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Meningeal cells organize the superficial glia limitans of the cerebellum and produce components of both the interstitial matrix and the basement membrane

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Summary

We have investigated the factors controlling both the morphological transformation of glial processes into endfeet and the deposition of extracellular matrix molecules into the overlying basement membrane by destroying meningeal cells over the hamster cerebellum by 6-hydroxydopamine administration on the day of birth. We report that within 24 h of destruction of meningeal cells, the concentrations of fibrillary collagens types I, III and IV in the glia limitans externa and the associated basement membrane molecules laminin, collagen type IV, and fibronectin are greatly diminished, resulting in the development of focal gaps in the basement membrane. The immunohistochemical integrity of the basement membrane is restored within 3 days over those surfaces of the folial apices where meningeal cells reappear. Likewise, the fibrillary collagens of the associated interstitial matrix are re-established in the same amounts as in controls. However, meningeal cells remain permanently absent from fissures and all extracellular matrix molecules tested disappear from rostral cerebellar folia covered by the anterior medullary velum. Moreover, the glial endfeet make up the superficial glia limitans only on folial apices, while they disappear from the fissural surfaces. In primary cultures, meningeal cells produce the fibrillary collagens type I, III, and VI, and the matrix molecules fibronectin and laminin, collagen type IV, nidogen, and heparansulphate proteoglycan. These findings indicate that meningeal cells (i) produce molecular components of both the interstitial matrix and the basement membrane, and (ii) are involved in the morphological transformation of glial fibres into the endfeet which constitute the superficial glia limitans.

Introduction

The neuroepithelium differs from all other mammalian epithelia in that cell division occurs at the apical poles of germinal cells. Proliferating cells have only a transitory contact with the basement membrane via a basal process that is elaborated during interkinetic migration of the nucleus in the primitive neural plate and neural tube, and also later, in the ventricular and subventricular zones (Sauer, 1938; Seymour & Berry, 1974). Exceptions to this general rule are found in the dentate gyrus and cerebellum where secondary proliferative zones are formed by transposition of germinal cells from the ventricular zone to the pial surface. For example, the secondary proliferative zone of the cerebellar external granular layer (EGL) is established at the glia limitans externa where all cells contact the basement membrane (Hausmann & Sievers, 1985). Thus, the glial limiting membrane abutting the basal lamina is initially formed by the endfeet of the primitive neuroepithelium and later by astroglial cells. The membrane is at first constituted by endfeet of the primordial radial glia, but later is augmented by those of the secondary glial scaffold, made up of the Bergmann glial fibres of Golgi epithelial cells which attach to the basement membrane (Rakic, 1971a,b; Rickmann & Wolff, 1985). It is generally accepted that epithelia secrete the components of their adjacent basement membrane, the basal lamina, whilst the surrounding mesenchymal cells probably insert constituents of the associated interstitial matrix (review in Trelstad, 1984; Bernfield *et al.*, 1984).

Astrocytes produce laminin and fibronectin, both *in vitro* (Liesi *et al.*, 1983, 1986; Price & Hines, 1985) and *in vivo* (Liesi *et al.*, 1984; Liesi, 1985), which are integral and/or associated constituents of the basal lamina.

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Moreover, there is morphological evidence that astrocytes form additional basal lamina molecules (Bernstein *et al.*, 1985), both in the presence of mesenchymal cells *in vivo* (Krüger *et al.*, 1986), and also after stimulation *in vitro* (Liesi *et al.*, 1983, 1986; Price & Hines, 1985). Thus, it can be assumed that the glial endfeet at the pial surface, together with the surrounding meningeal mesenchyme, form the overlying basal lamina and thereby stabilize the basal epithelial interface. There is no evidence that neuronal stem cells in the EGL form a basal lamina and thus their presence between astroglial endfeet could interrupt the continuity of production of the basal lamina, thereby destabilizing the neuroepithelial surface at the pia (Hausmann & Sievers, 1985).

We have previously shown that the superficial glia limitans and the overlying basement membrane of the newborn rat and hamster cerebellum become disrupted after the destruction of meningeal cells (Sievers *et al.*, 1981, 1983, 1986a, 1987; v. Knebel Doeberitz *et al.*, 1986), with the breaks in the basal lamina appearing preferentially over EGL cells. Moreover, this defect is elicited at ontogenetic stages when the superficial glia limitans covers less than 90% of the internal surface of the pial basal lamina (Sievers *et al.*, 1986a,b). At this time, the overlying meningeal cells probably have a role in stabilizing the cerebellar surface by either producing and depositing components of the basal lamina or stimulating adjacent glial endfeet to do this.

In the present paper, we report experiments designed to address the above issues which include an analysis of (i) the development of glial endfeet at the cerebellar surface using (a) qualitative and quantitative transmission electron microscopy, and (b) three immunohistochemical astroglial markers for vimentin, glial fibrillary acidic protein (GFAP) and S-100; (ii) the distribution of various molecules of the basal lamina and the pial interstitial matrix both in normal hamsters and after selective destruction of meningeal cells; and (iii) the synthesis and release of extracellular matrix molecules by meningeal cells grown in cell culture. Some of these findings have appeared in abstract form (Gude *et al.*, 1987a,b).

Materials and methods

Animals

Wistar rats and Syrian hamsters (*Mesocricetus auratus*) bred in our own colonies were mated overnight and the next day counted as day 1 of gestation (E1). The rats were born on E22, the hamsters on E16. The day of birth was counted as postnatal day zero (P0). Each ontogenetic group and experimental group contained 4–5 animals. The ontogenetic groups comprised: rats at E13, E15 and E17; hamsters at E12, E13, E15, P0–P7, P10, P15 and P30.

Treatment with 6-hydroxydopamine

Newborn hamsters received one injection of 20 μg 6-hydroxydopamine (6-OHDA) (free base) dissolved in 3 μl of 0.9% NaCl containing 1 mg ml^{-1} ascorbate. Controls were treated with the vehicle solution only. The animals were killed after various survival times and processed for either conventional light and transmission electron microscopy or immunohistochemistry (see below).

Light and transmission electron microscopy

Foetuses and postnatal animals were perfused through the left cardiac ventricle with 6% glutaraldehyde in a 0.05 M phosphate buffer for 10 min at room temperature. The brains were removed and left in the fixative overnight. The cerebellum was dissected the next day, postfixed in 2% aqueous OsO_4 for 2 h at 4° C, dehydrated in an ascending series of ethanol and embedded in araldite. Semithin and ultrathin sections were cut on a Reichert OMU2 ultramicrotome, stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmiskop I. Series of photographs were taken along the cerebellar surface, and the length of the contact of glial endfeet with the internal side of the pial basement membrane was measured using a semiautomatic image analysis system (IBAS 1, Kontron/Zeiss, Frankfurt, FRG).

Immunohistochemistry

All animals, except E12 foetuses, were perfused through the left ventricle with phosphate buffered saline (PBS) containing 4% paraformaldehyde, 0.2% picric acid and 5 mM $CaCl_2$ for at least 10 min. Their brains were postfixed for 1 h in the same solution at 4° C, washed for 3–4 h in Tris buffer, trimmed into small pieces containing the cerebellar vermis and embedded in paraplast. Sections, 20–40 μm thick, were deparaffinized, rehydrated and washed twice for 5 min in 0.01 M Tris buffer (pH 7.4) containing 0.9% NaCl, incubated for 3 min at room temperature in Ca^{2+}/Mg^{2+}-free PBS containing 0.05% trypsin (Seromed, Berlin, FRG), washed again twice for 5 min in Tris buffer, and then incubated with the primary antibody either for 24 h at 4° C or for 1 h at room temperature. The following polyclonal affinity purified antisera (raised in rabbits) were used as primary antibodies at the dilutions indicated: anti-laminin (1:250); anti-collagen type I (1:10); anti-collagen type IV (1:10); these antisera were generously supplied by Dr K. v.d. Mark (Max Planck Institut für Biochemie, Martinsried, FRG); anti-procollagen type III (1:3); anti-collagen type VI (1:3); these antisera were the kind gift of Dr D. Schuppan (Medizinische Klinik, Klinikum Steglitz, Free University of Berlin, FRG); anti-nidogen (1:20); anti-heparansulphate proteoglycan (1:20); these antisera were kindly given by Dr M. Paulssen (Max Planck Institut für Biochemie, Martinsried, FRG); anti-GFAP (1:250); anti-vimentin (1:20), both of Dakopatts (Hamburg, FRG).

After three washes in Tris buffer, the sections were incubated with the second antibody (swine anti-rabbit IgG coupled to fluorescein-isothiocyanate (FITC, Dako, Hamburg, FRG) diluted 1:30, for 1 h at room temperature. After three washes in Tris buffer, the sections were stained with the fluorescent nuclear dye bisbenzimide (Sigma, Munich, FRG), washed twice in Tris buffer, and then mounted in PBS/glycerol, 1:9 (pH 8.6) containing 2.5% sodium azide.

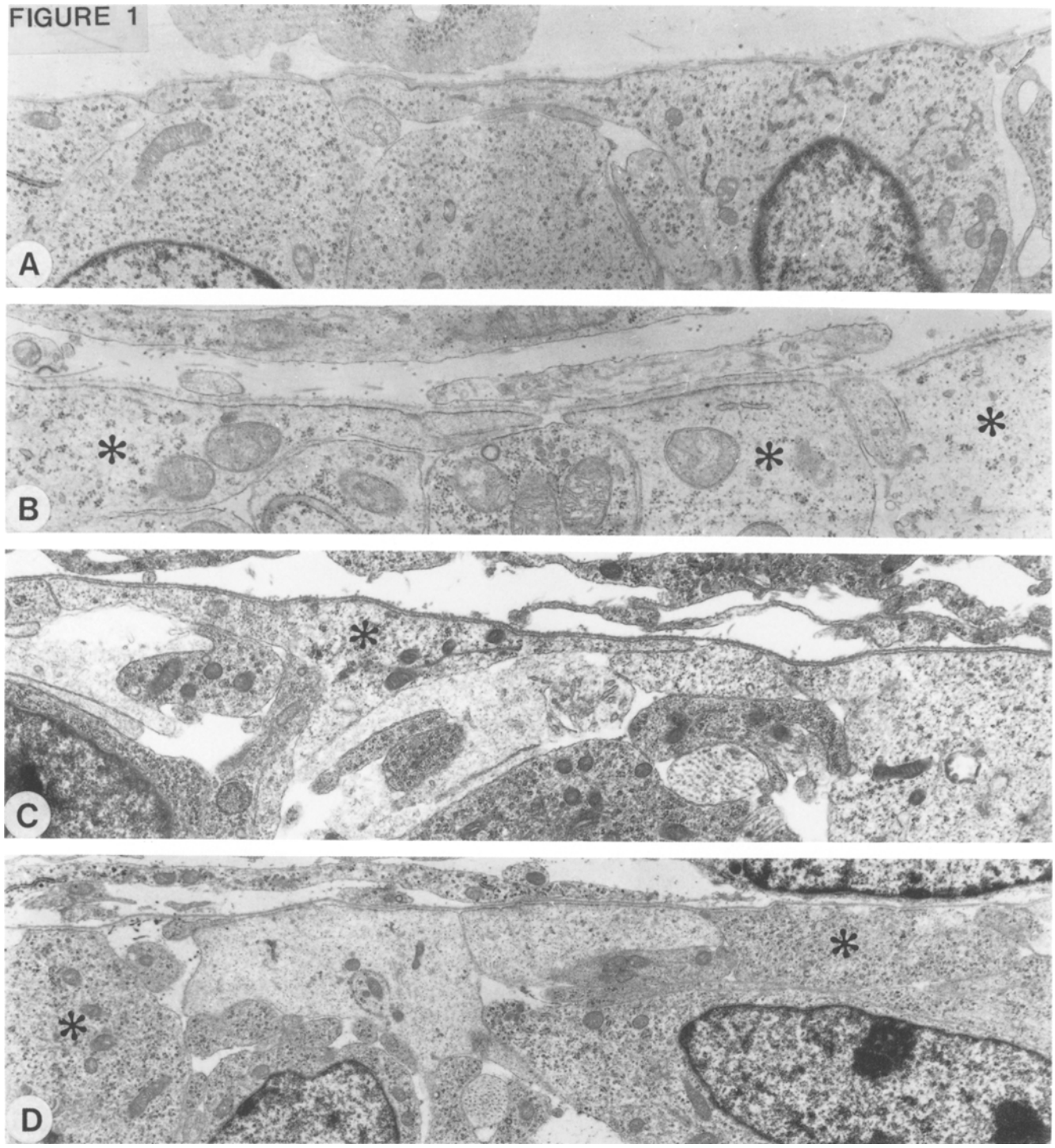

Fig. 1. Development of the superficial glia limitans at the cerebellar surface of E13 (A), E14 (B), E16 (C), and E17 (D) rat foetuses. The primitive neuroepithelial cells of the E13 cerebellar anlage are replaced by electron-lucent bulbous glial endfeet (asterisks in B) that contain monoribosomes in addition to scattered tubules and filaments. These plump endfeet transform into flattened elongated plates with a thin stem (asterisk in C), and contain predominantly monoribosomes. With the insertion of EGL cells and their processes (asterisks in D) among the electron lucent endfeet, the glia limitans is disrupted. Note the preponderance of monoribosomes within the glial endfeet which contain very few intermediate filaments. Magnification: (A) × 8000; (B) × 16 000; (C) and (D) × 7500.

Culture of meningeal cells

Meningeal membranes from newborn rat brains were carefully freed from attached tissue and choroid plexus, and incubated overnight in PBS containing 200 IU ml^{-1} collagenase type IV, Worthington (Biochrome, Berlin, FRG). After dissociation with a fire-polished Pasteur pipette, the cells were collected by centrifugation, washed several times, and seeded either onto cover slips in Leighton tubes or into culture flasks (Nunc, Mannheim, FRG) containing Earle's MEM without L-glutamine (Seromed F0323) to which were added 1% L-glutamine, and 10% newborn calf serum. The cultures were incubated at 37° C in an atmosphere of 5% CO_2. Two hours after inoculation, the medium was changed and 3 days after seeding, the cultures reached confluence. In order to either stimulate the synthesis of glycoproteins or inhibit their release, some cultures were treated with either 50 μg ml^{-1} ascorbate (Liesi *et al.*, 1983), or with 1 μM monensin (Ledger, 1980) 4 h before fixation.

For the immunohistochemical demonstration of intracellularly localized antigens, the cultures were washed twice with Hank's PBS, and then fixed with 3.5% paraformaldehyde and 0.2% picric acid in PBS (pH 7.4) for 15 min. After washing in PBS, the cells were permeabilized in methanol at 20° C for 15 min and then incubated with various antibodies (see above). For the demonstration of extracellular antigens, the cultures were washed twice in Hank's PBS, incubated with the primary and secondary antibodies for 30 min each, and then fixed in 3.5% paraformaldehyde (see above).

Results

Ultrastructural maturation of the cerebellar glia limitans externa in the rat

The cellular elements underlying the basal lamina of the glia limitans externa of the rat cerebellar anlage underwent distinct morphological changes in the course of development. In the earliest stages, when the cerebellar anlage first became discernible by the elongation of the pseudostratified neuroepithelium in the roof of the IVth ventricle, plump basal processes of immature epithelial cells were apposed to the basal lamina (Fig. 1A). When the first postmitotic neurons formed, and the cerebellar anlage became laminated by the emigration of neurons from the ventricular zone, a second, more mature type of process was seen intermingled with those of the primitive neuroepithelial cells (Fig. 1B). The basal ends of these presumptive primordial glial processes transformed rapidly into typical glial endfeet, with long, slender stems and a pyramidal base, drawn out into a thin sheet that contacted, and sometimes overlapped, adjacent endfeet (Fig. 1C).

In the caudal region of the cerebellar anlage, where the growing front of the EGL was spreading over the basal lamina in a rostral direction, the glial endfeet at first resembled those in other regions of the cerebellar anlage (Fig. 1D). However, the emigration of EGL cells from the caudolateral germinal trigone (Altman & Bayer, 1978), and their translocation over the basal lamina disrupted the continuity of the primitive superficial glia limitans. The EGL cells were attached to the basal lamina from the beginning of their tangential migration up to the end of their proliferative period (see also Hausmann & Sievers, 1985), and their contacts with the pial basal lamina broke up the continuity of the superficial glia limitans (Fig. 1D).

In quantitative terms, the disintegration of the glia limitans externa was substantial. Thus, at the time when EGL cells were spreading over the cerebellar surface, they occupied between one and two thirds of the inner aspect of the basal lamina. In the E17 rat foetus, for example, typical glial endfeet covered only $40.8 \pm 2.8\%$ (SEM) of this surface, while the complementary area was covered with EGL cells. We have proposed that the definitive glia limitans is ultimately established over the cerebellar surface from a second source of glial endfeet provided by astroglial cells within the EGL which form a secondary glia scaffold (see Sievers *et al.*, 1994a).

Immunohistochemical development of the hamster superficial glia limitans

The mature superficial glia limitans of the rodent cerebellum was formed by the endfeet of the radial Bergmann glial fibres of Golgi epithelial cells. These processes stained positively with antibodies against the glial filaments vimentin and GFAP, and also, the S-100 protein. The staining pattern of these three astroglial markers varied during development and was used to follow the molecular differentiation of the astroglial cells of the cerebellar anlage.

On E12 in the hamster, when the cerebellar anlage was comprised of neuroepithelial cells and the first postmitotic neurons, radial vimentin-positive processes spanned the whole thickness of the cerebellar wall; however, the immunoreactivity terminated below the superficial glia limitans which was not stained. Likewise, no GFAP or S-100 immunoreactivity was present at the pial surface (see Sievers *et al.*, 1994a). The vimentin-positive radial set of processes persists on E13 in the hamster when the EGL had started to spread across the basal lamina and, in caudal regions, GFAP immunoreactivity appeared for the first time underneath the pial surface, and rapidly extended rostrally during the next days (Fig. 2A). Immunoreactivity for GFAP was almost exclusively found near the pial surface in the cell bodies and plump basal processes of immature glial cells, yet was not present in typical glial endfeet (Fig. 2A,B). Numerous endfeet of immature Bergmann glial fibres were also stained with antisera against S-100 (Fig. 2C).

After P5 in the hamster, the superficial glia limitans exhibited a discontinuous band of GFAP-positive and S-100-immunoreactive endfeet (Fig. 2D,E). Beyond P10, the superficial glia limitans was seen as a

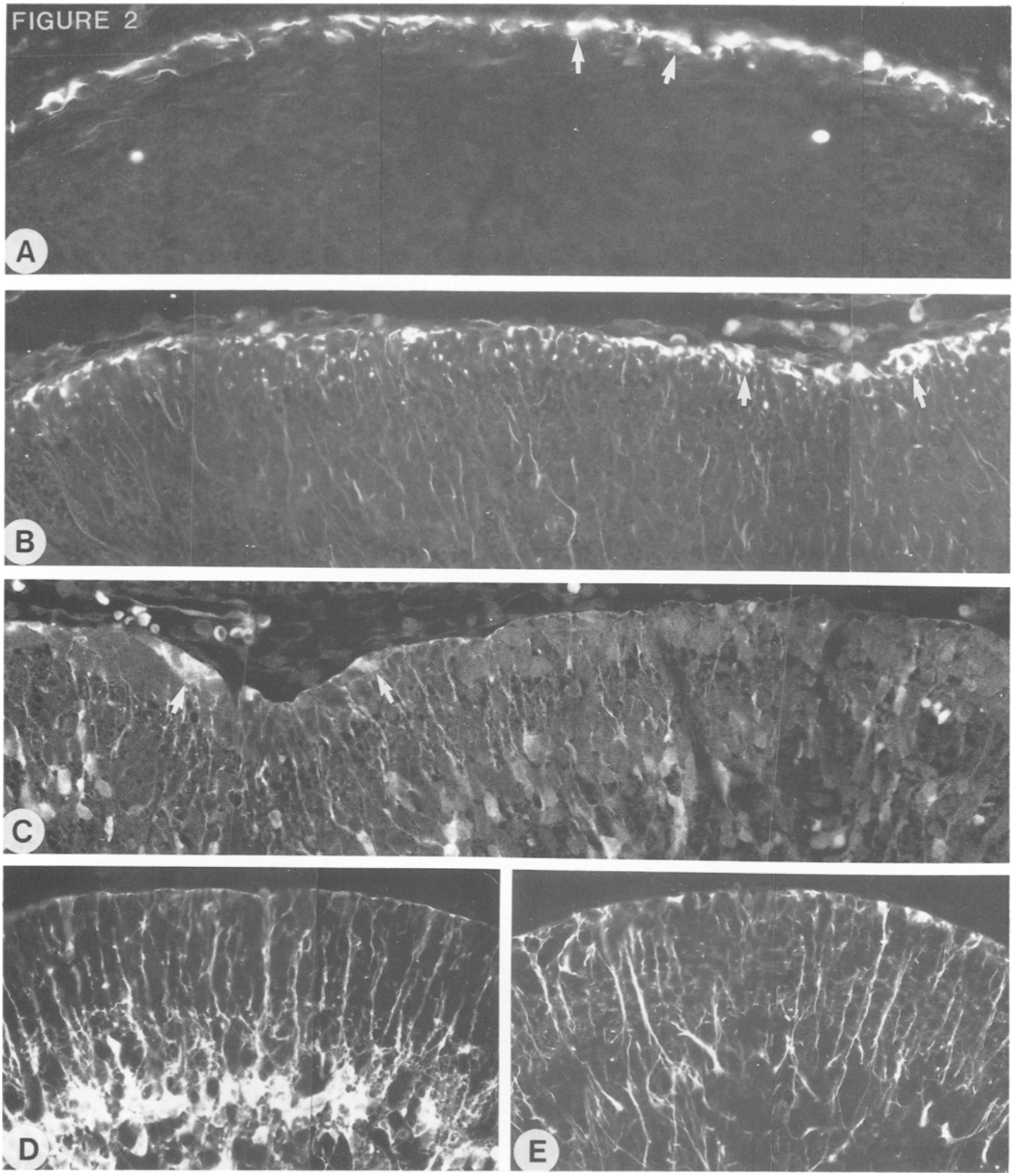

Fig. 2. Development of immunohistochemical astroglial markers at the cerebellar pial surface of E15 (A), P0 (B,C), and P5 (D,E) hamsters. At E15 the GFAP immunoreactivity is localized either in cell bodies (arrows) or tortuous processes aligned parallel to the pial surface, both of which belong to the EGL. Note the complete absence of GFAP immunoreactivity in the radial glial fibres and their endfeet. On P0, short thin glial endfeet and numerous intensely fluorescent bulbous varicosities scattered throughout the EGL which, in all probability, represent the tips of growing side branches of the Bergmann glial fibres. In S-100 preparations (C) the main stems of the Bergmann glial fibres and their endfeet are distinctly seen; however, it is apparent that the glia limitans is still incomplete (arrows mark glial cell bodies). Towards the end of the first week (P5), the glia limitans in S-100 preparation (D) is complete, being formed by a continuous row of intensely fluorescent conical endfeet. In GFAP preparations (E), however, there still are gaps at the pial surface which probably result from the low content of intermediate filaments. Magnification: (A–E) × 350.

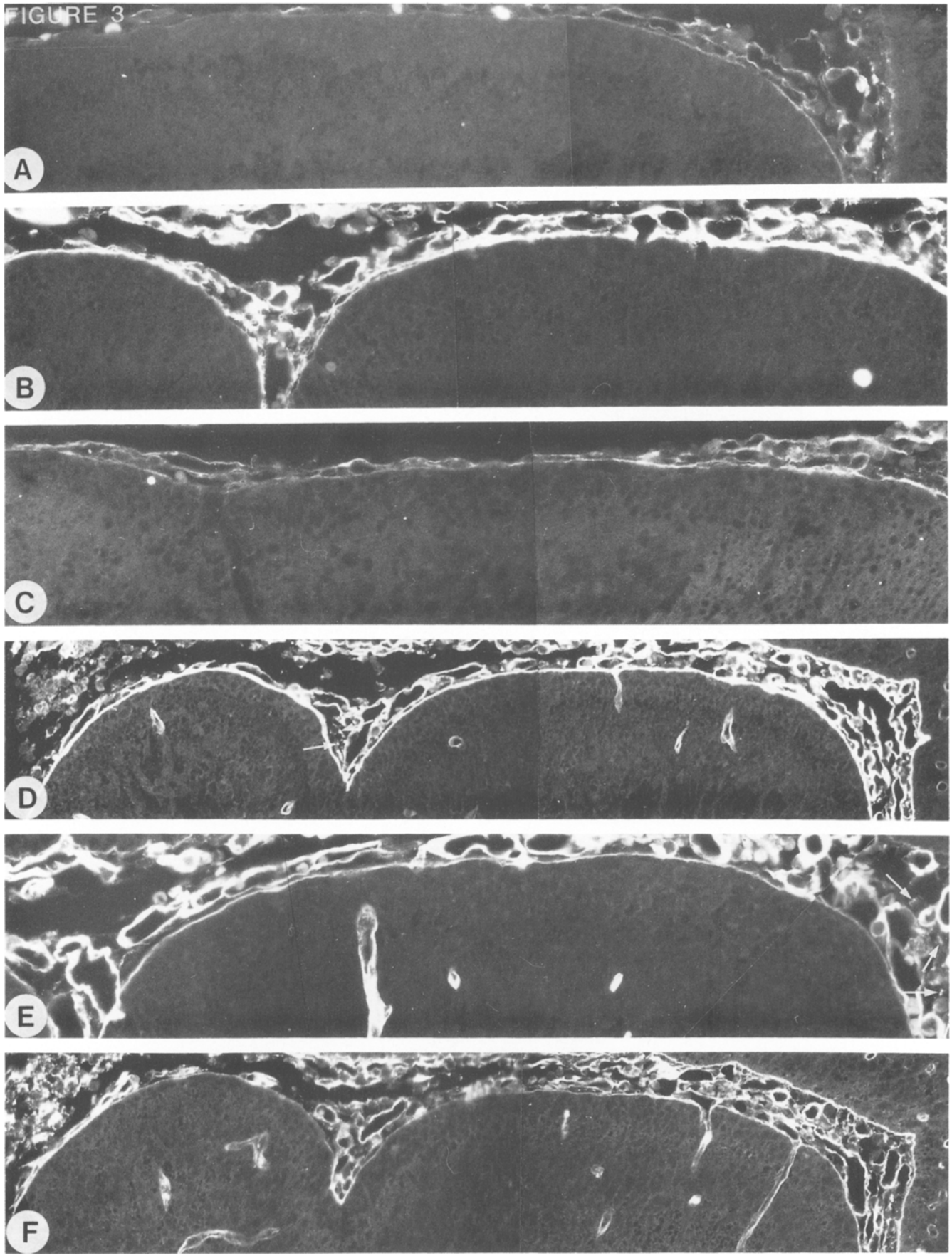

Fig. 3. Immunohistochemical demonstration of the distribution of fibrillary collagens (A–C) and integral or associated components of the basement membrane (D–E) at the cerebellar pial surface of newborn hamsters. Immunoreactivity against collagen type I (A) or type VI (C) associated with the pial surface, is faint but all components of the meninges stain heavily with antisera against procollagen type III (B). Intracerebral blood vessels are not stained. The antisera against laminin (D), collagen type IV (E), and fibronectin (F) stain not only the basement membrane of the pial surface and the extra- and intracellular blood vessels, but also small punctate deposits on the surface of meningeal cells (arrows in D and E), which may indicate that extracellular matrix molecules originate from these cells. Magnification: (A–C,E) × 350; (D,F) × 224.

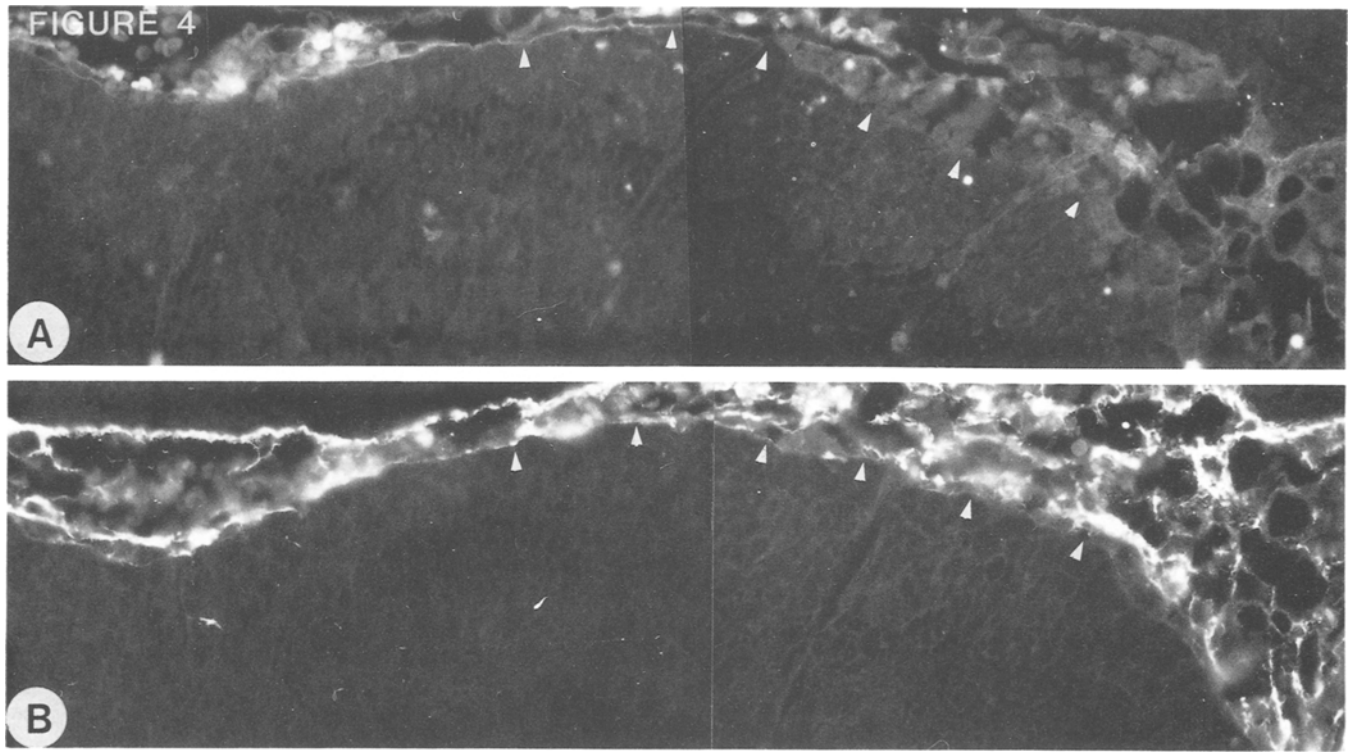

Fig. 4. Depletion of collagen type I (A) and procollagen type III (B) over the hamster cerebellar surface 24 h p.i. of 6-OHDA on P0. The pial surface is outlined by the arrowheads. Note that the immunoreactivity associated with the basement membrane has almost completely vanished, while there are still remnants of collagen fibrils within the subarachnoid space. Magnification: (A,B) × 350.

continuous band with punctate accentuations in both GFAP and S-100 preparations.

Distribution of components of the basal lamina and the interstitial matrix at the hamster cerebellar surface

Starting on P0 (the first time point studied) type I, III and VI fibrillary collagens were expressed over the hamster cerebellar surface. With increasing age, expression continuously increased (Fig. 3A–C). Weak immunoreactivity against collagen types I and VI was associated mainly with pial, but not intracerebral blood vessels, and only focally deposited along the pial basement membrane. Collagen type III was intensely stained with antibodies against procollagen type III both on blood vessels and on the pial basement membrane, as well as in the subarachnoid space.

The basement membrane constituents laminin and collagen type IV, and fibronectin were evenly distributed over the hamster cerebellar basement membrane, and the intensity of the immunoreactivities did not vary greatly over the period studied (Fig. 3D–F). Immunoreactivity against laminin, collagen type IV and fibronectin was also present as punctate deposits on meningeal cells in the cerebellar subarachnoid space (Fig. 3E–F).

Alterations of the extracellular matrix at the hamster cerebellar surface after destruction of meningeal cells

The sequelae of selective destruction of cerebellar meningeal cells after 6-OHDA injection has already been described (Sievers *et al.*, 1981, 1983, 1985, 1994b; Pehlemann *et al.*, 1985). In the present study, we have investigated the changes in both the composition of the extracellular matrix and the maturation of glial endfeet after meningeal cell destruction in the hamster.

Within 24 h after injection (p.i.) of 6-OHDA, the expression of fibrillary collagens (Types I, III and VI) at the cerebellar surface was reduced considerably (Fig. 4A,B), and the individual fibre bundles became dissociated from the pial surface. The immunoreactivity of the basal lamina molecules laminin, collagen type IV, and fibronectin was greatly reduced (Fig. 5A–C). Unstained discontinuities were seen focally in the basal lamina, which was not as smooth as in control animals having a ruffled and irregular appearance. In electron micrographs, thinning and rupturing of the basal lamina was seen (Sievers *et al.*, 1981, 1983; v. Knebel Doeberitz *et al.*, 1986).

Three days after 6-OHDA administration, the apical surface of the caudal cerebellar folia, where meningeal cells had begun to regenerate, was completely covered

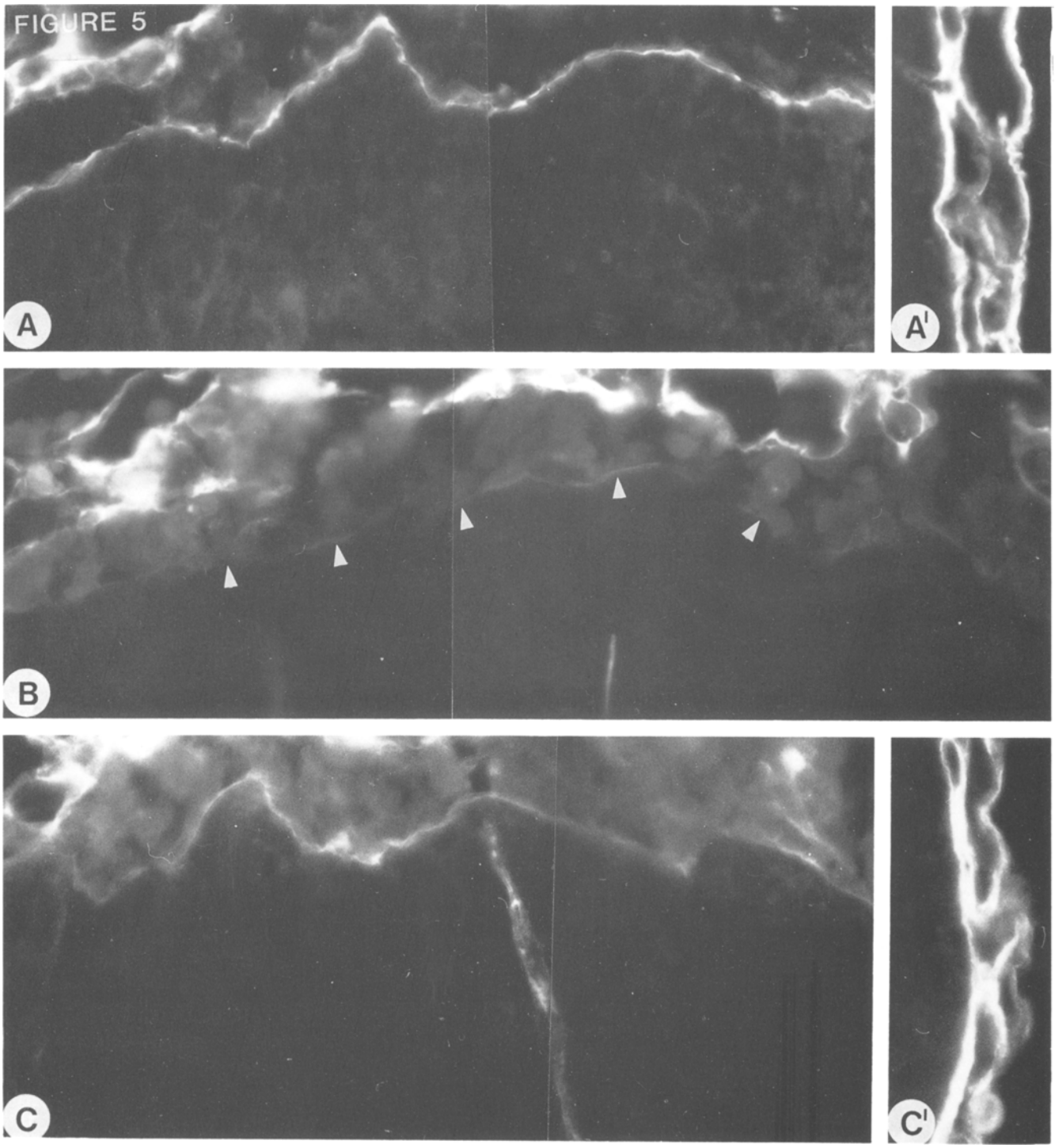

Fig. 5. Reduced immunoreactivity against laminin (A), collagen type IV (B), and fibronectin (C) at the hamster cerebellar basement membrane 24 h p.i. of 6-OHDA on P0. For comparison the intensity of the immune reaction against laminin (A') and fibronectin (C') in control animals of the same age is shown. In (B), the pial surface is outlined with arrowheads. It is unknown why collagen type IV is affected most by the destruction of meningeal cells. Note that the continuity of the pial basement membrane is disrupted in many places. Magnification: × 882.

with a basal lamina exhibiting the same patterns and intensity of immunofluorescent staining with all antibodies tested as in control animals (Fig. 6A,B). The same was true for the fibrillary collagens that had also reappeared to the same extent as in controls (Fig. 6C,D). However, within the rostral cerebellar folia, overlain by the anterior medullary velum (AMV), meningeal cells remained absent from fissures for the

next few days and the acute postinjection changes persisted and worsened. The basal lamina lining the surface of opposing folia became discontinuous, with large portions staining negative with antibodies against laminin, collagen type IV, and fibronectin (Fig. 6A,B). Similarly, the fibrillary collagens vanished from the fissures and were not replaced (Fig. 6C,D). Instead, a massive band of immunoreactivity against the fibrillary collagens traversed the entrance of the fissure, extending over the surface of the apices of adjacent sub-AMV folia (Fig. 6D). The molecules of both the interstitial matrix and the basal lamina remained permanently absent over sub-AMV fissures but reappeared, wherever a new pial surface with juxtaposed glia limitans and meningeal cells was formed.

These results show (i) that the destruction of meningeal cells is followed by a decrease in the amount of both the interstitial matrix and the basal lamina at the pial surface, and (ii) that these molecules are restituted only over those regions of the cerebellar surface repopulated with meningeal cells, while they remain absent, where meningeal cells are unable to regain contact with the glia limitans.

The superficial glia limitans is lost in those parts of the hamster cerebellar surface where meningeal cells remain absent

Within a few hours after the destruction of meningeal cells, strongly fluorescent punctate or bulbous varicosities appeared within the EGL which were not present in control animals (Fig. 7A). They probably represented both the somata of GFAP-positive cells which, in control animals, were restricted to the most superficial (proliferative) lamina of the EGL (see Sievers *et al.*, 1994a), and the swollen Bergmann glial fibres which had either detached from the basal lamina or did not ascend to the pial surface to form endfeet.

By 5 days p.i., the numbers of GFAP-positive processes were greatly reduced in the rostral sub-AMV cerebellum, especially within the fissures where a superficial glia limitans was absent (Fig. 7B), and, with the fusion of neighbouring folia, were not reconstituted later on (Sievers *et al.*, 1994b).

The disruption of the superficial glia limitans after destruction of meningeal cells was also obvious in S-100-immunohistochemical preparations. In the hamster, at 2 days after injection of 6-OHDA, the distribution of S-100 immunoreactivity did not differ significantly from controls, with a continuous S-100-positive glia limitans caudally and a punctate glia limitans rostrally. Starting at P3, the expression of this antigen decreased continuously. Thus, the distinct line of S-100 immunoreactivity at the cerebellar surface of controls was interrupted in the sub-AMV rostral regions of treated animals, and absent within these rostral cerebellar fissures. By P5, the superficial S-100-immunoreactivity that was by now very prominent in controls was almost completely absent in the rostral fissures of 6-OHDA-treated animals (Fig. 7C).

Thus, these findings show that neither glial endfeet nor a basal lamina is reformed in the sub-AMV fissures which were not repopulated by meningeal cells. The glial fibres which are present in these regions do not produce the extracellular matrix molecules which form the normal basement membrane.

Primary cultures of rat meningeal cells produce components of both the interstitial matrix and the basal lamina

The overwhelming majority of meningeal cells in tissue culture exhibited a fibroblast-like morphology and pattern of growth. Contaminating endothelial cells grew in an epithelial manner, forming small colonies of flat rounded cells which could be distinguished by their positive immunohistochemical reaction with antibodies against factor VIII (data not shown). The meningeal cells were GFAP-negative but produced fibrillary collagen types I, III and VI and components of the basal lamina including fibronectin (Fig. 8A,B), laminin (Fig. 8C), collagen type IV (Fig. 8D), nidogen (Fig. 8E) and heparansulphate proteoglycan (Fig. 8F). These latter molecules were seen both intracellularly and on the culture substrate (Fig. 8G–I). This indicates that they were both synthesized and released by the meningeal cells.

Discussion

The changes occurring in the basal lamina after destruction of meningeal cells as seen in the transmission electron microscope (Sievers *et al.*, 1983, 1986a,b, 1987; Pehlemann *et al.*, 1985; v. Knebel Doeberitz *et al.*, 1986) were investigated at the molecular level in the present study. Within 24 h, the intensity of the immunoreactivity against several integral and associated components of the basal lamina, like collagen type IV, laminin and fibronectin, was decreased. Concurrently, the immunoreactivities against the fibrillary collagens type I, III, and VI also were reduced. Although there was a recovery of the immunoreactivities against the constituents of both the basal lamina and the interstitial matrix over those regions of the cerebellum that were repopulated with meningeal cells, they further decreased and finally vanished from those regions that remained depleted of meningeal cells, i.e. the sub-AMV anterior cerebellar fissures. This latter phenomenon occurred in spite of the presence of Bergmann glial fibres, which indicates that meningeal cells not only produce extracellular matrix molecules themselves, but also induce endfeet formation and synthesis of basal lamina molecules by astroglial cells (see below).

Additional evidence for the above proposition is

provided by our results from meningeal cell cultures. Thus, primary cultures of rat meningeal cells synthesized and released not only the fibrillary collagens type I, III, and VI, but also laminin, collagen type IV (see also Rutka *et al.*, 1986a,b), nidogen, heparansulphate proteoglycan and fibronectin, all of which are either integral or associated constituents of the basal lamina. Meningeal cells over the cerebellar surface also exhibited punctate immunoreactivity against collagen type IV, laminin and fibronectin which indicates that they synthesized these molecules both *in vitro* and *in vivo*. Thus, it appears that meningeal cells, like muscle fibroblasts (Kühl *et al.*, 1982, 1984; Sanderson *et al.*, 1986) and Wi 38 fibroblasts (Alitalo, 1980), are capable of producing both the molecules of the interstitial matrix and the constituents of the basal lamina.

It is also possible that meningeal cells regulate the synthesis and release of basal lamina molecules from Bergmann glial fibres, and also modulate endfoot development. This assumption is based on the observations that after destruction of meningeal cells, established glial endfeet dedifferentiate and new ones are not formed. Moreover, the production of molecules of the basal lamina terminates, where Bergmann glial processes are present but meningeal cells remain absent, i.e. in the rostral sub-AMV cerebellar fissures. Indeed, there is evidence that astroglial endfeet at the pial surface produce basal lamina glycoproteins *in vitro* (Liesi *et al.*, 1983, 1986; Price & Hynes, 1985) and *in vivo* (Liesi *et al.*, 1984; Liesi, 1985) such as laminin, fibronectin and heparansulphate proteoglycan. It is, however, unknown at present what mechanisms are involved in this interaction between meningeal cells and Bergmann glial

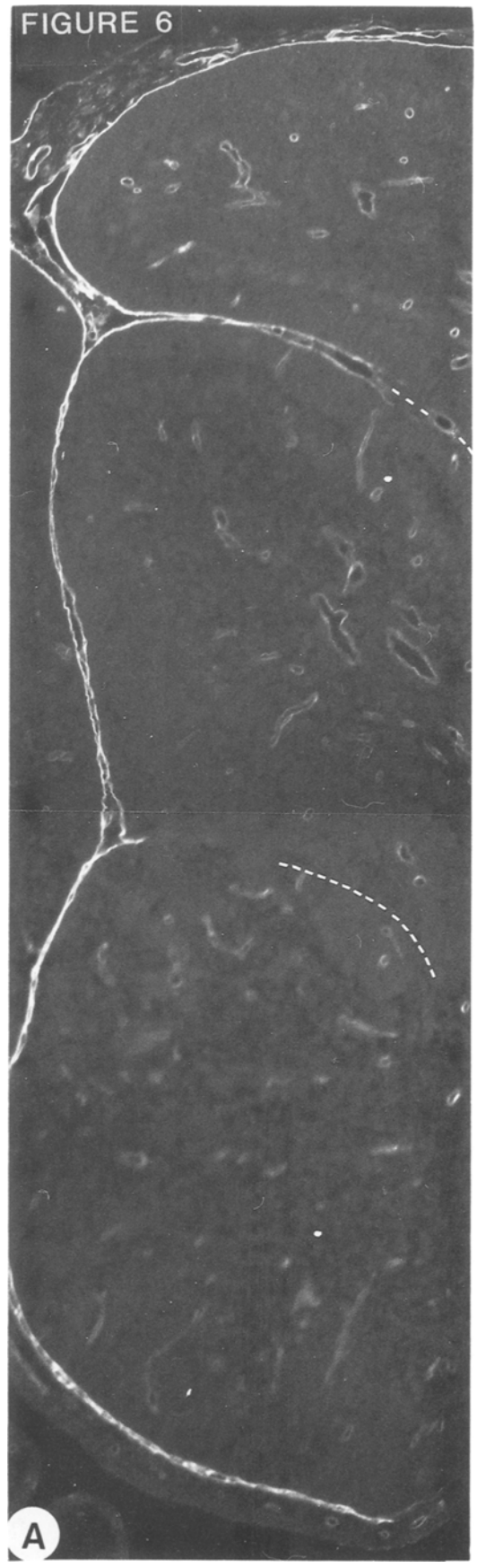

Fig. 6. Distribution of the immunoreactivity against laminin (A), collagen type IV (B,B'), type VI (C,C'), and procollagen type III (D,D') 3 days p.i. of 6-OHDA on P0 (A,B',C',D') and in control animals of the same age after 3 days p.i. vehicle solution (B–D). The continuity of the laminin positive basement membrane is restituted over the apices of the rostral sub-AMV cerebellar folia (A). However, the basement membrane does not reach into the anterior cerebellar fissures, but stops abruptly at their entrances and, with the absence of the basement membrane over adjacent folia, the latter have fused. The virtual surface of the respective fissures has been marked with a white broken line. Obliteration of the anterior cerebellar fissures is also demonstrated with the other markers of extracellular matrix molecules. Immunoreactivity against collagen type IV is continuously lost from the basement membrane in the depth of the primary fissure (compare B and B'), the fibrillary collagens type VI and III are also depleted from the fissures, but become restituted as a bridge of tissue traversing the entrance of the fissure (D', the arrowheads mark the pial surface of the adjacent folia). Magnification: (A) × 140; (B–D) × 350.

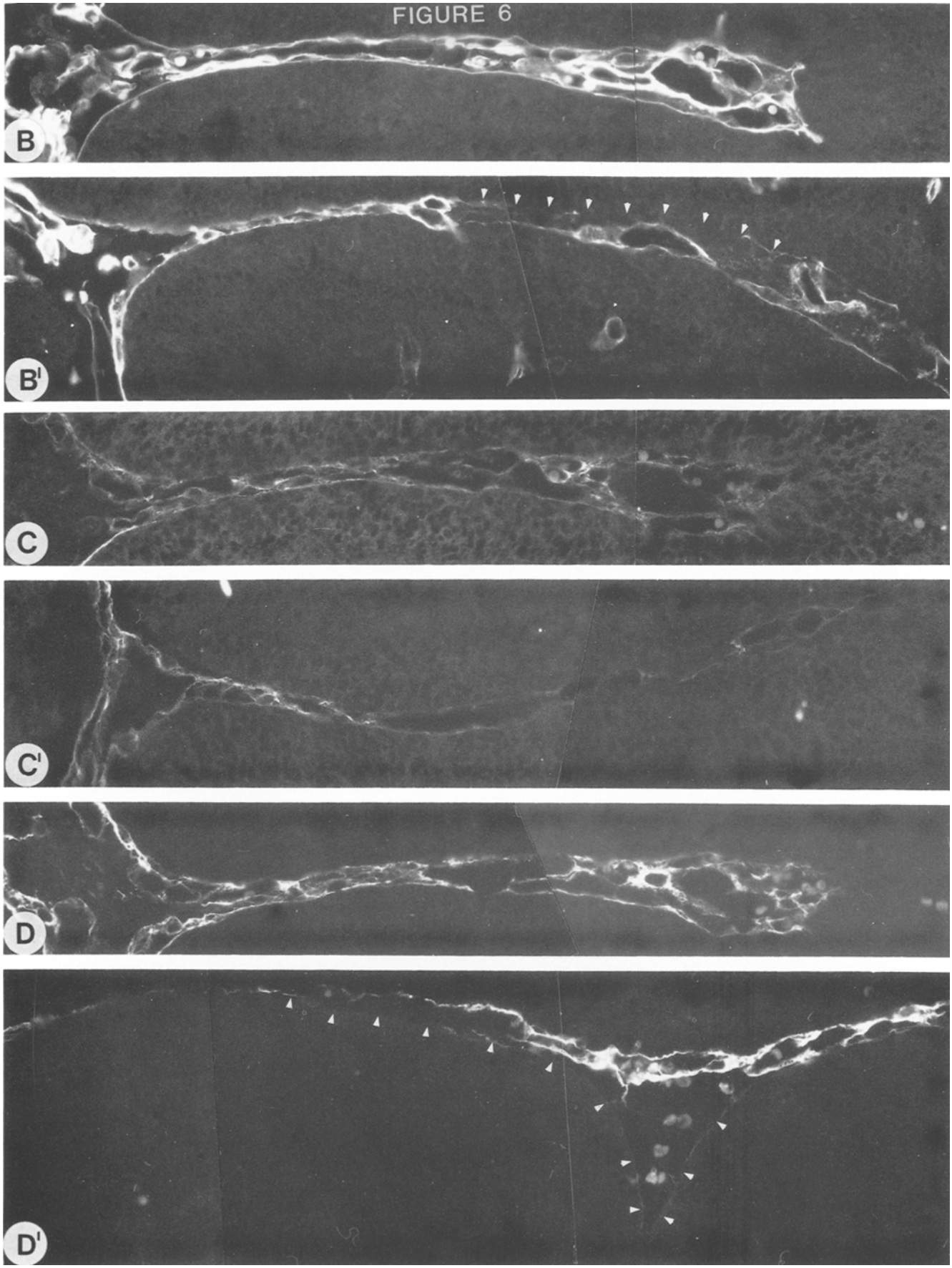
FIGURE 6
B
B'
C
C'
D
D'

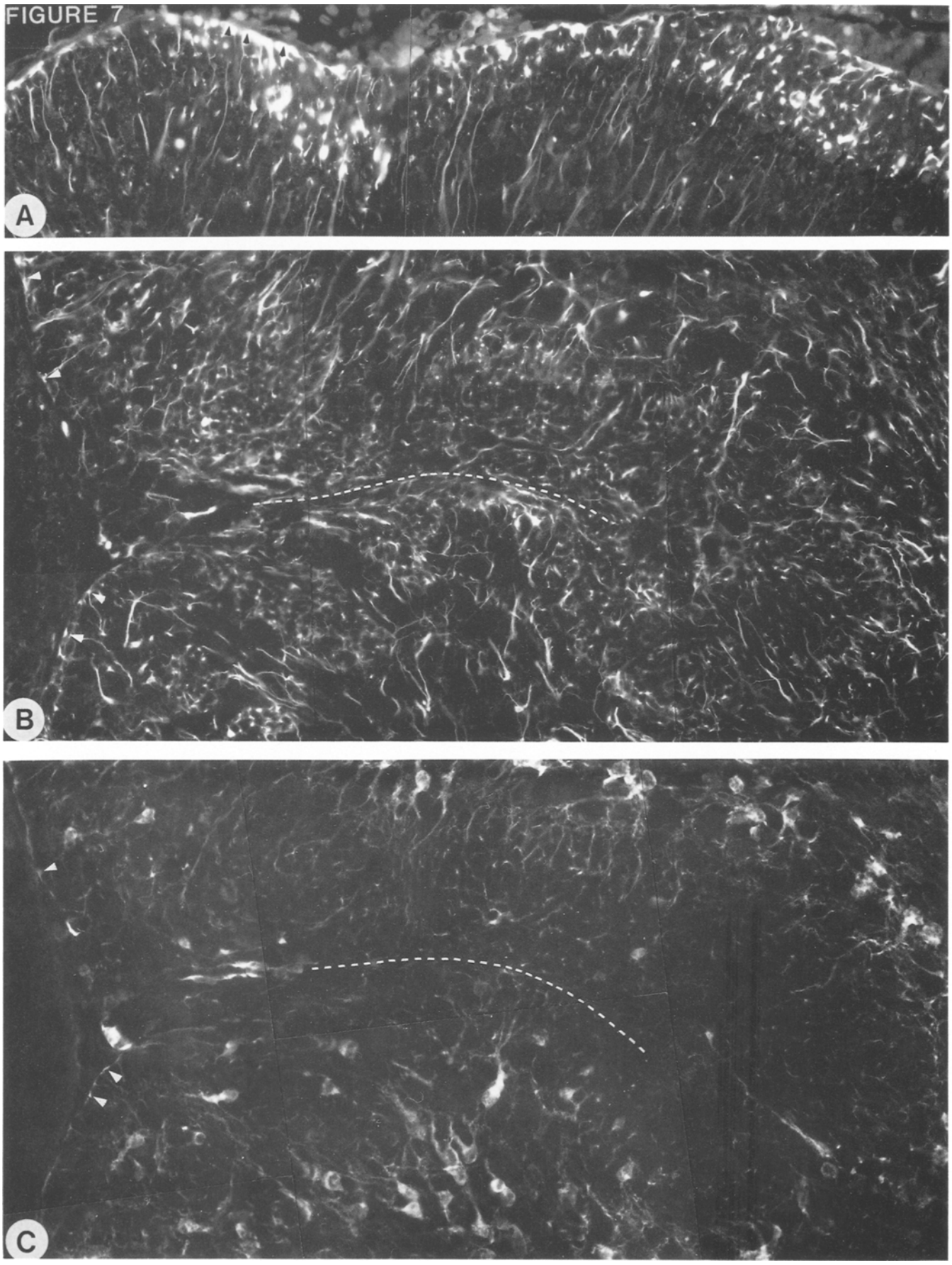

Fig. 7. Disturbance of the formation of endfeet in the anterior sub-AMV cerebellar fissures 1 (A) and 5 (B,C) days p.i. of 6-OHDA on P0. A dramatic increase in the amount and intensity of GFAP-immunoreactive varicosities within the EGL appears within 24 h (A) after destruction of meningeal cells (compare with Fig. 2B and E). The endfeet (arrowheads) likewise are intensely stained. However, they do not form a complete glia limitans. At 5 days p.i., endfeet are completely lacking at the surface of adjacent folia. Both in the GFAP (B) and the S-100 (C) preparations of adjacent sections of the preculminate fissure (whose virtual surface has been marked with a broken white line) the Bergmann glia fibres run tortuously towards the imaginary surface of the respective folia but do not form endfeet. At the anterior sub-AMV cerebellar surface, where meningeal cells have regenerated, the number of glial endfeet (marked with arrowheads) is also reduced in comparison to controls (compare with Fig. 2 D,E). Magnification: × 350.

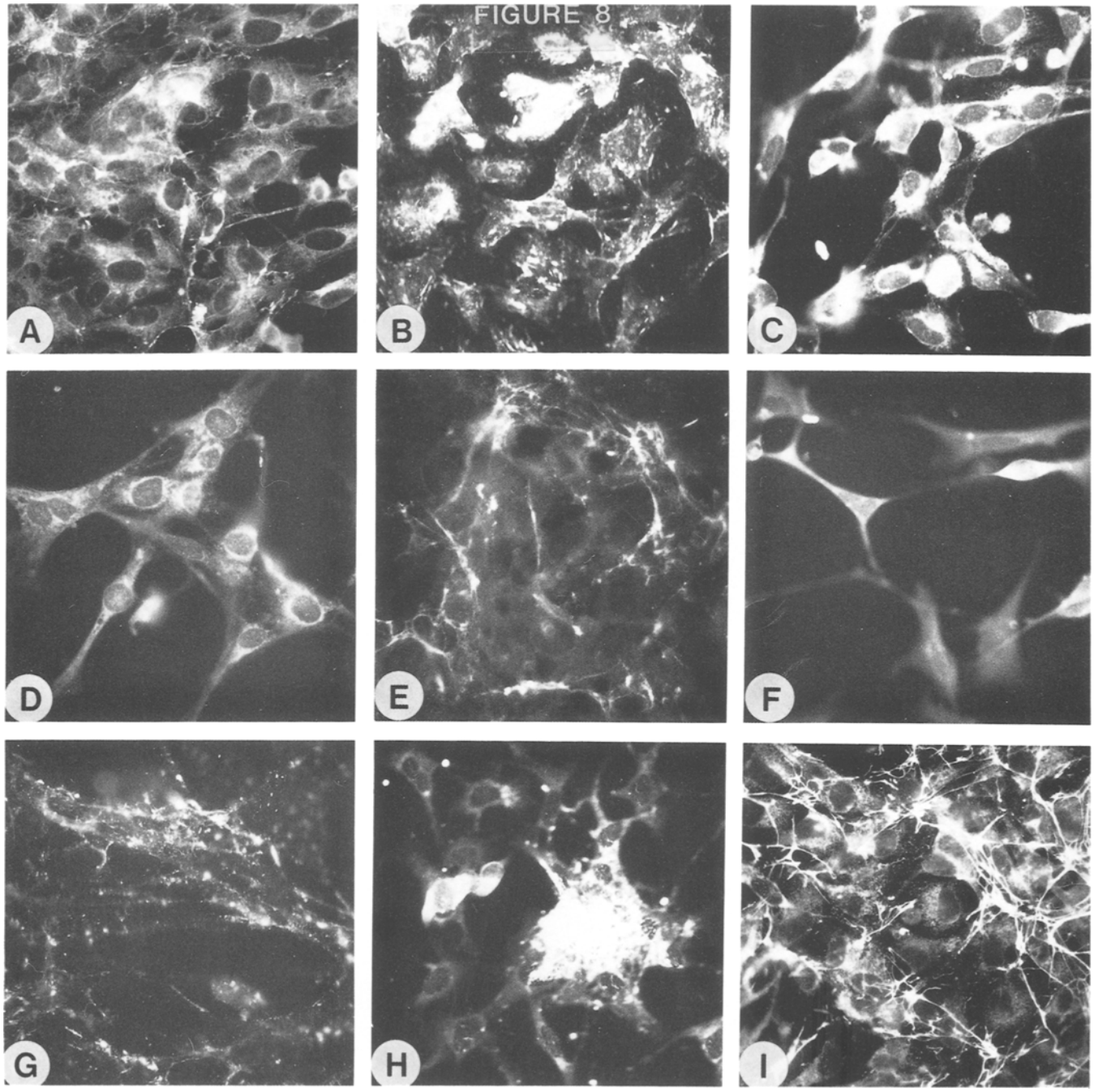

Fig. 8. Immunohistochemical demonstration of the presence of various molecules of the interstitial matrix and the basement membrane in primary cultures of meningeal cells from foetal hamsters. (A) Intracellular localization of procollagen type III; (B) intra- and extracellular localization of fibronectin; (C) intracellular localization of laminin; (D) intracellular localization of collagen type IV; (E) extra- and (F) intracellular localization of heparansulphate proteoglycan; extracellular localization of (G) laminin, (H) collagen type IV, and (I) procollagen type III. Magnification: × 350.

fibres with extracellular matrix molecules released by meningeal cells.

The intercalation of neuronal stem cells from E17 into the rat EGL amongst the primordial glial endfeet places greater demands on the latter to maintain the continuity of the glia limitans externa than in regions in which a secondary proliferative zone does not develop. Secondary glial endfeet do however develop and their numbers increase continuously from E17 up to birth both in the rat and in the hamster, when they cover two thirds of the internal surface of the basal lamina (Sievers *et al.*, 1981, 1986b). Thereafter, the coverage of the basal lamina by glial endfeet increases to a value of about 90% on P5 (Sievers *et al.*, 1986b),

and, finally to 100% as the EGL disappears. Thus, there is a long ontogenetic interval in which the superficial glia limitans is incomplete and over this period the glia limitans is vulnerable to destabilization if the cellular elements responsible for maintenance are depleted. The cerebellar surface becomes resistant to the effects of meningeal cell depletion when coverage by glial endfeet is about 90% (Sievers *et al.*, 1986b). Thus, as the area of the cerebellar surface increases after the EGL is established, glial endfeet proliferate as the glia limitans externa matures. We have provided evidence that glial stem cells resident in the EGL form a population of secondary radial glia whose endfeet provide the additional coverage for the expanding glia limitans (Sievers *et al.*, 1994a). The defects seen in the secondary radial glial scaffold in regions of the cerebellum developing without either meningeal cells or a basal lamina, strongly suggest that both these elements are essential to the normal ontogeny of secondary radial glial cells and their processes (Sievers *et al.*, 1994b).

A requirement for synchronization of basal lamina expansion and endfeet formation renders the superficial glia limitans of the cerebellar surface labile and probably explains the regular occurrence of neuronal ectopia within the cerebellar fissures of normal animals (Del Cerro *et al.*, 1976; Griffin *et al.*, 1980) derived from EGL cells that escape into the subarachnoid space (Hausmann *et al.*, 1985).

We have recently described maldevelopment of the dentate gyrus after destruction of the overlying meningeal cells. The alterations observed include disturbances in the formation of the glia limitans and, as a result, fusion of the dentate anlage with the underlying diencephalon, and derangement of the supra- and infrapyramidal blades of the dentate gyrus (Hartmann *et al.*, 1992). Destabilisation of the subpial secondary proliferative zone in the developing dentate gyrus is probably causally related to loss of contact with basal lamina (Hausmann *et al.*, 1987; Sievers *et al.*, 1992). Thus, the explanation for the sequelae of meningeal cell destruction in the dentate gyrus and cerebellum is similar (Hartmann *et al.*, 1992).

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