset*, A 3-day cut connective shows large lysosomes as well as hemocyte-like granules in a cell deep in the nervous tissue. Magnification × 8,650.

Figure 6. Cut connective examined 14 days after operation. Glial cells are observed with large amounts of matrix material in the adjacent extracellular space. This does not all have the fragmented appearance of disrupted matrix and so may represent freshly synthesized material. Magnification × 11,800.



Figures 7 to 10



Figures 11 to 16

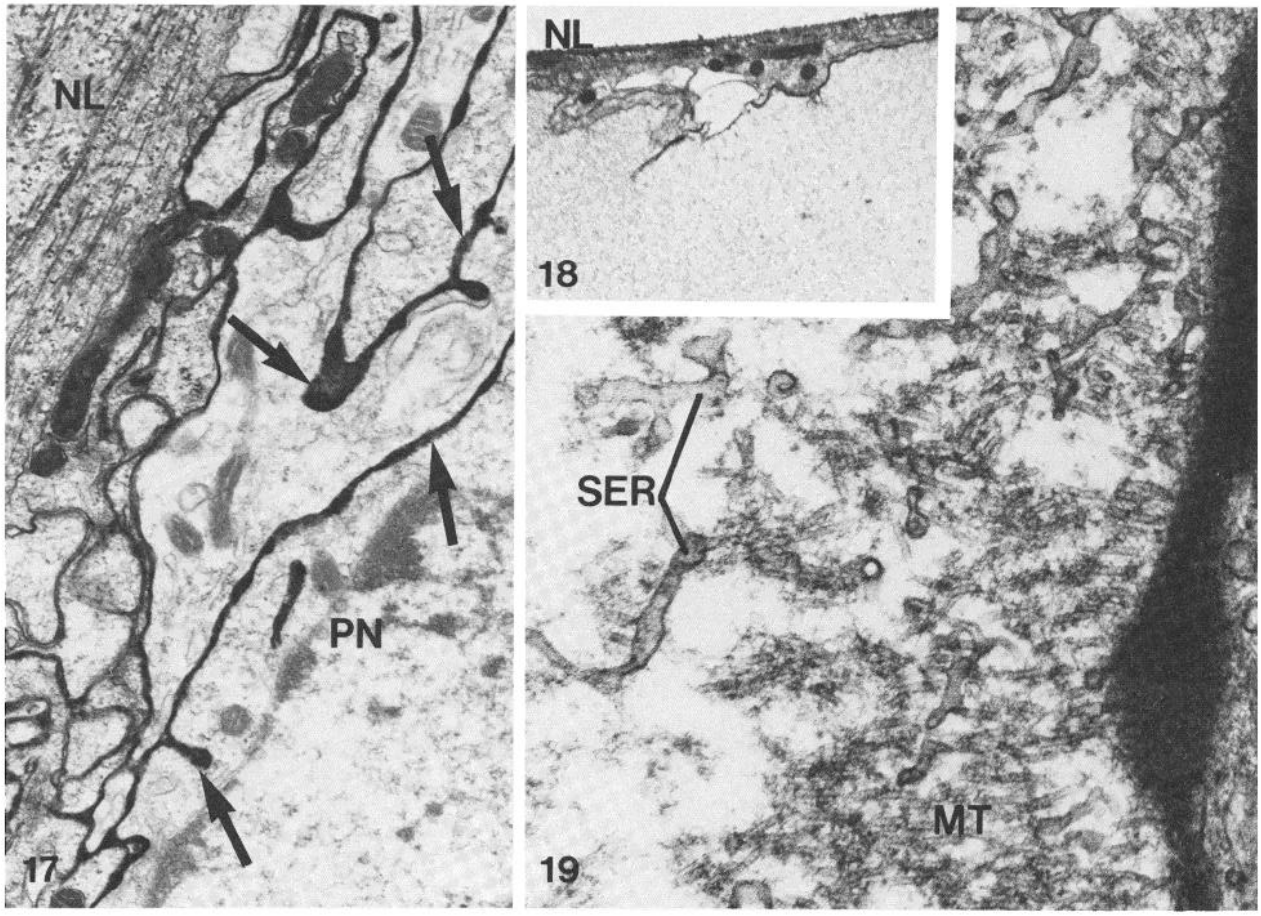


Figure 17. Five months after connective cutting, exogenous tracers, such as lanthanum (*arrows*), do not leak into the CNS beyond the perineurial barrier, indicating that all of the junctional components have been re-established. Magnification × 54,650.

Figures 18 and 19. Five months after cutting, some abnormal perineurial cells may still be found, in this case with an extensive fibrillar component (Fig. 18), becoming modified to possess unusual amounts of smooth endoplasmic reticulum and microtubules near the border with underlying glial cells. Magnification: Figure 18, × 8,000; Figure 19, × 52,560.

following ethidium treatment is associated with a speedy reorganization of the peripheral cellular elements in ethidium-treated preparations which, by 11 days, acquired some of the morphological characteristics of normal perineurial cells. These re-formed cells appeared to have been derived from granule-containing cells (Smith et al., 1984) which were also prominent in the surgically lesioned connectives used in this investigation.

Glial repair following surgical lesioning differs markedly from that observed after selective disruption with the glial toxin. In the latter case glial repair is a well-ordered process in which the granule cells may rapidly assume a characteristic glial morphology (Smith et al., 1984). In the cut connectives used in this investigation, however, relatively limited glial damage is accompanied by complex and prolonged responses in which, for

Figure 7. Twenty-one days after the cutting, glial cells may still be in a very disorganized state with enlarged extracellular spaces and fragmented matrix components. Magnification × 14,650.

Figure 8. Cut connective left for 24 days before examination. Glial cells appear to be producing extracellular matrix material (*arrows*) (see *inset*) which is gradually filling up the intercellular clefts and dilatations. Magnification × 21,550; *Inset*, × 36,000.

Figure 9. Lysosome in the glial cytoplasm in connectives operated on 11 days earlier. Magnification × 10,600.

Figure 10. Granule-laden hemocyte-like cells which appear to be entering the nervous system and phagocytosing debris (*arrow*) in a connective cut 24 days earlier. Magnification × 14,600.

Figure 11. In a connective cut 40 days before, glial lysosomes may assume gigantic proportions, presumably due to the enhanced phagocytic activity the cells has experienced. Magnification × 31,250.

Figures 12 and 13. Glial cells in the perineurial region, 24 days after cutting the connective, undergoing some reorganization as well as becoming coupled with each other by gap junctions (Fig. 12) or associated by septate junctions (Fig. 13). Magnification: Figure 12, × 42,700; Figure 13, × 89,600.

Figure 14. Twenty-eight days after connective severance, some axons still exhibit disordered smooth endoplasmic reticulum, while the perineurial tight junctions are not yet established as lanthanum (*arrows*) leaks into interglial and axoglial clefts. Magnification × 42,700.

Figure 15. Neural lamella with the perineurium being established, 28 days after connective cutting. Note the enlarged extracellular spaces, in this case full of a fine fibrous material, perhaps coagulated blood protein that leaked in earlier. Magnification × 26,200.

Figure 16. Glial cells in connective cut 40 days earlier, showing relatively normal glial packing around axons but atypical interglial septate junctions, such as usually only occur between perineurial cells. Magnification × 47,000.

example, there is only a slow restoration of repaired perineurial glia. Clearly, therefore, the presence of undamaged axons and extracellular matrix following selective glial disruption must exert a profound influence on the mechanisms of glial repair. It is interesting to note in this connection that the extracellular matrix has been shown to be important in the positioning and orientation of both neurons and glial elements in the developing insect central nervous system (Bate and Grunewald, 1981; Swales and Lane, 1984) and in other mammalian systems as well (Montesano et al., 1983).

References

Adrian, E. K., and R. L. Schelper (1981) Microglia, monocytes and macrophages. In *Glial and Neuronal Cell Biology, 11th International Congress of Anatomy*, E. A. Vidro and S. Federoff, eds., Part A, pp. 113–124, Alan R. Liss, Inc., New York.

Aguayo, A. J., S. David, and G. M. Bray (1981) Influence of the glial environments on the elongation of axons after injury: Transplantation studies in adult rodents. J. Exp. Biol. *95:* 231–240.

Bate, C. M., and E. B. Grunewald (1981) Embryogenesis of an insect nervous system. II. A second class of neuron precursor cells and the origin of the intersegmental connectives. J. Embryol. Exp. Morphol. *61:* 317–330.

Billingsley, M. L., N. Hall, and G. H. Mandel (1982) Trauma-induced glial proliferation: Possible involvement of the immune system. Immunopharmacology *5:* 95–101.

Bodenstein, D. (1957) Studies on nerve regeneration in *Periplaneta americana*. J. Exp. Zool. *136:* 89–115.

Boulton, P. S. (1969) Degeneration and regeneration in the insect central nervous system. I. Subtitle. Z. Zellforsch. *101:* 98–118.

Boulton, P. S., and C. H. F. Rowell (1969) Degeneration and regeneration in the insect central nervous system. II. Subtitle. Z. Zellforsch. *101:* 119–134.

Brockes, J. P., K. J. Fryxell, and G. E. Lemke (1981) Studies on cultured Schwann cells: The induction of myelin synthesis and the control of their proliferation by a new growth factor. J. Exp. Biol. *95:* 215–230.

Brockes, J. P., G. E. Lemker, and D. R. Balzer, Jr. (1980) Purification and preliminary characterization of a glial growth factor from the bovine pituitary. J. Biol. Chem. *255:* 8374–8377.

Fontana A., A. Grieder, S. Arrenbrecht, and P. Grob (1980) *In vitro* stimulation of glial cells by a lymphocyte-produced factor. J. Neurol. Sci. *45:* 55–62.

Fugita, S., Y. Tsuchihashi, and T. Kitamura (1981) Origin, morphology and function of microglia. In *Glial and Neuronal Cell Biology*, E. A. Vidrio and S. Federoff, eds., pp. 141–170, Alan R. Liss Inc., New York.

Gilmore, S. A., and R. C. Walls (1981) Patterns of labelling of intraspinal reactive cells in rats injected with [H^3] thymidine prior to or following sciatic axotomy. Brain Res. *218:* 1–13.

Lane, N. J. (1981) Invertebrate neuroglia: Junctional structure and development. J. Exp. Biol. *95:* 7–33.

Lane, N. J., and J. E. Treherne (1970) Uptake of peroxidase by the cockroach central nervous system. Tissue Cell *2:* 413–425.

Lane, N. J., and J. E. Treherne (1972) Studies on perineurial junctional complexes and sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. Tissue Cell *4:* 427–436.

Lane, N. J., and J. E. Treherne (1980) Functional organization of arthropod neuroglia. In *Insect Biology in the Future*, M. Locke and D. S. Smith, eds., pp. 765–795, Academic Press, Inc., New York.

Lane, N. J., H. le B. Skaer, and L. S. Swales (1977) Intercellular junctions in the central nervous system of insects. J. Cell Sci. *26:* 175–199.

Leech, C. A., and J. E. Treherne (1984) Growth and ion-specificity of excitability in regenerating cockroach giant interneurones. J. Exp. Biol. *101:* 311–318.

Meiri, H., M. E. Spira, and I. Parnas (1981) Membrane conductance and action potential of a regenerating axonal tip. Science *211:* 709–712.

Meiri, H., A. Dormann, and M. E. Spira (1983) Comparison of ultrastructural changes in proximal and distal segments of transected giant fibres in the cockroach *Periplaneta americana*. Brain Res. *263:* 1–12.

Montesano, R., L. Orci, and P. Vassalli (1983) *In vitro* rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J. Cell Biol. *97:* 1648–1652.

Pitman, R. M., and K. A. Rand (1982) Neural lesions can cause dendritic sprouting of an undamaged adult insect motoneurone. J. Exp. Biol. *96:* 125–130.

Roederer, E., and M. J. Cohen (1983a) Regeneration of an identified central neuron in the cricket. I. Control of sprouting from soma, dendrites, and axon. J. Neurosci. *3:* 1835–1847.

Roederer, E., and M. J. Cohen (1983b) Regeneration of an identified central neuron in the cricket. II. Electrical and morphological response of the soma. J. Neurosci. *3:* 1848–1859.

Schofield, P. K., and J. E. Treherne (1984) Localization of the blood-brain barrier of an insect: Electrical model and analyses. J. Exp. Biol. *109:* 319–331.

Sears, T. A. (1982) *Neuronal-glial Cell Interrelationships*, p. 374, Springer-Verlag, Berlin.

Smith, P. J. S., C. A. Leech, and J. E. Treherne (1984) Glial repair in an insect central nervous system: Effects of selective glial disruption. J. Neurosci. *4:* 2698–2711.

Swales, L. S., and N. J. Lane (1984) Embryonic development of glial cells and their junctions in the locust central nervous system. J. Neurosci., in press.

Treherne, J. E. (1981) *Glial-Neurone Interactions*. Review Volume, J. Exp. Biol., Vol. 95.

Treherne, J. E., and P. K. Schofield (1981) Mechanisms of ionic homeostasis in the central nervous system of an insect. J. Exp. Biol. *95:* 61–73.