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Radial glia and cell debris removal during lesion-regeneration of the lizard medial cortex

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Summary. Intraperitoneal injection of the neurotoxin 3-acetylpyridine (3AP) induces a rapid degeneration of the medial cerebral cortex (lizard fascia dentata) granular layer and of its zinc enriched axonal projection (lizard mossy fibres). After 6-8 weeks post-lesion the cell debris have been removed and the granular layer is repopulated by neurons generated in the subjacent ependyma. Both processes, neuron incorporation and debris removal, seem to be crucial for successful regeneration. Scavenging processes in the lesioned mammalian CNS are usually carried out by microglia and/or astrocytes. In the lizard cerebral cortex there are no free astrocytes and the only glial fibrillary acid (GFAP) immunoreactive cells are radial glia-ependymocytes, similar to those present during mammalian CNS development. Ependymocytes, in addition to their help in vertical migrations of just generated immature neurons, built the cortical glial scaffold, insulate the blood capillaries, form the outer glial limiting membrane, thus playing an essential role in the lizard cortical blood-brain barrier. In this study, by means of GFAP-immunocytochemistry and electron microscopy, we have shown that radial glial cells participate actively in the removal/phagocytosis of cellular debris generated in the lesion process: mainly degenerated synapses, but interestingly, also some neuronal somata. Cell debris taken up by ependymocyte lateral processes seem to be progressively transported to either distal (pial) or proximal (ventricular) poles of the cell, where they result in lipofuscin accumulations. The hypothetical subsequent exchange of debris from ependymoglia by microglia/macrophages and Kolmer cells is discussed.

Key words: Ependyma, Phagocytosis, Hippocampus, Postnatal neurogenesis, 3-acetylpyrydine

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Introduction

The lizard cerebral cortex has been considered as a reptilian hippocampus on the basis of its connectivity, cytoarchitectonics and development (López-García et al., 1992). The medial cortex, a region resembling the mammalian fascia dentata (Molowny and López-García, 1978; López-García et al.,1983; López-García and Martínez-Guijarro, 1988), undergoes postnatal neurogenesis (López-García et al., 1988) and is capable of regenerating itself if lesioned specifically with the neurotoxin 3-acetylpyridine (3AP) (Font et al., 1991). The new neurons that replace the lesioned ones are generated in the subjacent neuroepithelium (sulcus) which shows cell proliferative activity throughout the lizard life span (Kirsche, 1967; López-García et al., 1988).

In mammals, nervous tissue damage is usually followed by a characteristic gliotic response in which both microglia and astroglia participate (see Landis, 1994). An injury to the mammalian CNS usually implies the formation of a glial scar, mainly constituted of reactive astrocytes; this scar impedes correct growth of new axon branches and thus prevents neuronal circuitry repair (Berry et al., 1983; Reier, 1986).

Debris removal seems to be crucial to achieving a successful restoration of damaged adult nervous tissue. Glial cells are beneficial for neural repair, removing degenerating debris (Pow et al., 1989) and providing structural support in regions undergoing substantial degeneration and cell death (Gentschev and Sotelo, 1973). Nevertheless, the neuronal debris is rather inefficiently removed (Sloviter et al., 1993, 1996) and this could be one factor that prevents neuronal regeneration in the mammalian CNS.

In lizards, microglia cells transiently disappear from the medial cortex during the first days following the 3AP-lesion, re-invading it afterwards (López-García et al., 1994). The participation of these cells in debris removal is delayed and unexpected. Moreover, in the lizard cerebral cortex there are no free astrocytes. The only glial fibrillar acidic protein (GFAP) immunoreactive cells are ependymocyte-radial glia cells, closely

similar to those of embryonic mammalian nervous tissue (Rakic, 1982; Rickmann et al., 1987; Yanes et al., 1992). Ependymocyte-radial glia cell somata are localised in the ependyma lining the cerebral ventricles. Long radial processes arise from these somata; these radial shafts traverse the cortex reaching the pial surface, where their end-feet form the limiting glial membrane of the brain parenchyma. Abundant varicose processes arising from the ependymocyte-radial glia shafts cover, on one side, the blood vessel surface forming part of the blood-brain barrier (García-Verdugo et al., 1981) and, on the other side, partially cover synaptic contacts (perisynaptic glial processes) and the rest of neuronal processes. Moreover, the initial segment of these ependymocyte shafts may guide the newly generated neurons to their final location in the medial cortex (Garcia-Verdugo et al., 1986).

In order to study the role of radial glia in the debris scavenging after lizard medial cortex lesion, we have used GFAP immunocytochemistry as well as electron microscopy. Our results confirm that radial glia participates in the debris removal.

Materials and methods

56 Healthy adult lizards (4-5,5 cm snout-vent) of the species *Podarcis hispanica*, captured in the surroundings of Burjassot, Valencia (Spain) and maintained in terraria simulating their environmental conditions were used in this study. Experimental protocols were carried out according to the guidelines concerning animal care and protection in our institution.

An intraperitoneal injection of the neurotoxin 3-acetylpyridine (3AP) (dose: 150 mg Kg^{-1} b/w) was performed in 32 lizards. Non injected lizards (n=7) were used as negative controls. At various days post-lesion (1, 2, 4, 7, 15, 30 and 42 days), animals under ether anaesthesia were transcardially perfused with either 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2-7.4 (n=18) for light microscopy, or with 2% paraformaldehyde, 2% glutaraldehyde in 0.1M phosphate buffer (n=21) for electron microscopy. Then the brains were removed and postfixed in the same fixative for 6 hours at 4 °C. After dehydrating, the brains were either embedded in Polywax (BDH, Poole) and serially sectioned (parallel series of 10 μm thick transverse sections) or transversely sliced (75-100 μm thick slices) using a vibratome. Glutaraldehyde fixed slices were postfixed in 1% osmium tetroxide and embedded in epoxy resin (TAAB, Aldersmarton). Semithin sections were toluidine blue stained and observed under the light microscope to asses the lesion extent of the neurotoxin. Selected sections were re-embedded; ultrathin sections obtained from them were lead stained and observed under the electron microscope.

In addition, some lizard cortex Golgi impregnated sections belonging to the laboratory collection were also used in this study.

GFAP immunocytochemistry

After Polywax removal with alcohol or resin removal with etoxide, sections were immunostained as described previously (Nacher et al., 1996). Briefly, sections were incubated overnight in a polyclonal anti-GFAP antibody (Sigma, St. Louis, MO) 1:500 dilution. The second layer was a goat anti-rabbit IgG (Sternberger-Meyer, Baltimore) 1:100 dilution, for 30 minutes, followed by the third layer: Rabbit peroxidase-antiperoxidase (PAP) complex (Sternberger-Meyer, Baltimore) 1:100 dilution for 30 minutes. 3,3'-diaminobenzidine 4HCl (Sigma, St. Louis) and H_2O_2 were used for colour development. Controls without primary antibody were done for assessment of correct immunostaining.

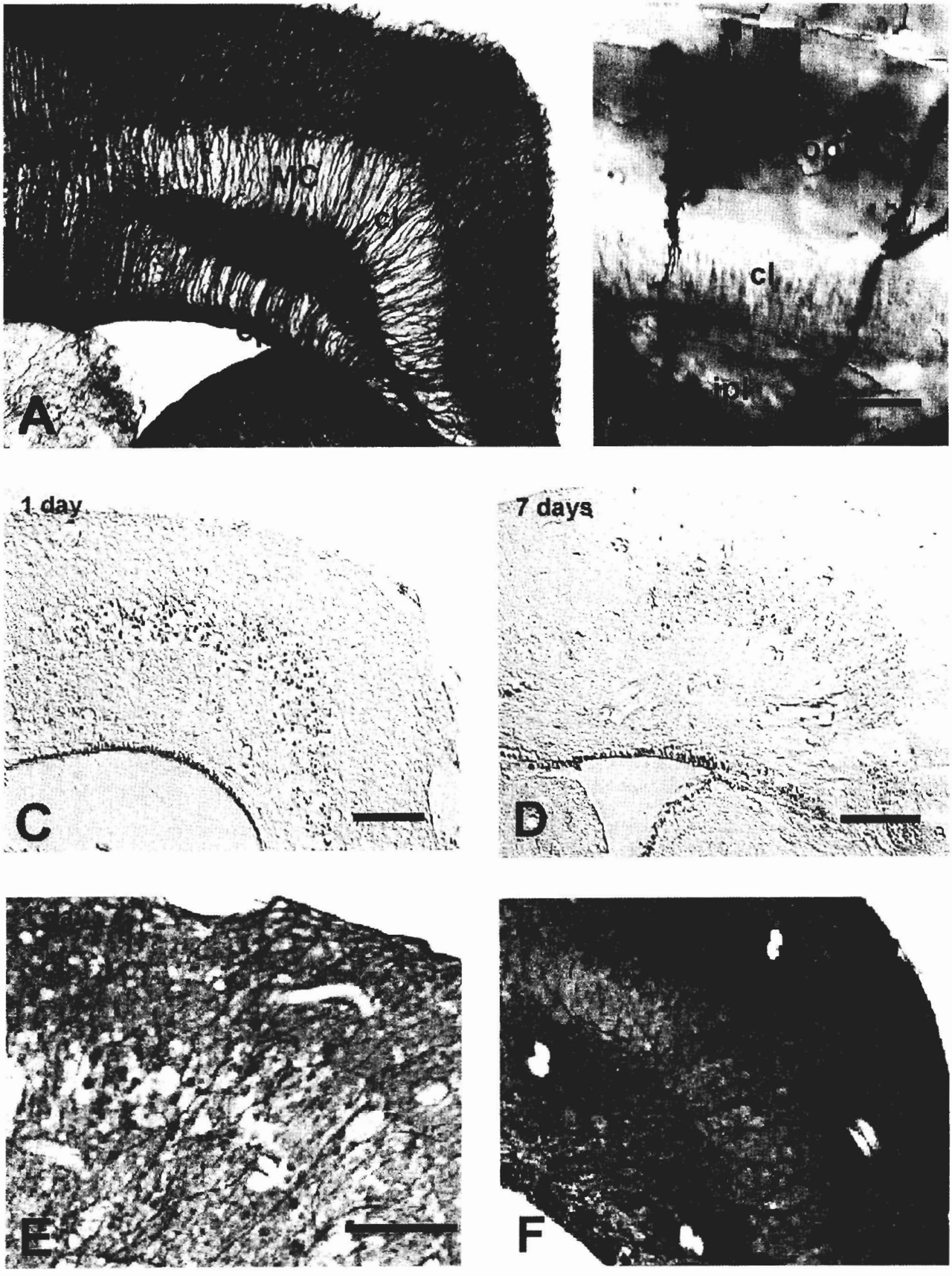
Results

GFAP immunostaining of slices revealed the ependymocyte-radial glia scaffold of the lizard cerebral cortex (Fig. 1A) and also showed the conspicuous lamination pattern of the medial cortex. Silver chromate impregnation of isolated ependymocyte-radial glia cells (Fig. 1B) also revealed the different segments of the radial glial shafts, i.e., a deep or proximal-slender segment close to the ependyma, a second varicose segment while crossing the inner dendritic layer beneath the cell layer, a third-slender segment while crossing the cell layer and finally some outer plexiform layer branches reaching the pial surface. Ramification of ependymocyte-radial glial branches in the outer plexiform layer of the medial cortex was frequent.

Time-course of morphological changes induced by 3AP lesion

In semithin and Polywax sections, necrotic nuclei (seen as pyknotic in toluidine blue stained sections and assessed under electron microscopy), swollen somata and cytoplasmatic processes were clearly recognised after 3AP injection (Fig. 1C, D). The lesion degree was

Fig. 1. A. Radial glia scaffold in the medial and dorsomedial cortices of a control lizard. Vibratome section (75 μm thick) immunostained for GFAP. DMC: dorsomedial cortex; MC: medial cortex; ep: ependyma; ipl: inner plexiform layer; cl: cell layer; opl: outer plexiform layer. **B.** Photomontage of Golgi impregnated radial glia cells in the lizard medial cortex. The somata are located in the ependyma and their long and spinous radial process traverse every cortex layer to the outer surface of the brain. ipl: inner plexiform layer; cl: cell layer; opl: outer plexiform layer. **C and D.** Toluidine blue stained Polywax sections of the medial cortex. C: 1 day after lesion; note the presence of abundant pyknotic nuclei in the cell layer and the vacuolized aspect of the plexiform layers. D: 7 days after lesion; the number of pyknotic nuclei in the cell layer is scarce when compared with that of figure C. **E and F.** Polywax sections immunostained for GFAP. E: 1 day after lesion. Weak labelled processes can be seen traversing the plexiform and cell layers. F: 7 days after lesion. The radial glia processes and endfeet appear intensely labelled, especially in the outer plexiform layer. Scale bars: 50 μm.


A
cl
1 day
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7 days
D
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F

highly variable among 3AP injected animals; usually the percentage of pyknotic versus normal nuclei in the medial cortex cell layer was different among animals sacrificed at the same time postlesion. In all cases this percentage progressively decreased as the regeneration progressed and the observed morphological changes after 3AP lesion agree with those described in previous studies (López-García et al., 1992, 1994). In short: twelve hours after 3AP injection, some neuronal somata of the medial cortex displayed initial symptoms of necrosis, including swelling of the cytoplasm and chromatin condensation. One day after 3AP injection, many neuronal somata of the medial cortex cell layer had pyknotic nuclei and swollen cytoplasm, features which are symptomatic of cell death; nevertheless, a few neuronal somata resisted the lesion. From days 2-8 post-lesion, many pyknotic nuclei, residual debris and non-lesioned somata coexisted in the medial cortex cell layer. From days 8-30, immature neuronal somata appeared there. Sometimes, they displayed fusiform or polarised cytoplasm and fine grain dispersed chromatin inside their nuclei which is similar to the morphology of migratory neurons that could be frequently seen in the inner plexiform of these animals. Then, the numbers of pyknotic nuclei in the medial cortex cell layer progressively decreased, and that of new immature plus normal somata increased. Several months later the lizard medial cortex appeared indistinguishable from that of a control lizard.

Although there was a huge amount of neuronal death in the medial cortex cell layer, we have only observed some scarce isolated pyknotic nuclei in the cortical ependyma of lesioned lizards.

The dorsal and dorsomedial cortex plexiform layers were also affected by the 3AP injection, especially those regions occupied by the zinc enriched axonal projection of the granular neurons of the medial cortex (principal targets of 3AP neurotoxin). Interestingly, the degenerative changes in these areas appeared clearly delayed in time with respect to those degenerative changes observed in the medial cortex; they only affected a small fraction of cell somata in these cortical areas.

GFAP immunocytochemistry and electron microscopy

Medial Cortex

GFAP immunostained sections showed the profiles of radial glia shafts cytoskeleton (Fig. 1E,F), especially their end-feet onto the pial surface, and allowed the discrimination of the density of these profiles in semithin sections throughout the lesion-regeneration process. The numerical density of these profiles varied strongly between individuals, independently of the survival time, but the thickness of the profiles varied (2.6±0.75 μm in controls; 4±1.46 μm in 15 days postlesion animals; 3.14±0.7 μm in 150 days postlesion animals) and was visually detected as an increment of immunostaining from day 7-15 postlesion.

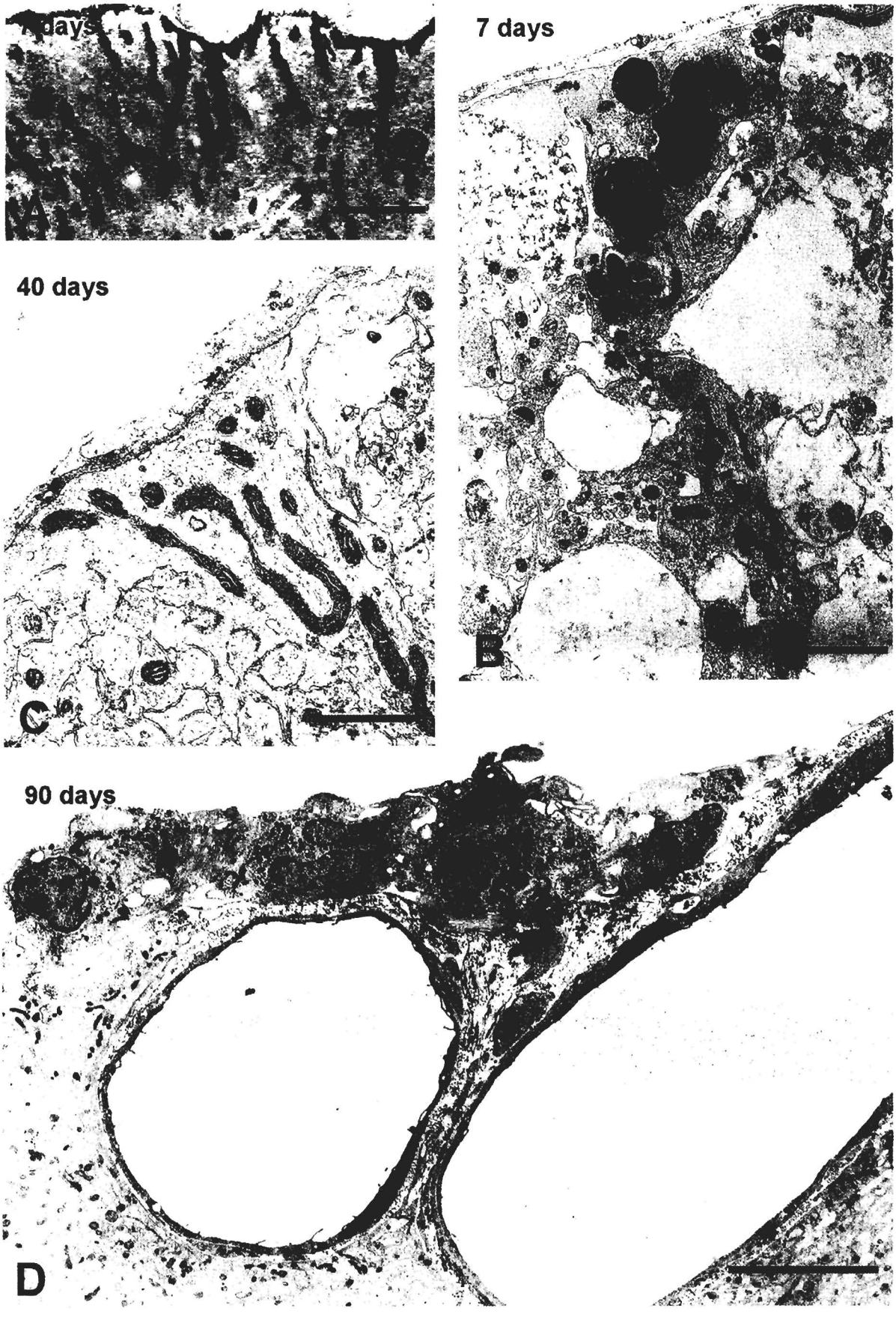
As soon as day one postlesion, GFAP positive profiles became more sinuous and displayed thickenings with vacuole like structures inside, especially in the segments located in the outer plexiform layer. Seven days after the 3AP injection these profiles were more prominent (Fig. 2A). Under the electron microscope these thickenings appeared occupied by electron-dense structures (Fig. 2B) which subsequently disappeared in regenerated animals (Fig. 2C). At 7 days post-lesion, abundant degenerated synaptic contacts could be observed in the plexiform layers of the medial, dorsomedial and dorsal cortex, especially in the zinc enriched areas (Fig. 3A). Some of these degenerating synapses persisted two weeks after the lesion, and could be seen surrounded by ependymocyte-radial glia profiles (Fig. 3B,C). These profiles appeared as ring-like structures in the GFAP immunostained semithin sections (Fig. 3B). GFAP positive rings and thickenings persisted in the outer plexiform layer of 7-10 days postlesion animals but these structures progressively shifted to the radial glia segments crossing the cell layer; they were especially abundant in 15-20 days postlesion animals (Fig. 4 A-C). At this time, 15-20 days postlesion, similar structures appeared in the inner radial glia segments crossing the inner plexiform layer and the juxta-ependymary alveus layer.

The electron microscope showed that GFAP positive rings in the cell layer were ependymocyte-radial glia processes surrounding bigger cell debris including pyknotic nuclei (Fig. 4D,E).

In the radial glia of 30-40 days postlesion animals almost every distal GFAP positive segment located in the outer plexiform layer, and its corresponding end-feet, displayed a completely normal aspect. Nevertheless, there were many thickenings in the inner plexiform layer and numerous degenerating cells were still surrounded by GFAP immunoreactive processes in the cell layer.

After long survival periods (90-150 days), occasional thickenings and cell debris could still be seen in the inner ependymocyte-radial glia segments located in the inner plexiform layer and close to the ependyma (Fig. 5) while the distal radial cytoplasmatic shafts and pial end-feet displayed a normal appearance. Interestingly, granular-macrophage-like cells were abundant in the

Fig. 2. Micrographs showing the aspect of radial glia endfeet located in the outer limiting membrane of the medial cortex. **A.** 7 days post-lesion. Semithin section where the GFAP immunolabelled endfeet appear inflated. **B.** 7 days post-lesion, electron microscopy. Observe the presence of degenerated structures inside the endfeet and the terminal process. **C.** 40 days postlesion. Endfeet absolutely devoid of degenerating structures. **D.** 90 days post-lesion. The glial endfeet are free of cellular debris; observe two blood vessel profiles and some microglia/macrophage-like cells located in the meninges with abundant lipofuscin inclusions. Scale bars: 10 μm in A, 1 μm in B and C, 5 μm in D.


4 days
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juxta-pial meningeal space at this time (Fig. 2D). Looking at the ventricular surface, the cell somata of ependymocytes accumulated abundant lipofuscin electron dense residual bodies (Fig. 5E).

Dorsomedial and dorsal cortices

Although delayed in time, intense ultrastructural and immunocytochemical changes in radial glia processes located in the dorsal and dorsomedial cortices of 3AP lesioned animals were also observed. Disorganised-ramified GFAP immunoreactive processes, showing abundant thickenings in their endfoot, were observed in the dorsal and dorsomedial cortices of lesioned animals after 7 days. In contrast, such changes were seen in the medial cortex radial glia even at 4 days after 3AP injection. Simultaneously, the electron microscope demonstrated these structures filled with cellular debris; which persisted for a prolonged period in these areas. Lizards surviving up to three months still displayed many residual thickenings and cell debris inside the radial glia apical shafts of the dorsal and dorsomedial cortices.

Discussion

The results presented here suggest that radial glia cells are actively involved in the removal of cellular debris generated during the lesion of the lizard medial cortex. These cells participate in the phagocytosis of both degenerated neurites and neuronal somata.

The glial cytoarchitectonics in the lizard cortex

The lizard cerebral cortex is populated by three types of glial cells: ependymocytes or radial glia cells, oligodendrocytes and microglia. Oligodendrocytes are scarce in the lizard cortex and are usually located in a zone of myelinated fibres above the ependyma, the alveus (Molowny et al., 1972; Yanes et al., 1992). Microglia cells populate the medial, dorsomedial, dorsal and lateral cortices although some regional differences can be detected (Berbel et al., 1981; Castellano et al., 1991).

As has been demonstrated for other lizard species, no free GFAP immunoreactive cells resembling astrocytes can be detected in the cerebral cortex of control lizards (Yanes et al., 1990). Some electron microscopic studies also support the absence of astrocytes (García-Verdugo et al., 1981). Nevertheless, the presence of glutamine synthetase immunoreactive cells resembling astrocytes suggests that some free astroglia cells could exist in the brain of other lizard species (Monzon-Mayor et al., 1990).

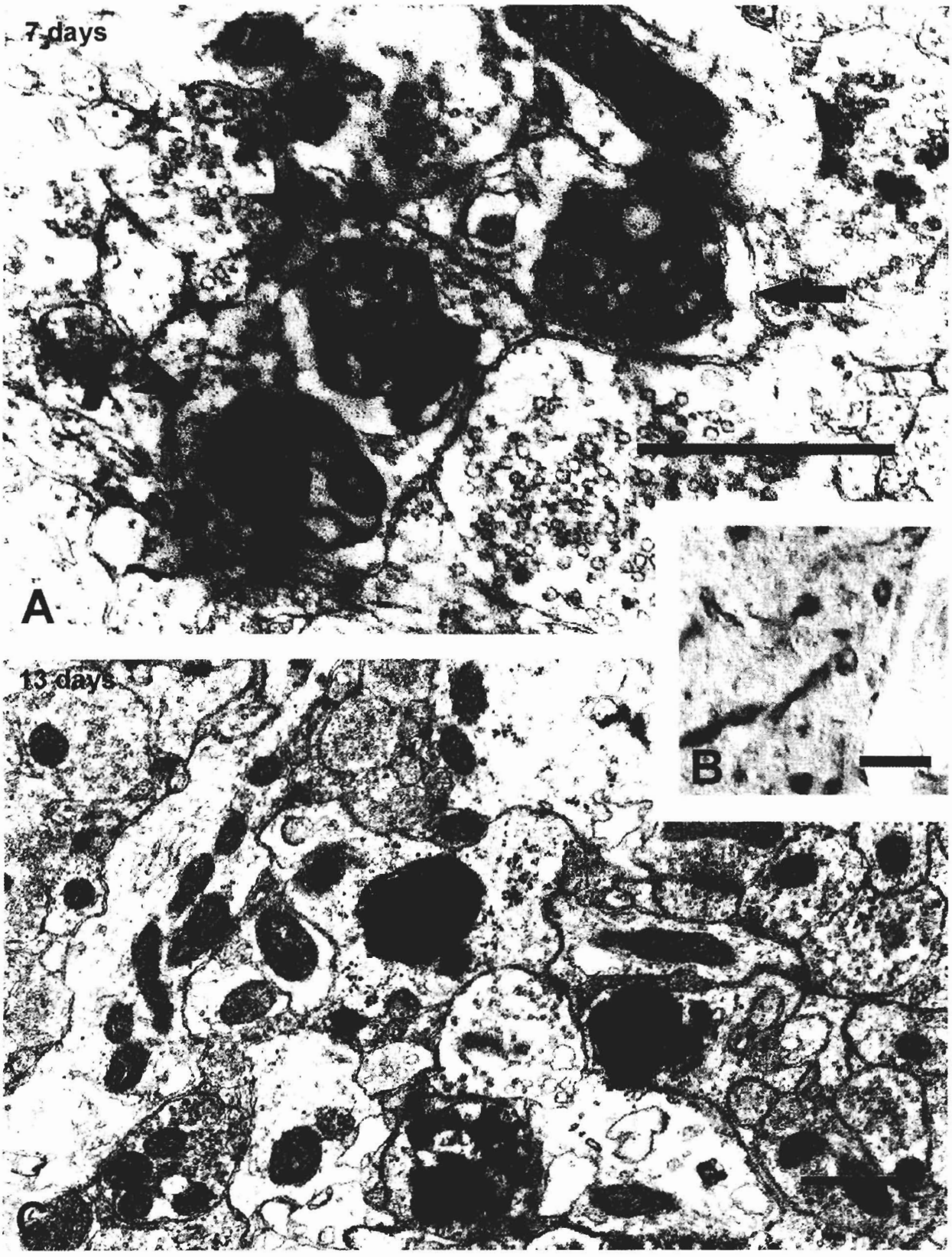
The lizard ependyma lining the telencephalic ventricles appears formed of a single layer of cells, the ependymocytes that emit thick vertical shafts reaching the pial surface (radial glia). Some ependymal areas, the sulci, display a pseudostratified appearance and are considered as zones of residual embryonic neuroepithelium (Kirsche, 1967) and are also populated by radial glia. Radial glia cells have their somata located in the ventricular wall and extend long vertical cytoplasmatic prolongations that reach the cerebral surface. The extremities of the radial glia (end-feet) form the glial limiting membrane. They also have processes that surround the blood vessels forming the blood-brain barrier (García-Verdugo et al., 1981). Almost every radial glial cell has gliofilaments of GFAP, from which they are considered of astrocytic lineage (Levitt and Rakic, 1980; Onteniente et al., 1983; Benjelloun-Touimi et al., 1985; Dahl et al., 1985). Under normal conditions, no GFAP immunostaining is usually seen in the varicosities that emerge from the main cytoplasmatic processes, probably due to the absence of filamentous GFAP in these structures. However, these varicosities, which phagocytose the neuronal debris, can be visualised in the electron microscope or after GFAP immunostaining in 3AP-lesioned animals, thus indicating some functional/mechanical activation after the lesion.

Previous electron microscopic studies have shown morphological heterogeneity in the lizard cortex ependymocytes (García-Verdugo et al., 1981); this raises the question of whether distinct types of ependymocytes may exist in the lizard cortex. If so, some of them could act as stem cells (neuroblasts/glioblasts) as has already been pointed out (López-García et al., 1988; Molowny et al., 1995), others could participate in the cortical glial scaffold and blood brain barrier, and some others could participate in the post-lesion scavenging phenomena. The other possibility is that the ultrastructural differences between ependymocytes only reflect the differential activity of the same cellular type. In fact, the subsequential changes following 3AP lesion include a dramatic ultrastructural homogenisation of the ependymocyte somata of the lizard cortex (unpublished observations)

Radial glia and neurogenesis

In the developing mammalian CNS, radial glial cells guide neurons generated in the neuroepithelium to their final destination in the brain parenchyma (Rakic, 1972, 1982; Anton et al., 1996). In many areas of the

Fig. 3. Lesioned boutons in the outer plexiform layer of the dorsomedial cortex. **A.** Electron micrograph showing some lesioned synaptic boutons with acute (arrowheads) or mild (arrow) symptoms of degeneration; other boutons appear unaffected (7 days post-lesion). **B.** Semithin section immunolabelled for GFAP; observe labelled processes with ring-like shape (13 days postlesion). **C.** Electron micrograph showing various degenerating and non degenerating boutons and synapses which appear dark and surrounded by glial processes with glycogen granules inside the glial cytoplasm. Scale bars: 1 μm in A and C, 5 μm in B.


7 days
A
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mammalian CNS radial glia are transitory elements which disappear and become astrocytes after the neuronal migration period is over (Ramón y Cajal, 1911; Levitt and Rakic, 1980; Marín-Padilla, 1995). Nevertheless, some centres in the adult mammalian CNS have radial glia: pituycites-tanycites in the hypothalamus and neurohypophyseal system (Scott et al., 1995), Bergman glia in the cerebellum (Choi and Lapham, 1980) or radial glia proper in the hippocampus (Gould et al., 1992). Radial glia also persist during adulthood in the CNS of birds (Alvarez Buylla et al., 1987), frogs (Chetverukhin and Polenov, 1993) and fishes (Achucarro, 1915; Ebner and Colonnier, 1975). The CNS of reptiles presents a well developed radial glial scaffold, particularly in the telencephalon. These glial cells can be found in lizards (Ramón y Cajal, 1917; Regidor et al., 1974; García-

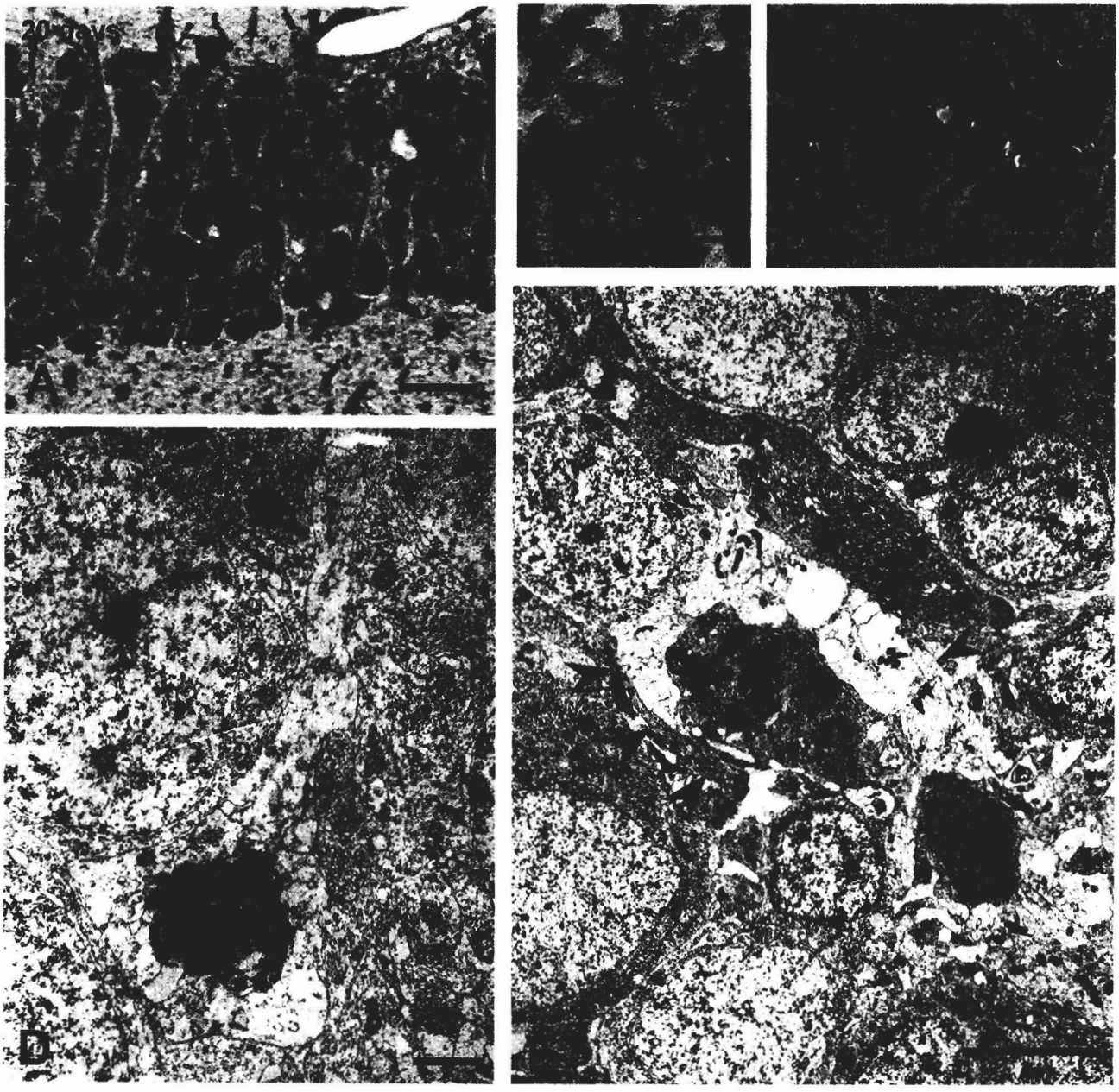

Fig. 4. Aspects of GFAP immunolabelled processes in the medial cortex cell layer and degenerating pyknotic nuclei; 20 days postlesion. **A-C**: Micrographs of semithin sections showing GFAP immunolabelled processes surrounding degenerating granular neurons. **D and E**. Electron micrographs of degenerated nuclei surrounded by glial processes (arrowheads). D corresponds with the squared immunolabelled process in A. Scale bars: 20 μm in A, 10 μm in B and C, 1 μm in C, 5 μm in D, 0,5 μm in E.

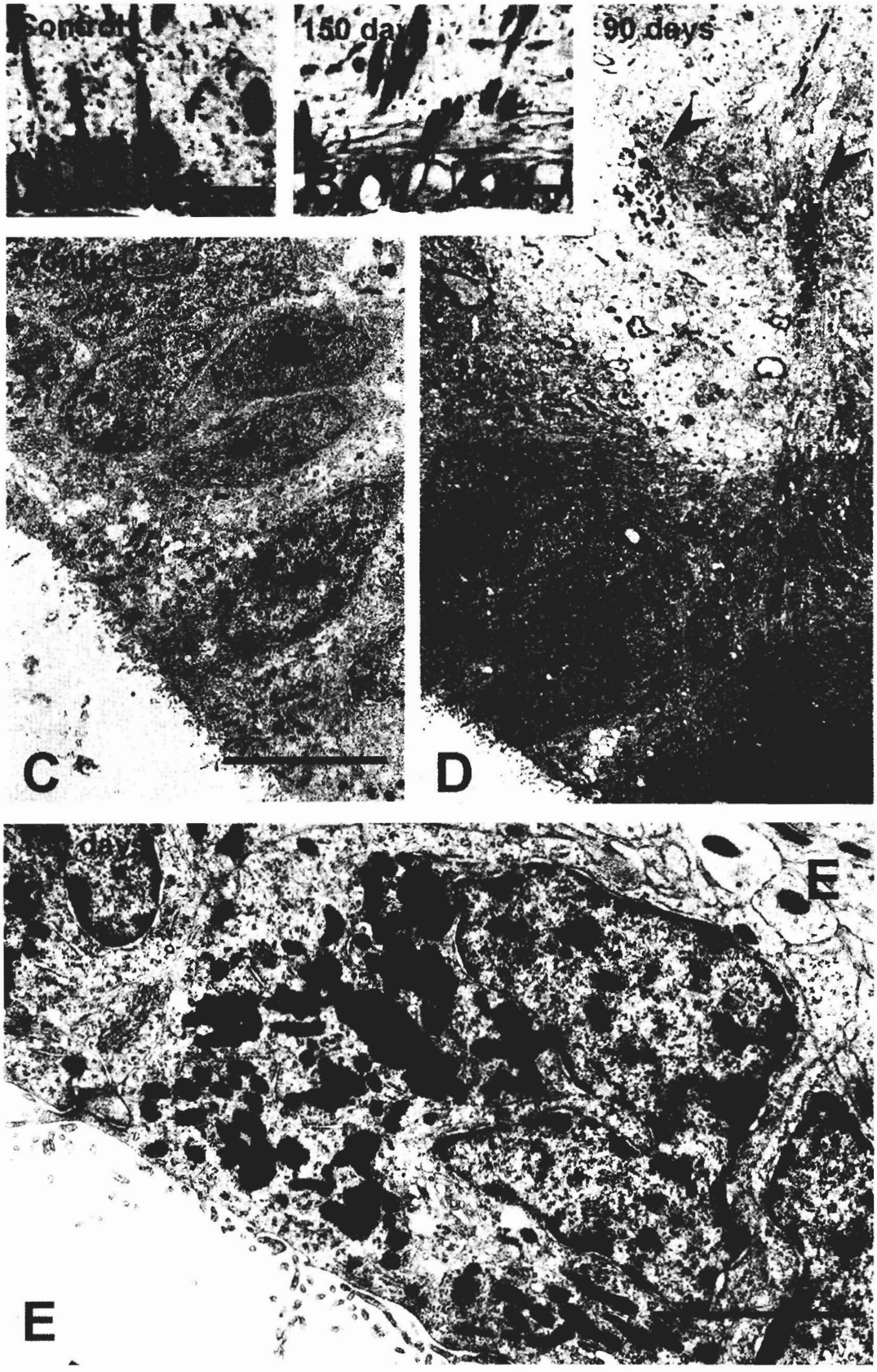

Fig. 5. Medial cortex inner plexiform layer and ependyma.
A and B. Semithin sections immunolabelled for GFAP: A: Contro. The asterisk signals a juxtaependymary neuronal somata; B: 150 days post-lesion. Note the presence of cytoplasmatic inclusions inside the ependymal somata (arrowheads). **C.** Electron micrograph showing the ependyma of the medial cortex in a control non-lesioned lizard. **D.** 90 days post-lesion; cellular debris traversing the inner plexiform layer inside radial glia processes (arrowheads); observe that the ependymal somata still appear devoid of degenerating structures. **E.** 150 days post-lesion, detail of an ependymocyte (radial glia) soma showing abundant lipofuscin bodies inside its cytoplasm. Scale bars: 10 μm in A and B, 5 μm in C, 5 μm for D, 0.5 μm for E

Verdugo et al., 1981; Yanes et al., 1990) and also in turtles (Ebner and Colonnier, 1975; Kriegstein et al., 1986) and snakes (Onteniente et al., 1983). Surprisingly, all of these vertebrate CNS areas in which radial glia persist after birth also show postnatal neurogenesis (see Alvarez Buylla and Lois (1995) for review): fish brain (Raymond and Easter, 1983; Zupanc and Horschke, 1995), bird vocal centre (Goldman and Nottebohm, 1983) mammalian hypo-thalamus (Seress, 1985), cerebellum (Altman, 1972), hippocampus (Altman and Das, 1965; Angevine, 1965; Bayer, 1980) and the lizard medial cortex (López-García et al., 1988), cerebellum (López-García et al., 1990) and nucleus sphericus (Perez-Sanchez et al., 1989). The olfactory bulbs of both lizards (García-Verdugo et al., 1989) and mammals (Hinds, 1968) also show postnatal neurogenesis. Nevertheless, the newly generated neurons in the subventricular zone of mammals (Lois and Alvarez-Buylla, 1994) and the ventricular zone of the lizard rostral telencephalon (Peñafiel et al., 1996) follow a tangential migration mechanism in which radial glia do not seem participate.

Postnatal neurogenesis still occurs in the adult fascia dentata of mammals (Bayer et al., 1982; Kaplan and Bell, 1984). Radial glia also persist in the adult hippocampus, and probably guide new neuron migration (Cameron et al., 1993). Moreover, these adult radial glial cells proliferate (Cameron et al., 1993) and could be the source of the new neurons as has been suggested for the canary brain (Alvarez Buylla et al., 1990) and the lizard medial cortex (Molowny et al., 1995).

Reactive gliosis after lesion: a comparative insigh

Glial cells may remove degenerative debris (Pow et al., 1989) and provide structural support in regions undergoing substantial degeneration and cell death (Gentschev and Sotelo, 1973). In the mammalian CNS astrocytes undergo a number of structural and functional changes after brain injury (Latov et al., 1979; Nathaniel and Nathaniel, 1981; Duchen, 1984; Reier, 1986; Landis, 1994). The reactive response of astrocytes is characterised by proliferation, hypertrophy and a dramatic increases in the number of intermediate glial filaments and in the levels of GFAP and its encoding message (Bignami and Dahl, 1976; Latov et al., 1979; Nathaniel and Nathaniel, 1981; Torre et al., 1993; Landis, 1994; Stringer, 1996). All these events lead to the formation of a glial scar, which interferes with axon regrowth (Reier, 1986).

During the lizard medial cortex lesion-regeneration, radial glial cells, in some way have a similar behaviour to mammalian astrocytes i.e., increased GFAP immuno-reactivity, ramification, phagocytosis and accumulation of cellular debris. Due to the absence of astrocytes in the lizard cerebral cortex, gliotic response must be exclusively carried out by radial glia and macrophages, as occurs during Wallerian degeneration of the optic nerve of fishes (Springer and Wilson, 1989) and amphibians (Lazar, 1979). Radial glia phagocytose degenerating fibres debris produced during Wallerian degeneration in fishes, frogs and salamanders (Naujoks-Manteuffel and Niemann, 1994). Moreover, supporting cells in the olfactory mammalian neuroepithelium also play a significant role in phagocytosis after bulbectomy and during postnatal development (Suzuki et al., 1996). Nevertheless, after Wallerian degeneration of the rat optic nerve, astroglial cells apparently do not participate in phagocytic phenomena, although some cytoplasmatic inclusions, for example lipid droplets, suggest some implication in the local metabolisation of some tissue degradation products (Carbonell et al., 1991). In rats, adrenalectomy results in a specific lesion of the granule cells of the fascia dentata (Sloviter et al., 1989) (a lesion similar to that of 3AP in the granule cells of the lizard medial cortex). In the lesioned rat fascia dentata, astrocytes filled with neuronal debris are observed around the lesioned granular layer and appear to phagocytose the pyknotic nuclei. Adrenalectomy also results in increased production of granule cells and radial glia (Gould et al., 1992; Cameron and Gould, 1994). Radial glia are more evident and express nestin following kainic acid hippocampal lesions (Clarke et al., 1994).

In rats, 3AP intraperitoneal injection mainly affects the inferior olivary nuclei. Marked astrogliosis occurs within 12 hours and appears to be essential for neuronal somata fragmentation and disintegration; the climbing axons in the cerebellar cortex (coming from inferior olivary neurons) also suffer degenerative changes and are phagocytosed by Bergman glia in about 7 days (Anderson and Flumerfelt, 1980).

In the lizard dorsomedial and dorsal cortex (the main axonal projection areas of the lesioned neurons) the radial glia response is as high as that in the medial cortex. As Bergman glia phagocytose degenerating axon terminals in their projection areas, it is likely that lizard radial glia cells phagocytose the degenerating boutons of lesioned medial cortex granule neurons. The plexiform layers of the dorsal and dorsomedial cortices are the zinc-enriched axonal projection fields of the medial cortex granule neurons (López-García and Martínez-Guijarro, 1988). It is, perhaps, indirect evidence or a consequence of this phagocytic activity that lizard radial glia cells, (whose shafts intermingle with zinc enriched axonal boutons) display c-Fos immunostaining in 3AP lesioned lizards during this period of intense phago-cytosis (Blasco-Ibañez, 1992); however, radial glia cells whose vertical shafts do not intermingle with zinc-lesioned boutons do not express c-Fos immunostaining (Blasco-Ibañez, 1992).

As occurs in the 3AP lesioned olivary nuclei (Ogawa et al., 1993), the lesioned lizard medial cortex also has a microglial response, which shows a considerable delay in lizards (López-García et al., 1994). The presence of macrophage like cells filled with debris in the vicinity of the endfeet at long times after lesion suggests that there could be an exchange of degenerating material between

the radial glia and the macrophages, similar to what happens with perineurial glia and macrophages in the insect embryonic nervous system (Sonnenfeld and Jacobs, 1995).

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