Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Neuroscience Vol. 51, No. 1, pp. 137–148, 1992
Printed in Great Britain

0306-4522/92 $5.00 + 0.00
Pergamon Press Ltd

DEMYELINATION, AND REMYELINATION BY SCHWANN CELLS AND OLIGODENDROCYTES AFTER KAINATE-INDUCED NEURONAL DEPLETION IN THE CENTRAL NERVOUS SYSTEM

I. DUSART,* S. MARTY and M. PESCHANSKI†
INSERM CJF 91-02, Neuroplasticité et Greffes Intracérébrales, Faculté de Médecine, 8 rue du Général Sarrail, 94010 Créteil, France

Abstract—Excitotoxins are thought to kill neurons while sparing afferent fibers and axons of passage. The validity of this classical conclusion has recently been questioned by the demonstration of axonal demyelination. In addition, axons are submitted to a profound alteration of their glial environment. This work was, therefore, undertaken to reassess axonoglial interactions over time after an excitotoxic lesion in the rat. Ultrastructural studies were carried out in the ventrobasal thalamus two days to 18 months after neuronal depletion by *in situ* injections of kainic acid. In some cases, lemniscal afferents were identified by using anterograde transport of wheatgerm agglutinin conjugated to horseradish peroxidase from the dorsal column nuclei.

Two and four days after kainate injection, numerous dying axons displaying typical signs of Wallerian degeneration were observed in a neuropile characterized by the loss of neuronal somata and dendrites, an increase in number of microglia/macrophages and the disappearance of astrocytes. Ten and 12 days after kainate injection, degenerating axons were no longer observed although myelin degeneration of otherwise unaltered axons was ongoing with an accumulation of myelin remnants in the neuropile. At 16 and 20 days, the demyelination process was apparently complete and axons of different diameters were sometimes packed together. One and two months after kainate injection, the axonal environment changed again: remyelination of large-caliber axons occurred at the same time as reactive astrocytes, oligodendrocytes and numerous Schwann cells appeared in the tissue. Schwann cell processes surrounded aggregates of axons of diverse calibers, ensheathed small ones and myelinated larger ones. Axons were also remyelinated by oligodendrocytes. Horseradish peroxidase-labeled lemniscal afferents could be myelinated by either of the two cell types. After three months, the neuropile exhibited an increase in number of hypertrophied astrocytes and the progressive loss of any other cellular or axonal element. At this stage, remaining Schwann cells were surrounded by a glia limitans formed by astrocytic processes.

These data indicate that although excitotoxins are sparing the axons, they are having a profound and complex effect on the axonal environment. Demyelination occurs over the first weeks, accompanying the loss of astrocytes and oligodendrocytes. Axonal ensheathment and remyelination takes place in a second period, associated with the reappearance of oligodendrocytes and recruitment of numerous Schwann cells, while reactive astrocytes appear in the tissue at a slightly later time.

Over the following months, astrocytes occupy a greater proportion of the neuron-depleted territory and other elements decrease in number. These successive stages in alteration of axonoglial interactions seem to evolve in parallel to the changes in density and terminal morphology that we described earlier for myelinated afferent fibers to the excitotoxic lesion.

Axons afferent to a CNS area subjected to an excitotoxic agent are not directly damaged, structurally or functionally, in sharp contrast to neuronal somata and dendrites that are rapidly destroyed.[15,17,47,60] After a few weeks, however, axonopathy has been described in many cases, probably resulting in a dying-back process leading to a decrease in the number of axons that have been deprived of their targets[33,37,59] (see discussion in Ref. 16). In parallel to these regressive events, we have observed structural changes that resemble attempts at axonal regeneration by myelinated fibers (lemniscal and corticothalamic afferents,[43] thalamostriatal axons,[39] cortico- and rubrospinal fibers[40]) while unmyelinated afferents to the same areas, either monoaminergic[44] or not,[40] remained unaltered.

This distinction made between the fate of axons on the basis of their normal glial ensheathment suggested that excitotoxins may secondarily affect axons by interfering with axonoglial relationships. As a result of the excitotoxic lesion, profound glial and vascular changes are observed. Leakage of the blood–brain barrier occurs rapidly,[19] together with activation of resident microglia.[35] Recruitment and proliferation of microglia/macrophages lead to a major increase in the number of glial cells over the first weeks following neuronal loss[35] while astrocytes are no longer observed.[20] Later, the area is filled with

*Present address: Brain Research Institute, University of Zurich, Switzerland.
†To whom correspondence should be addressed.
Abbreviations: BDHC, benzidine di-hydrochloride; HRP, horseradish peroxidase; PNS, peripheral nervous system.

reactive astrocytes that progressively form the so-called glial scar.[20] Two recent observations have suggested that these changes may alter axonoglial interactions. Coffey *et al.*[12,13] have observed that an injection of ibotenic acid into the septum provoked a massive loss of immunocytochemical markers of myelin within days, indicating a demyelination of axons passing through the area. This observation was in keeping with previous suggestions of a rapid demyelination process after kainate injection.[37,59] Analysis of the fine structure of the thalamic neuropile, one month after kainate injection, has additionally revealed the presence of Schwann cells.[18] The abnormal location of these peripheral nervous system (PNS) cells in a CNS area deep in the brain, and their involvement in axonal ensheathment and myelination are clear signs of remyelination[8,22,24,38,55] and, therefore, of the previous occurrence of axonal demyelination. Since it is well established that pathological changes of myelin ensheathment of central axons is responsible not only for functional but also for structural axonal alteration (see References and Discussion in Ref. 49), excitotoxins may have a secondary deleterious effect on afferent systems to a neuron-depleted area.

The present study has therefore been undertaken to precisely analyse the changes in axonoglial relationships over time after an excitotoxic lesion.

EXPERIMENTAL PROCEDURES

Twenty-seven female Sprague–Dawley albino rats (200 g at the beginning of the experiments; Charles River, France) were used. They were anesthetized with chloral hydrate (400 mg/kg, i.p.) and received a slow-pressure injection of 5 nmol of kainic acid (in 0.15 μl of saline, over 12 min). The injection was aimed at the lateral border of the rostral thalamus as previously described,[43] in order to obtain a large area of total neuronal depletion in the thalamic reticular and ventrobasal nuclei.

Following various survival times (two, four, 10, 12, 16 and 20 days, one, two, three, six, 12 and 18 months), 24 animals were prepared directly for standard electron microscopy. Rats were perfused with warm heparinized phosphate-buffered saline (0.1 M; pH 7.4) followed by cold fixative containing 2% glutaraldehyde, 2% paraformaldehyde and 4% sucrose in the same buffer. The brain was taken and postfixed for 24 h. The diencephalon was trimmed out and cut serially in coronal sections (50 μm thick) on a Vibratome (Oxford Inst.). Sections were fixed with osmium (OsO_4, 2% in phosphate buffer, 0.2 M, pH 7.4; 1:1 solution), dehydrated through progressive alcohols and embedded flat in Epon. Areas of interest were chosen by analysing the flat-embedded sections with a light microscope. As previously described,[43] the lesion was conspicuous in the osmicated section because of the colorless aspect of the area as compared to the dark color of the rest of the tissue. The lesioned area, and in some cases the intact opposite thalamus, were trimmed out and further sectioned on an ultramicrotome before staining with lead citrate. In order to define quantitatively the thickness of the myelin, the ratio of the inner (axon) and outer (axon + myelin) diameters was calculated to facilitate comparison of fibers in the area of neuronal depletion to those in the opposite (unlesioned) thalamus in two animals, two months after kainate injection. Statistical analysis was carried out using the Student's *t*-test.

Three other rats were used for analysis of specifically identified somatosensory afferents to the ventrobasal thalamus two months after kainate injection. Two days before perfusion, rats were anesthetized and wheatgerm agglutinin conjugated to horseradish peroxidase (HRP) (10% aqueous solution) was injected by pressure, using a 1-μl Hamilton syringe into the left dorsal column nuclei at the level of the obex, under visual guidance after opening of the cisterna magna. Rats were perfused according to the technique described above, except for the postfixation which lasted only 2 h. After sections were cut on the Vibratome, they were treated for HRP histochemistry using a previously described technique with benzidine di-hydrochloride (BDHC) as a chromogen.[45] Briefly, sections were incubated for 20 min in a solution containing 80 ml acetate buffer (pH 6.2), 100 mg sodium nitroprusside, BDHC (100 mg) previously dissolved in 20 ml warm absolute alcohol and 0.03% hydrogen peroxide. After several washes with the acetate buffer, sections were post fixed with warm (45°C) osmium (phosphate-buffered solution as described above) for 1 h then prepared for electron microscopy as described above.

RESULTS

In embedded sections, the area of neuronal loss that was to be trimmed out for our study was conspicuous because of its colorless aspect related to a decreased osmication. When compared to adjacent Nissl-stained sections, this area comprised a large part of the ventrobasal thalamus but never extended to the large fiber tracts around it, the internal capsule and medial lemniscus, that were as well stained as on the opposite unlesioned side. Preservation of the osmication of these white matter areas was observed even when the kainate lesion had extended in the gray matter on both sides of the tracts, i.e. to the striatum and to the zona incerta, respectively. At the ultrastructural level, the identification of the axons was easily made when axons were surrounded by layers of myelin. In other cases, for unmyelinated or totally demyelinated axons, we relied upon the commonly accepted criteria.[46] Axons, in the CNS neuropile, are characterized as profiles having smooth contours and a cylindrical shape, display a clear cytoplasm that contains neurofilaments, arrays of microtubules and a few mitochondria and that, mostly, contains no ribosomes. Analysis of the neuropile between two days and 18 months after kainate injection allowed us to characterize four different periods according to specific features of axonoglial injections: (i) a few days during which Wallerian degeneration predominated; (ii) the second week, characterized by profuse demyelination of axons; (iii) the third week in which the neuropile was devoid of myelinated axons; and (iv) subsequent periods in which large axons were remyelinated.

Demyelination process

Two and four days after kainate injection, the neuropile was characterized by the presence of cellular debris and numerous microglia/macrophagic cells. In contrast, astrocytes were noticeably less numerous than in the intact tissue (Fig. 1). Dendrites and

Fig. 1. Four days after kainate injection, the neuropile is characterized by the presence of degenerating dendrities exhibiting remnants of synaptic organizations (D) and axons (A1, A4) whose cytoplasm is abnormally either electron-lucent or electron-dense and contains altered mitochondria. All axons in this picture exhibit alteration of myelin sheaths although axonopathy is not conspicuous in some of them (A2, A3). Note the absence of astrocytic endfeet around the capillary. Scale bar = 1 μm.

neuronal somata displaying a normal morphology were totally absent and membranes surrounded apparently empty spaces that sometimes contained a few organelles that exhibited various signs of degeneration. The identification of these structures as formerly dendritic or somatic could be made because of the persistence of postsynaptic densities (Fig. 1). Axonal profiles could still be clearly

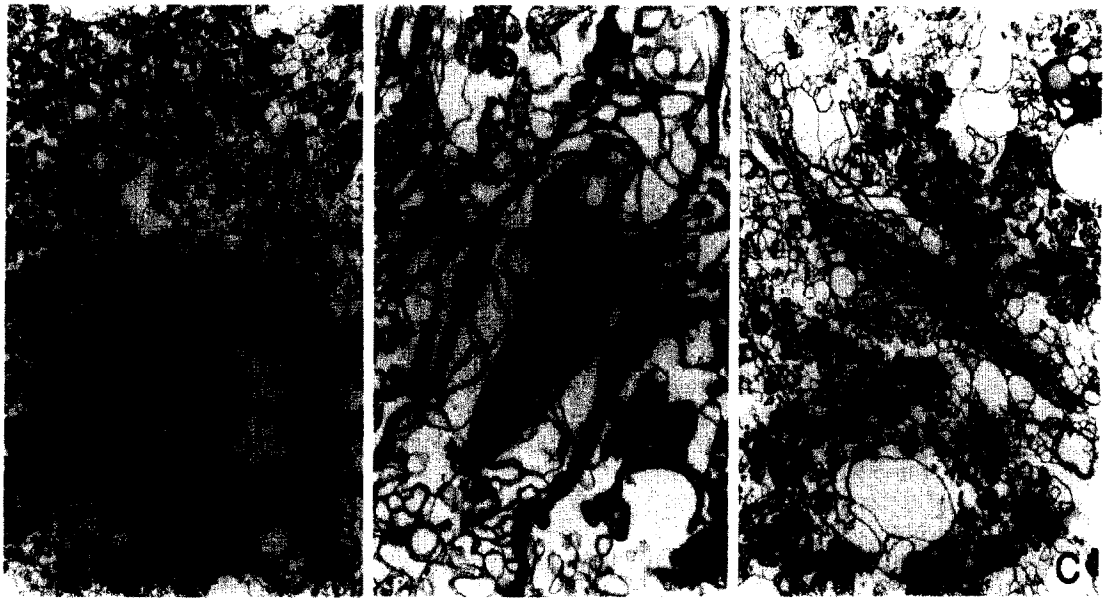

Fig. 2. Ten days after kainate injection. (a) The neuropile contains cellular and myelin debris together with numerous macrophages (M). Degenerating axons are not conspicuous at this stage although demyelination is clearly continuing. (b) At higher magnification, inner layers of myelin appear dismantled before outer ones. (c) A large axon, totally demyelinated, is surrounded by myelin debris that is sometimes still attached to its membrane. Scale bar in c = 2 μm for a, 0.5 μm for b and 1.5 μm for c.

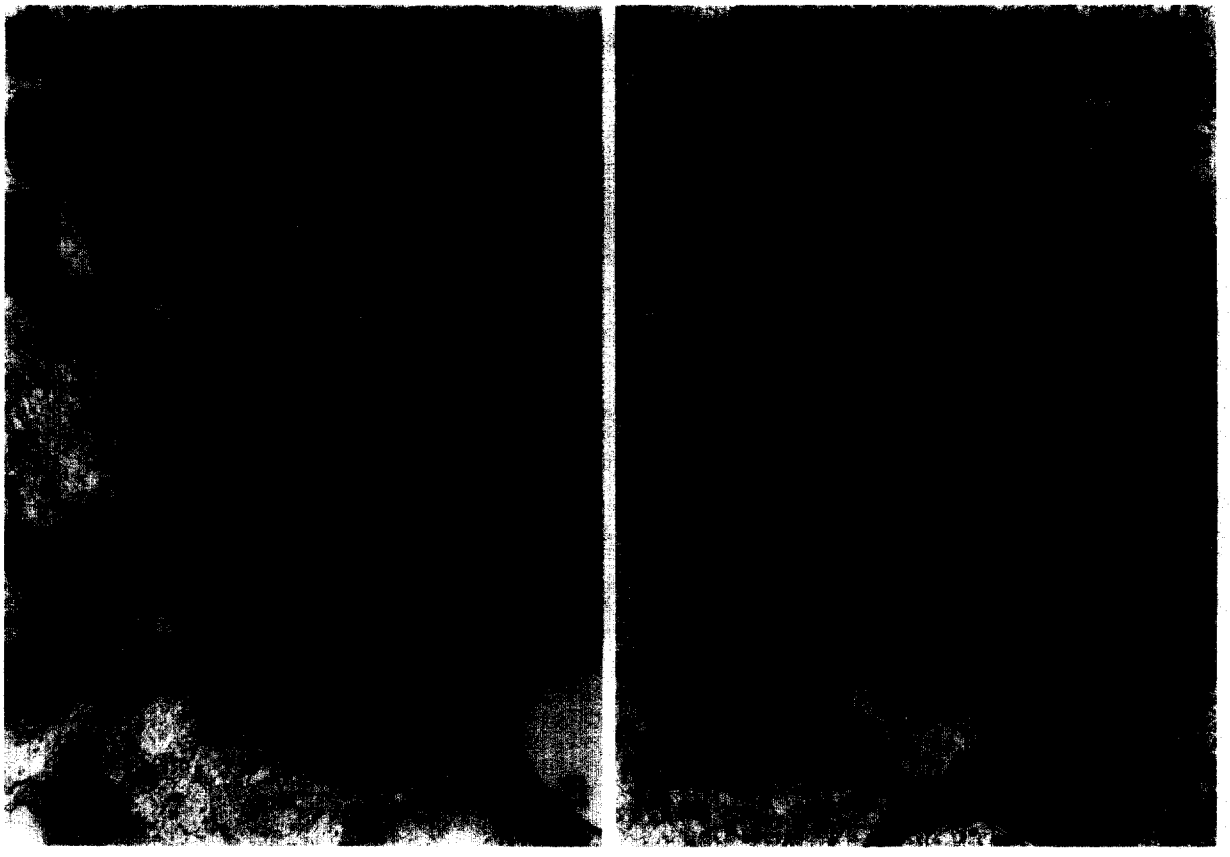

Fig. 3. Sixteen days after kainate injection. (a) The neuropile contains little myelin. In this field, unmyelinated axons of various calibers are packed together. Some cells extend processes between axons (stars); macrophages (M) are also visible. (b) Enlarged view of the area boxed in panel a showing the apposition between the membranes of a cell process and axons. Scale bar in b = 5 μm for a and 2 μm for b.

identified. Many of them exhibited signs of Wallerian degeneration with morphologically altered mitochondria—enlarged with widened cristae—and an increase of the axoplasmic electron density. Other axons, however, either myelinated or not, did not display these degenerative features and looked healthy. One striking characteristic of the tissue, however, was the widespread abnormality of myelin sheaths surrounding the normal and degenerating axons (Fig. 1). Myelin sheaths were dismantled and the lamellae appeared loosely arranged around the axons, sometimes there was better preservation of the outermost lamellae when the axons looked normal (See Figs 1, 2a, b).

Ten and twelve days after kainate injection, the alteration of the neuropile had progressed with the almost total disappearance of any structures reminiscent of dendrites or somata. Small numbers of synaptic boutons were still attached to an electron-dense postsynaptic membrane and formed what have been called "moustache" contacts.[29] Macrophages, often loaded with debris, were widespread in the tissue (Fig. 2a) and the extracellular space was expanded and contained myelin debris (Fig. 2a–c). Astrocytes were not present in significant numbers. As far as the axons are concerned, this period was characterized by the disappearance of morphological signs of Wallerian degeneration. Axons exhibited neither an electron-dense axoplasm nor contained enlarged mitochondria (Fig. 2b, c). The abundance of unmyelinated, less than 0.5 μm diameter, or demyelinated axons of large caliber (Fig. 2c) was striking.

Sixteen and 20 days after lesion induction, the neuropile exhibited yet another set of characteristics. Any remnants of synaptic contacts had disappeared at this stage. Macrophages, loaded with debris, were observed mostly close to the blood vessels (Fig. 3a). The neuropile was characterized by the near disappearance of myelin, except for a few remnants (Fig. 3b). Axons appeared, therefore, systematically unmyelinated, whatever their caliber. In some cases (as in Fig. 3), axons were packed together in large numbers. Some unidentified cells extended long processes between axons in these clusters (Fig. 3b).

Remyelination process

One and two months after the lesion had been induced, the general features of the neuropile changed again, due to the reappearance of numerous myelinated axons and to the presence of astrocytes,

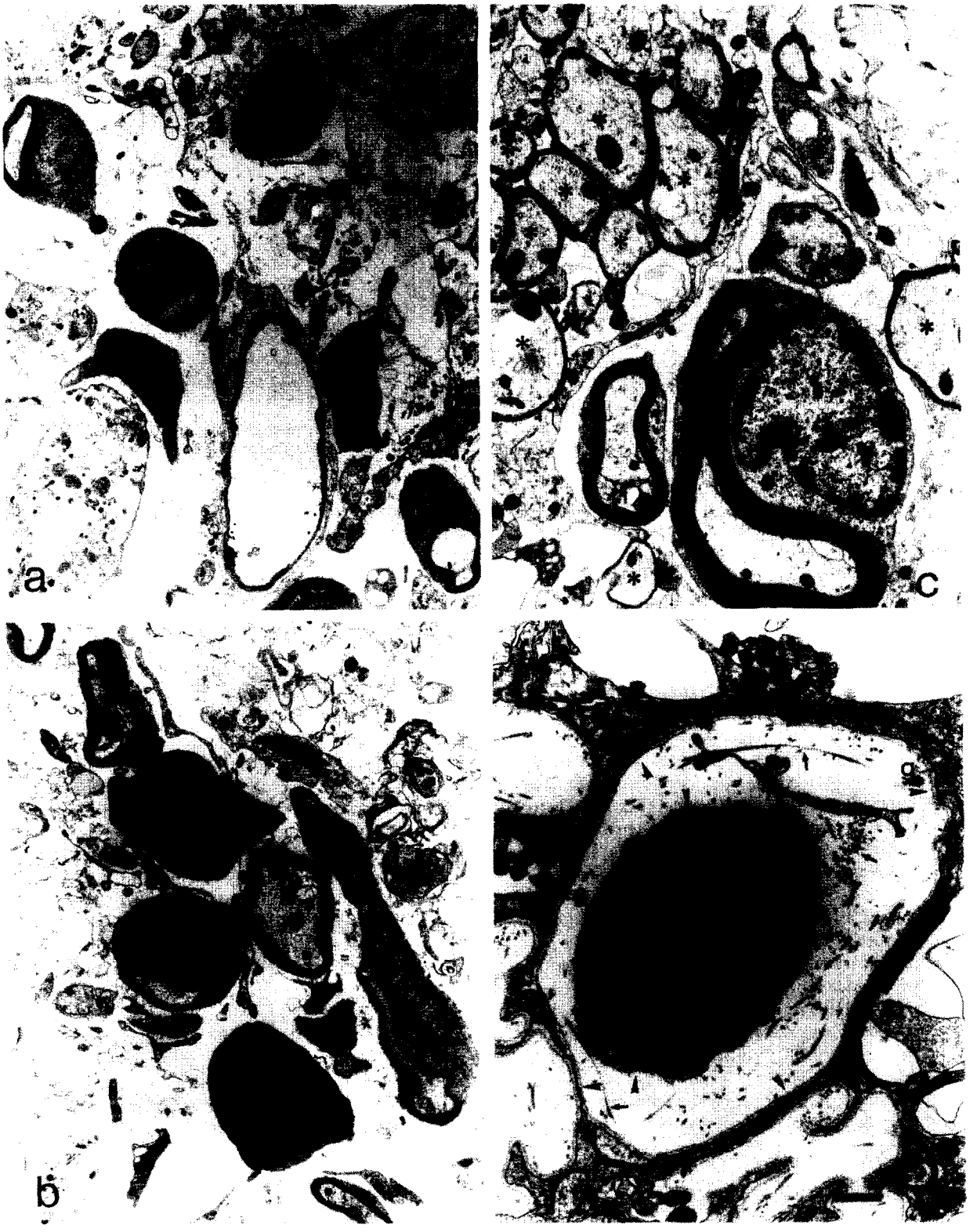

Fig. 4. Areas containing Schwann cells in one- (b), two- (a, c) and six- (d) month-old kainate lesions. (a–c) In the earlier periods, Schwann cells are numerous and interspersed with macrophages (M) (a, b), or with axons myelinated by oligodendrocytes (stars, c). (d) At later stages, Schwann cells are few in number and appear surrounded by astroglial processes, containing gliofilaments (g), that are bordered by a continuous basal lamina (arrowheads). Note the presence of collagen fibrils (arrows) around the Schwann cell. Scale bar in d = 3.3 μm for a, 2 μm for b, 1 μm for c and 0.6 μm for d.

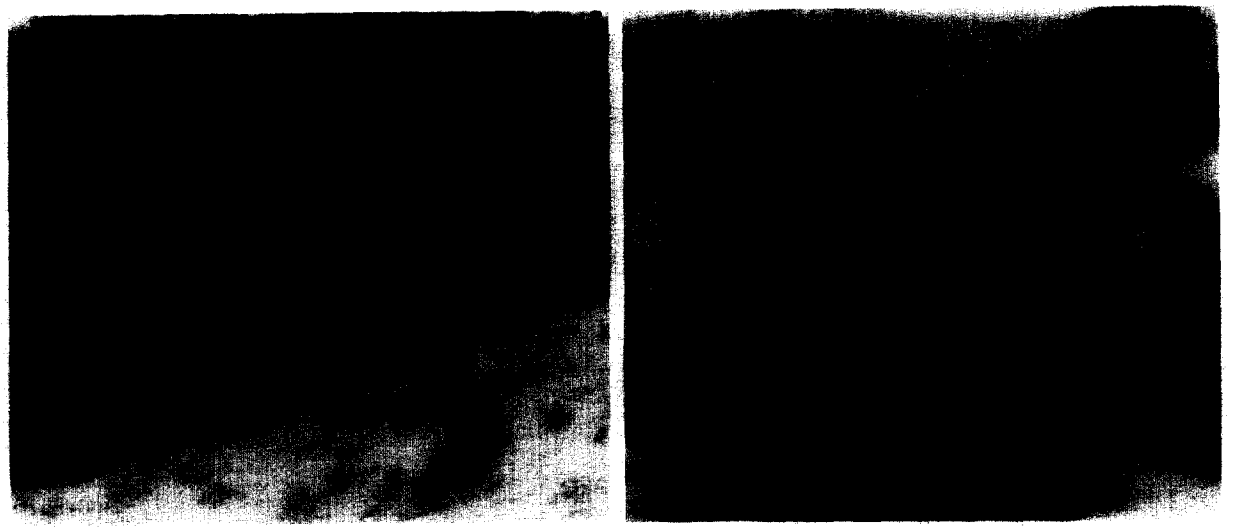

Fig. 5. Two months after kainate injection, high-magnification electron photomicrographs showing myelin formed by a Schwann cell (a) and oligodendrocytes (b). Scale bar = 75 nm.

oligodendrocytes and numerous Schwann cells. Schwann cell myelin was differentiated from oligodendrocytic myelination on the basis of three main morphological criteria:[46] (i) a thin rim of cytoplasm around the entire perimeter of the ensheathed axon; (ii) the presence of the nucleus and cytoplasm in the sheath surrounding the axon; and (iii) a basal lamina and collagen fibrils around the Schwann cells. Many areas in the tissue appeared to contain numerous Schwann cells, some of which myelinated axons (Fig. 4a, b). Many clusters of Schwann cells were close to blood vessels (Fig. 4a), but this was not always the case (Fig. 4b). Axons myelinated by oligodendrocytes were also often grouped together (Fig. 4c). There was, however, no strict delineation of territories between Schwann cells and oligodendrocytes and adjacent axons could be myelinated by either one (Fig. 4c).

Qualitative observation of the myelin sheaths formed by oligodendrocytes or Schwann cells showed no specific characteristics (Fig. 5a, b). Quantification demonstrated that the new sheaths of myelin formed by oligodendrocytes in the lesion were especially thin since the ratio between axon and axon + myelin diameters was 0.874 ± 0.043 ($n = 27$) as compared to 0.687 ± 0.08 ($n = 22$) in the intact thalamus ($P < 0.001$). There was no statistically significant difference, in contrast, between the ratios calculated for oligodendrocytes in the intact thalamus and Schwann cells in the lesion (0.731 ± 0.097, $n = 20$, $P > 0.1$) whereas the difference was significant between oligodendrocytes in the lesion and Schwann cells ($P < 0.001$).

In the lesioned thalamus, as well as in the intact nucleus, unmyelinated axons ensheathed by profiles that could be identified as oligodendroglial were not observed. In contrast, Schwann cells ensheathed many of them according to arrangements that seemed to depend upon the diameter of the axons. Large axons were myelinated by Schwann cells according to a one-to-one relationship (Fig. 6a and see also Fig. 4a, b). Medium-caliber axons were often, but not always, isolated in a separate trough of a Schwann cell, even though the same cell ensheathed other unmyelinated axons (Fig. 6b, c). In some cases, medium-caliber axons were surrounded by several interdigitating processes of the same Schwann cell (Fig. 6c), suggesting the appearance of a mesaxon and the potential start of a myelination process. Smaller axons were not isolated but, quite the contrary, ensheathed together by one cytoplasmic process (Fig. 6b). Morphological features atypical for the CNS also appeared at the level of nodes of Ranvier, in particular when the nodes were surrounded by cytoplasmic processes of the two Schwann cells that myelinated adjacent axonal segments (Fig. 6d).

In the animals in which lemniscal axons had been anterogradely labeled from the dorsal column nuclei, crystals of BDHC reaction product were observed not only in axons myelinated by oligodendrocytes (Fig. 7a) but also in axons myelinated (Fig. 7b) or simply ensheathed, without myelination, by Schwann cells (Fig. 7c). Limitation of the technique prevented a strict quantification of the different types of ensheathment to be made but there did not seem to be a particular preference of lemniscal fibers to be associated with either oligodendrocytes or Schwann cells.

At later stages, no further modification of axonoglial relationships occurred and the overall features of the neuropile progressively evolved toward a loss of all elements except for hypertrophied astrocytes containing bundles of gliofilaments that formed a dense network. Remaining Schwann cells myelinating axons were totally surrounded by astroglial processes that were lined up with a basal lamina on their side facing the PNS element (Fig. 4d).

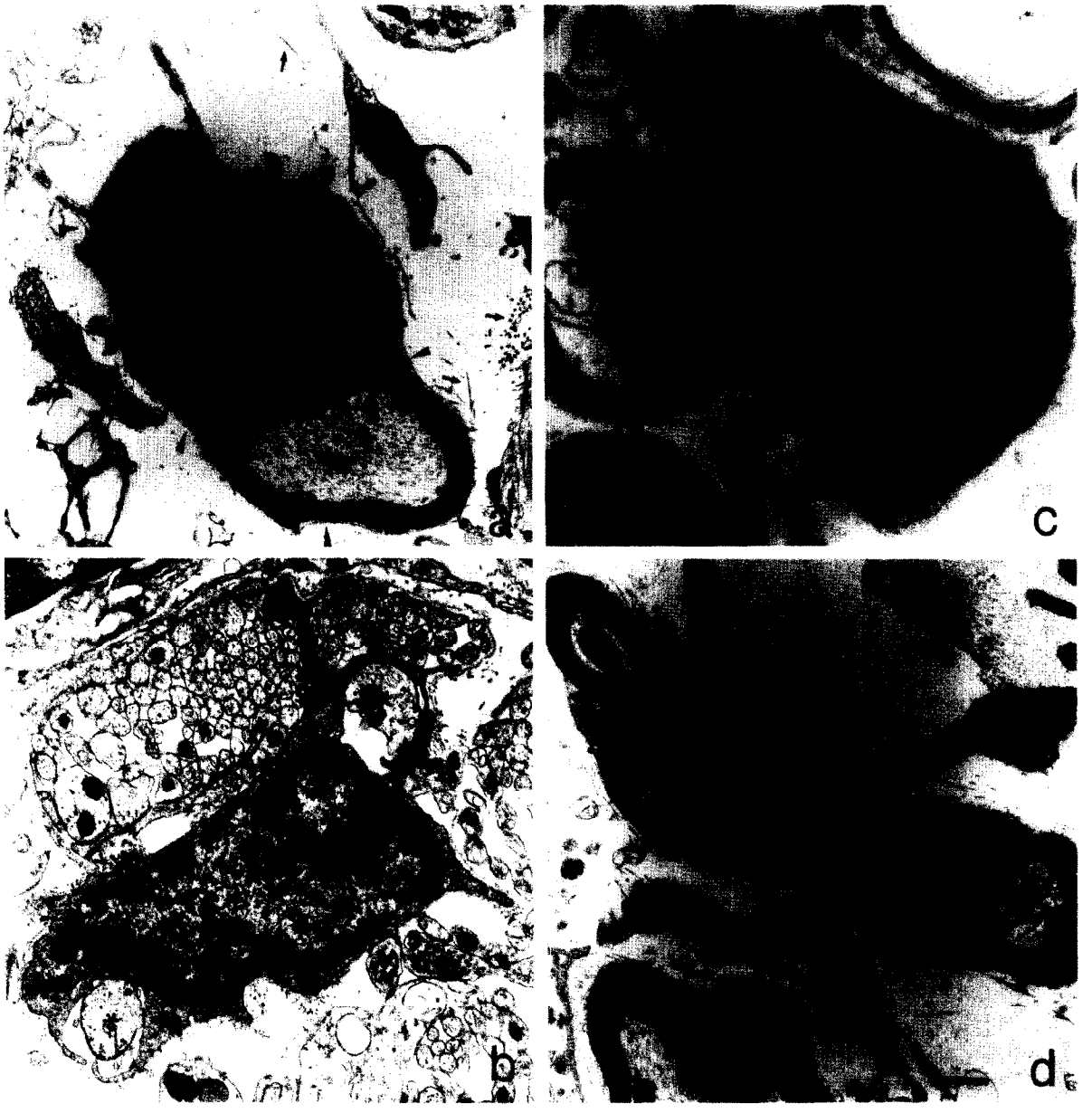

Fig. 6. Axonal ensheathments by Schwann cells in the one- (c) and two- (a, b, d) month-old kainate lesion. (a) Typical one-to-one relationship between a Schwann cell and a myelinated axon. Note that the axon–Schwann cell complex is totally surrounded by a basal lamina (arrowheads) and that collagen fibrils are present in the neuropile arrows. (b) A Schwann cell simultaneously ensheaths numerous unmyelinated axons of fine and medium calibers, without myelinating them. Fine-caliber axons, together with one medium-size axon, are contained in a single trough while two other medium-sized and caliber axons are contained in separate troughs (asterisk). (c) Schwann cell ensheathing small and medium-sized axons. Note the formation of a mesaxon (m) around one of the medium-sized axons, suggesting the start of a myelination process. (d) Node of Ranvier formed by two myelinating Schwann cells. Note the presence of cytoplasmic processes uncovering the node, a feature that is typical of peripheral nerve myelination. Scale bar in d = 0.8 μm for a and d, 0.6 for b and 0.45 for c.

DISCUSSION

This study confirms and extends previous results that had indicated profound alteration of axonoglial interactions in an area excitotoxically depleted of neurons. Two major issues are raised by these results. First, the demyelination process leads, within two weeks, to an almost complete disappearance of myelin sheaths. This suggests that the excitotoxic lesion provides conditions that initiate demyelination which may be related to the death of oligodendrocytes. Second, remyelination and axonal ensheathment are carried out by Schwann cells and oligodendrocytes that are recruited into the lesion area.

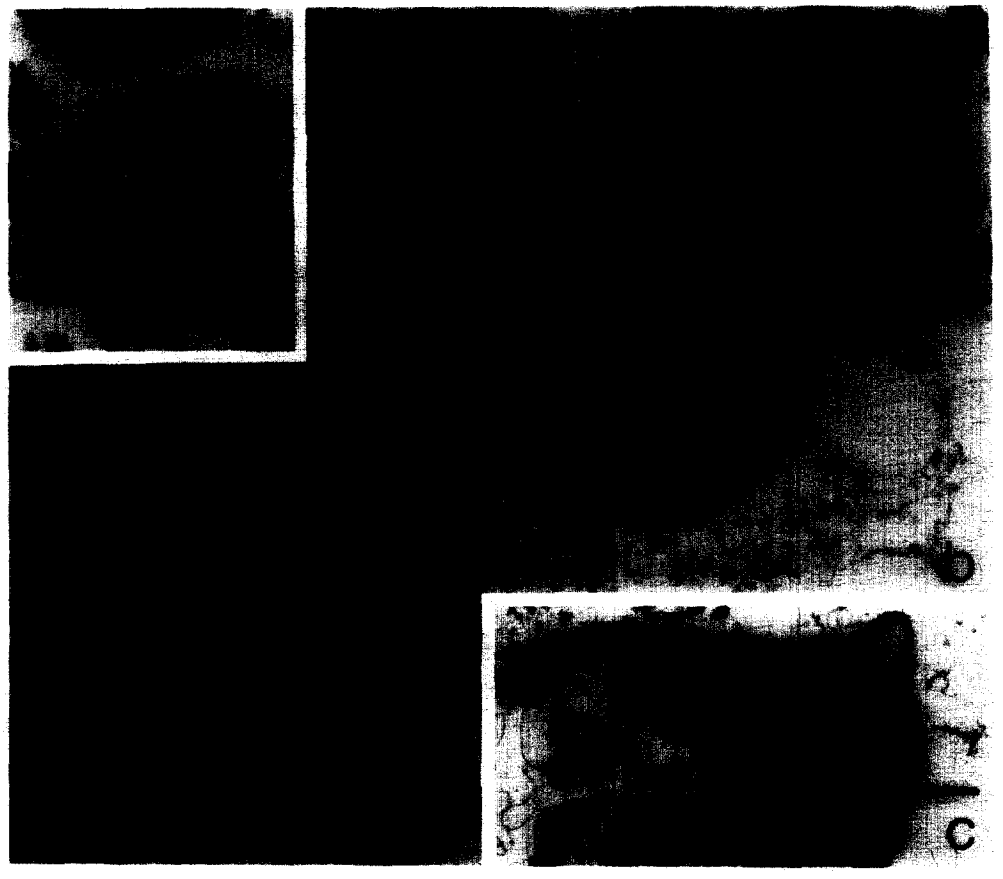

Fig. 7. Lemniscal afferents anterogradely labeled using wheatgerm agglutinin–HRP in the two-month-old kainate lesion. These identified CNS axons are ensheathed either by oligodendrocytes (a) or by Schwann cells that may (b) or may not (c) myelinate them. Arrows point to crystals of BDHC reaction product. Scale bar in c = 0.3 μm for a and 0.8 μm for b and c.

Demyelination of unlesioned axons accompanies massive neuronal loss

Demyelination of axons of passage or afferent fibers has been reported in only a few studies of excitotoxic lesions.[12,13,59] An indirect confirmation of such a process was given by our observation that numerous Schwann cells ensheath and myelinate axons in the one-month-old kainate-lesioned thalamus.[18] The results of the present study strongly support the existence of such a demyelinating process. Direct evidence of demyelination of surviving axons was difficult to obtain in short-term lesions because many myelinated axons were undergoing Wallerian degeneration. However, as early as ten days after the kainate injection demyelination was apparent with otherwise healthy axons surrounded by debris of myelin lamellae. The existence of this process was further supported by the observation of grouped unmyelinated axons of all calibers at a later stage. Large (greater than 0.5 μm in diameter), apparently intact, axons were devoid of a myelin sheath which is uncommon in the intact CNS but has been described in demyelinating experimental lesions.[55] In addition, large axons myelinated by oligodendrocytes in long-term lesions displayed a myelin sheath much thinner than in intact tissue. Such thin myelin sheaths are typical of remyelination,[5,26,41,48] and thus indirectly demonstrate a prior demyelination.

Demyelination of afferent fibers, and probably of fibers of passage in the neuron-depleted area could be the result of direct injury to the oligodendrocytes by kainate, comparable to that observed with specific gliotoxic agents such as lysolecithin.[4,25] However, even though oligodendrocytes have glutamate receptors,[31] kainate is not toxic for them when applied for 1 min on acute brain slices (Kettenmann, personal communication). In addition, there is no demyelination in the internal capsule, a structure exposed to kainate injected at the border of the internal capsule, and sometimes spreads across it to the striatum. It is interesting to mention that, in sharp contrast to what occurs in the thalamic nucleus, there is no major recruitment or proliferation of microglia/macrophages in the internal capsule,[35] where the leakage of the blood–brain barrier is weak (unpublished observations) and reactive astrocytes are present in large numbers[20] in the first weeks after kainate injection. This indicates that glial changes in the white matter in which kainate is infused are quite different from

those observed in areas in which it induces neuronal degeneration, suggesting that those observed in the thalamus are not related to a direct injury of oligodendrocytes by kainate. Alternatively, the leakage of the blood–brain barrier[19] and the macrophagic invasion[35,36] that are observed in the thalamic lesion may lead to a progressive accumulation of oligodendrogliotoxic substances and, therefore, to a long-lasting demyelination process. Several recent *in vitro* studies suggest that blood-borne complement factors as well as cytokines produced by activated macrophages may be toxic to oligodendrocytes and CNS myelin.[11,51,52,58] Thus, mature oligodendrocytes would be under attack as complement factors leak through the blood–brain barrier, or when the brain parenchyma contains activated macrophages. This hypothesis is supported by the demonstration that demyelination can be blocked shortly after the lesion by irradiation that decreases inflammatory response and reduces leakage of the blood–brain barrier.[13] If one or both mechanisms are involved, it is most likely that the nature of the agent leading to neuronal death plays little role in the additional demyelination since any neuronal death of consequence will induce both a profuse leakage of the blood–brain barrier and an inflammatory response.

Schwann cells are recruited in the CNS

In demyelinating lesions produced in the spinal cord, Schwann cells have been observed mostly in superficial regions, close to the dorsal root entry zones, suggesting that they may have penetrated the CNS neuropile due to a leakage of the CNS–PNS barrier.[14,24,53–55] Such an origin is highly unlikely in our experimental conditions, given the distance separating the dorsal thalamus from the CNS–PNS border. A more likely explanation comes from the demonstration that Schwann cells are normally present in the wall of small arteries, associated with innervation of the vessels.[10,23] Using tritiated thymidine, Harrison and Pollard[27] have demonstrated that Schwann cells located in perivascular spaces, rather than those in the dorsal roots, proliferate following a demyelinating lesion of the spinal cord. Schwann cells penetrating the experimentally-demyelinated spinal cord have been observed in clusters surrounding capillaries,[27,28,30,55] possibly in close proximity to a suitable extracellular matrix.[6] A similar observation was made in our study. This suggests that, in the neuron-depleted area, capillary walls may act as the substrate along which Schwann cells can migrate out of their original location in the perivascular walls of small arteries. The extent of this phenomenon is probably increased by the reactive angiogenesis that takes place after kainate lesion.[19]

Astrocytes, bordered by a basal lamina, form a glia limitans wherever the CNS neuropile is in contact with peripheral elements.[5,53,54] It has been clearly established that damage to the glial limiting membrane is an important prerequisite to Schwann cell invasion of the CNS in demyelinating lesions of the spinal cord and the retina.[5,8,28,42] The results obtained in the present study are in agreement with those summarized by Blakemore[5] concerning the remyelination by Schwann cells of spinal cord demyelinated axons. It has been observed in the present ultrastructural study that kainate injection is followed first by swelling of astrocytic processes leading to a disruption of the tight layer formed around capillaries by astrocytic endfeet. This is in keeping with our previous light-microscopic analysis that showed that reactive astrocytes are not present in the neuron-depleted area during the first weeks following kainate injection.[20] These results clearly underline the transitory failure of astrocytes to oppose the invasion of Schwann cells. Astrocytes, however, rapidly increase in number after one month, becoming the prevailing elements after three months. In the kainate lesion, as in other experimental conditions in which a breach appears in the glia limitans, astrocytes tend to repair it, thereafter excluding Schwann cells,[5,7,21,53] forming, in the excitotoxic lesion, a tight glia limitans around every Schwann cell.

Several studies have demonstrated that Schwann cells do not myelinate central axons unless these axons are demyelinated[7] or in a growth process[1,32,50,57]. The demyelination process observed in the neuron-depleted area provides a clear opportunity for Schwann cells to identify available axons. It is interesting to mention, however, that large (probably demyelinated) axons are not the only targets of Schwann cells. Numerous small axons are additionally ensheathed. The axonoglial relationships observed in the neuron-depleted thalamus are, in that sense, demonstrative of the fact that Schwann cells are not just replacing missing elements but acting with CNS axons as they would have done, had they been ensheathing PNS axons.

Structural alteration and possible growth of axons as a consequence of altered axonoglial relationships

In the excitotoxin-lesioned thalamus, terminals of lemniscal fibers exhibit structural changes and possible growth.[43] Time-course analysis of these changes has revealed that large varicosities formed over the first three weeks. Over the following months, thin processes grew out of these varicosities and extended into the neuron-depleted area, suggesting attempts at growth and regeneration (Refs 43, 44 and unpublished observations). The fact that altered afferents only arise from myelinated axons[44] and that the formation of terminal varicosities is concurrent with the period of demyelination observed here, suggest that there may be a relationship betweeen these axonal changes and the alteration of axonoglial interactions.

The remyelination of central axons by Schwann cells may aid both axonal survival as well as the growth of the thin processes out of the varicosities.[43,44] Schwann cells indeed produce neurotrophic

factors which promote survival and growth of both peripheral and central axons.[2,3,9,56] It has been proposed[16] that target-deprived fibers rapidly retract from areas of neuronal depletion due to the loss of maintenance factors released by target cells. Following the same reasoning, the observation of a long-term survival of target-deprived axons suggests some recovery of trophic influences in the target zone. The release of trophic factors in an excitotoxically lesioned area has been demonstrated biochemically[34] and Schwann cells appear to be good candidates for the production of some of these factors. When Schwann cells are involved in the demyelination–remyelination process, it may lead to local changes that sustain survival and regenerative attempts. The subsequent action of astrocytes, systematically confining Schwann cells, would then appear to be quite a negative process that induces a complete sterilization of the neuron-depleted area which, in turn, could result in an extension of the neuronal loss to areas containing the neurons of origin of the then retracting axons.

Acknowledgements—The authors wish to thank Dr Joanne Sweeney, Dr Salvatore Rapisardi and Dr Brigitte Onténiente for their critical review of the manuscript, Dr Janine Breton-Gorius, Josette Guichard (U 91) and Françoise Roudier (U 161) for their help with electron microscopy. Supported by Ministère de la Recherche et de la Technologie (MRT 106728) and ARSEP.

REFERENCES

1. Aguayo A. J., Charron L. and Bray G. M. (1976) Potential of Schwann cells from unmyelinated nerves to produce myelin: a quantitative ultrastructural and autoradiographic study. *J. Neurocytol.* **5,** 565–573.
2. Assouline J. G., Bosch P., Lim R., Kim I. S., Jensen R. and Pantazis N. J. (1987) Rat astrocytes and Schwann cells in culture synthesize nerve growth factor-like neurite-promoting factors. *Devl Brain Res.* **31,** 103–118.
3. Assouline J. G., and Pantazis N. J. (1989) Localization of the nerve growth factor receptor on fetal human Schwann cells in culture. *Expl Cell Res.* **182,** 499–512.
4. Blakemore W. F. (1979) Observations on remyelination in the rabbit spinal cord following demyelination induced by lysolecithin. *Neuropath. appl. Neurobiol.* **4,** 47–59.
5. Blakemore W. F. (1983) Remyelination of demyelinated spinal cord axons by Schwann cells. In *Spinal Cord Reconstruction* (eds Kao C. C., Bunge R. P. and Reier P. J.), pp. 281–291. Raven Press, New York.
6. Blakemore W. F. (1984) Limited remyelination of CNS axons by Schwann cells transplanted into the sub-arachnoid space. *J. neurol. Sci.* **64,** 265–276.
7. Blakemore W. F., Crang A. J. and Curtis R. (1986) The interaction of Schwann cells with CNS axons in regions containing normal astrocytes. *Acta neuropathol., Berlin* **71,** 295–300.
8. Blakemore W. F. and Patterson R. C. (1975) Observations on the interactions of Schwann cells and astrocytes following X-irradiation of neonatal rat spinal cord. *J. Neurocytol.* **4,** 573–585.
9. Bosch E. P., Zhong W. and Lim R. (1989) Axonal signals regulate expression of glia Maturation Factor-Beta in Schwann cells: an immunohistochemical study of injured sciatic nerves and cultured Schwann cells. *J. Neurosci.* **9,** 3690–3698.
10. Büssow H. (1978) Schwann cell myelin ensheathing CNS axons in the nerve fibre layer of the rat retina. *J. Neurocytol.* **7,** 207–214.
11. Cammer W., Bloom B. R., Norton W. T. and Gordon S. (1978) Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: a possible mechanism of inflammatory demyelination. *Proc. natn. Acad. Sci. U.S.A.* **75,** 1554–1558.
12. Coffey P. J., Perry V. H., Allen Y., Sinden J. and Rawlins J. N. P. (1988) Ibotenic acid induced demyelination in the central nervous system: a consequence of a local inflammatory response. *Neurosci. Lett.* **84,** 178–184.
13. Coffey P. J., Perry V. H., Rawlins J. N. P. (1990) An investigation into the early stages of the inflammatory response following ibotenic acid-induced neuronal degeneration. *Neuroscience* **35,** 121–132.
14. Collins G. H., West N. R. and Parmely J. D. (1986) The histopathology of freezing injury to the rat spinal cord. A light and electron microscope study. II. Repair and regeneration. *J. Neuropath. exp. Neurol.* **45,** 742–757.
15. Coyle J. T., Molliver M. E. and Kuhar M. J. (1978) *In situ* injection of kainic acid: a new method for selectively lesioning neuronal cell bodies while sparing axons of passage. *J. comp. Neurol.* **180,** 301–324.
16. Coyle J. T. and Schwarcz R. (1983) The use of excitatory amino acids as selective neurotoxins. In *Handbook of Chemical Neuroanatomy* (eds Björklund A. and Hökfelt T.), Vol. 1, pp. 508–527. Elsevier, Amsterdam.
17. Divac I., Markowitsch H. J. and Pritzel M. (1978) Behavioral and anatomical consequences of small intrastriatal injections of kainic acid in the rat. *Brain Res.* **151,** 523–532.
18. Dusart I., Isacson O., Nothias F., Gumpel M. and Peschanski M. (1989) Presence of Schwann cells in neurodegenerative lesions of the central nervous system. *Neurosci. Lett.* **105,** 246–250.
19. Dusart I., Nothias F., Roudier F., Besson J. M. and Peschanski M. (1989) Vascularization of fetal cell suspension grafts in the excitotoxically lesioned adult rat thalamus. *Devl Brain Res.* **48,** 215–228.
20. Dusart I. Marty S. and Peschanski M. (1991) Glial changes following an excitotoxic lesion in the CNS II. Astrocytes. *Neuroscience* **45,** 541–549.
21. Fishman P. S., Nilaver G. and Kelly J. P. (1983) Astrogliosis limits the integration of the peripheral nerve grafts into the spinal cord. *Brain Res.* **277,** 175–180.
22. Feigin I. and Popoff N. (1966) Regeneration of myelin in multiple sclerosis: the role of mesenchymal cells in such regeneration and in myelin formation in the peripheral nervous system. *Neurology* **16,** 364–372.
23. Gabella G. (1976) *Structure of the Autonomic Nervous System.* Chapman and Hall, London.
24. Gilmore S. A. and Duncan D. (1968) On the presence of peripheral-like nervous and connective tissue within irradiated spinal cord. *Anat. Rec.* **160,** 675–690.

25. Gilmore S. A., Sims, T. J. and Heard J. K. (1982) Autoradiographic and ultrastructural studies of areas of spinal cord occupied by Schwann cells and Schwann cell myelin. *Brain Res.* **239,** 365–375.
26. Gledhill R. F. and McDonald W. I. (1977) Morphological characteristics of central demyelination and remyelination. *Ann. Neurol.* **1,** 552–560.
27. Harrison B. M. and Pollard J. D. (1984) Pattern of Schwann cell remyelination in a spinal cord lesion. *Neurosci. Lett.* **52,** 275–280.
28. Harrison B. M. (1987) Schwann cells divide in a demyelinating lesion of the central nervous system. *Brain Res.* **409,** 163–168.
29. Herndon R. M., Addick E. and Coyle J. T. (1980) Ultrastructural analysis of kainic acid lesions to the cerebellar cortex. *Neuroscience* **5,** 1015–1026.
30. Hirano A., Zimmerman H. M. and Levine S. (1969) Electron microscopic observations of peripheral myelin in a central nervous system lesion. *Acta neuropath., Berlin* **12,** 348–365.
31. Kettenmann H., Blankerfeld G. V. and Trotter J. (1991) Physiological properties of oligodendrocytes during development. *Ann. N.Y. Acad. Sci.* **633,** 64–77.
32. Katzman R., Broida R. and Raine C. S. (1977) Reinnervation, myelination and organization of iris tissue implanted into the rat midbrain—an ultrastructural study. *Brain Res.* **138,** 423–443.
33. Krammer E. B., Lischka M. F., Karobath M. and Schonbeck G. (1979) Is there a selectivity of neuronal degeneration induced by intrastriatal injection of kainic acid? *Brain Res.* **177,** 577–582.
34. Manthorpe M., Nieto-Sampedro M., Skaper S. D., Lewis E. R., Barbin G., Longo F. M., Cotman C. W. and Varon S. (1983) Neuronotrophic activity in brain wounds of the developing rat. Correlation with implant survival in the wound cavity. *Brain Res.* **267,** 47–56.
35. Marty S., Dusart I. and Peschanski M. (1991) Glial changes following an excitotoxic lesion in the CNS. I. Microglia/macrophages. *Neuroscience* **45,** 529–539.
36. Murabe Y., Ibata Y. and Sano Y. (1981) Morphological studies on neuroglia. III. Macrophage response and "microgliocytosis" in kainic acid-induced lesions. *Cell Tiss. Res.* **218,** 75–86.
37. Nadler J. W., Perry B. W., Gentry W. and Cotman C. W. (1981) Fate of the hippocampal mossy fibres projection after destruction of its postsynaptic targets with intraventricular kainic acid. *J. comp. Neurol.* **196,** 549–569.
38. Nagashima K., Wege H., Meyermann R. and Meulen ter V. (1979) Demyelinating encephalomyelitis induced by a long-term Corona virus infection in rats. *Acta neuropath., Berlin* **45,** 205–213.
39. Nothias F., Wictorin K., Isacson O., Björklund A. and Peschanski M. (1988) Morphological alteration of thalamic afferents in the excitotoxically lesioned striatum. *Brain Res.* **461,** 349–354.
40. Nothias F., Cadusseau J., Dusart I. and Peschanski M. (1991) Fetal neural transplants into an area of neurodegeneration in the spinal cord of the adult rat. *Restor. Neurol. Neurosci.* **2,** 183–188.
41. Périer O. and Grégoire A. (1965) Electron microscopic features of multiple sclerosis lesions. *Brain* **88,** 937–952.
42. Perry V. H. and Hayes L. (1985) Lesion-induced myelin formation in the retina. *J. Neurocytol.* **14,** 297–307.
43. Peschanski M. and Besson J. M. (1987) Structural alteration and possible growth of afferents after kainate lesion in the adult thalamus. *J. comp. Neurol.* **258,** 185–203.
44. Peschanski M. and Besson J. M. (1987) Kainic acid induced neurodegeneration in ventrobasal complex of the rat thalamus: growth cone-like endings of functional afferent fibers. In *Effects of Injury on Trigeminal and Spinal Somatosensory Systems*, pp. 331–338. Alan R. Liss, New York.
45. Peschanski M., Roudier F., Ralston H. J. III and Besson J. M. (1985) Ultrastructural analysis of the terminals of various somatosensory pathways in the ventrobasal complex of the rat thalamus: an electron-microscopic study using wheatgerm agglutinin conjugated to horseradish peroxidase as an axonal tracer. *Somatosensory Res.* **3,** 75–87.
46. Peters A., Palay S. L. and Webster H. de F. (1991) *The Fine Structure of the Nervous System.* Oxford Univ. Press, New York.
47. Peterson G. M. and Moore R. Y. (1980) Selective effects of kainic acid on diencephalic neurons. *Brain Res.* **202,** 165–182.
48. Prineas J. W. and Connell F. (1979) Remyelination in multiple sclerosis. *Ann. Neurol.* **5,** 22–31.
49. Prineas J. W. (1985) The neuropathology of multiple sclerosis. In *Handbook of Clinical Neurology: Demyelinating Diseases* (ed. Koestsier J. C.), pp. 213–257. Elsevier, Amsterdam.
50. Richardson P. M., McGuinness U. M. and Aguayo A. J. (1982) Peripheral nerve autografts to the rat spinal cord: studies with axonal tracing methods. *Brain Res.* **237,** 147–162.
51. Scolding N. J., Morgan B. P., Houston W. A. J., Linington C., Campell A. K. and Compston D. A. S. (1989) Vesicular removal by oligodendrocytes of membrane attack complexes formed by activated complement. *Nature* **339,** 620–622.
52. Selmaj K. W. and Raine C. S. (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage *in vitro*. *Ann. Neurol.* **23,** 339–346.
53. Sims T. J. and Gilmore S. A. (1983) Interactions between intraspinal Schwann cells and the cellular constituents normally occurring in the spinal cord: an ultrastructural study in the irradiated rat. *Brain Res.* **276,** 17–30.
54. Sims T. J., Gilmore S. A., Waxman S. G. and Klinge E. (1985) Dorsal–ventral differences in the glial limitans of the spinal cord: an ultrastructural study in developing normal and irradiated rats. *J. Neuropath. exp. Neurol.* **44,** 415–429.
55. Snyder D. H., Valsamis M. P., Stone S. H. and Raine C. S. (1975) Progressive demyelination and reparative phenomena in chronic experimental allergic encephalomyelitis. *J. Neuropath. exp. Neurol.* **3,** 209–221.
56. Taniuchi M., Clark B., Schweitzer J. B. and Johnson E. M. (1988) Expression of nerve growth factor receptors by Schwann cells of axotomized peripheral nerves: ultrastructural location, suppression by axonal contact, and binding properties. *J. Neurosci.* **8,** 664–681.
57. Weinberg E. L. and Raine C. S. (1980) Reinnervation of peripheral nerve segments implanted into the rat central nervous system. *Brain Res.* **198,** 1–11.

58. Wren D. R. and Noble M. (1989) Oligodendrocytes and oligodendrocyte/type-2 astrocyte progenitor cells of the adult rats are specifically susceptible to the lytic effects of complement in absence of antibody. *Proc. natn. Acad. Sci. U.S.A.* **86,** 9025–9029.
59. Wuerthele S. M., Lovell K. M., Jones M. Z. and Moore K. E. (1978) A histological study of kainic acid induced lesions in the rat brain. *Brain Res.* **147,** 489–497.
60. Zaczeck R., Schwarcz R. and Coyle J. T. (1978) Long-term sequelae of striatal kainate lesion. *Brain Res.* **152,** 626–632.

(*Accepted* 1 *June* 1992)