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Distribution patterns of vimentin-immunoreactive structures in the human prosencephalon during the second half of gestation

NORBERT ULFIG[1], FRANK NEUDÖRFER[1] AND JÜRGEN BOHL[2]

[1] *Neuroembryonic Research Laboratory, Department of Anatomy, University of Rostock, and*
[2] *Department of Neuropathology, University of Mainz, Germany*

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ABSTRACT

Neuronal migration is guided by long radially oriented glial fibres. During late stages of development radial glial cells are transformed into astrocytes. A predominant intermediate filament protein within radial glial cells and immature astrocytes is vimentin. In this study fetal brain sections were used to demonstrate the transient features of vimentin-positive radial glia. In the lower half of the cerebral wall of the 6th gestational month bundles, curvature, and crossing of vimentin-positive fibres are regularly seen. Moreover, fibres terminating on vessels are observed. In the upper half fibres are radially oriented; when ascending towards the pial surface the number and diameter of fibres appears conspicuously decreased. Radially aligned fibres display numerous varicosities. In the 8th month the bulk of vimentin-positive fibres is encountered next to the ganglionic eminence and below isocortical cerebral fissures. The dentate gyrus is conspicuous due to its high amount of immunolabelled fibres. Furthermore, densely packed fibres are visible within the internal and external capsule and in the vicinity of the anterior commissure. Radial glial somata are found in the proliferative areas as well as in the adjacent white matter. In the latter location bipolar, monopolar and stellate vimentin-positive cells are present. The results demonstrate an area-specific distribution pattern of vimentin-positive structures which can be correlated with migrational events. Areas maturing late in development for instance, reveal dense immunolabelling in the 8th month. The orientation and position of radial fibres point to an additional developmental role of these fibres, i.e. their involvement in the guidance of growing axons. Moreover, the arrangement and morphology of vimentin-positive fibres, such as retraction of fibres or occurrence of varicosities, are indicative of degenerative events. Accordingly, a transformation of radial glial somata, their displacement towards the white matter and finally the growth of stellate processes can clearly be demonstrated.

Key words: Radial glia; astrocytes; axon guidance; ganglionic eminence; neuronal migration.

INTRODUCTION

Nerve cells are generated within the neuroepithelium which lines the ventricular cavities. These proliferative zones are located at distances of up to several centimetres from the target positions of the neurons. Neuronal migration comprises the time interval between the departure of postmitotic neuroblasts from the proliferative zones and their settling at their final destination. Migration from the proliferative zones to the target regions (for instance, the cortical plate) is guided by long glial fibres which are radially oriented. These radial glial cells which span their processes along the full width of the cerebral wall provide scaffolding for migrating neurons (Misson et al. 1991; Rakic, 1995*a*), the latter possessing a bipolar shape follow the radial pathways by means of surface-mediated interactions. Electron microscope studies have demonstrated the presence of close membrane appositions and specialised junctions between migrating neurons and radial glial fibres (Rakic, 1972). Postmitotic neurons, born in a definite sector of

Correspondence to Professor N. Ulfig, Neuroembryonic Research Laboratory, Department of Anatomy, University of Rostock, Gertrudenstr. 9, D-18055 Rostock, Germany.

the proliferative zone, are eventually found within a distinct column-like compartment of the cortical plate. During late stages of development radial glial cells gradually disappear.

In addition to providing a guide for migrating neurons the radial glial cells are associated with other developmental events. Probably, the radial fibres represent a guiding cue for growing axons (Norris & Kalil, 1991; Silver et al. 1982). In particular, axons of the corpus callosum may be directed by the radial glial fibres. Observations in migrational disorders underline this assumption: gyral abnormalities as a consequence of disturbed migration are frequently accompanied by hypoplasia or agenesis of the corpus callosum (Volpe, 1995). The elongated radial glial fibres may also be involved in the signalling of information between the proliferative zones and the distant target regions (Rakic, 1995*a*). Later in development, the radial glial cells fulfil another role: proliferation and differentiation into astrocytes and probably into oligodendrocytes. In the monkey brain transitional forms between radial glial cells and astrocytes can first be observed at around midgestation. At this period some radial glial somata become detached from the proliferative zone and are displaced to the intermediate zone. Then a morphological transformation of radial glial cells into mature astrocytes can be observed (Voigt, 1989).

Vimentin has been established as a predominant intermediate filament protein within immature astrocytes and radial glial cells (Bovolenta et al. 1984; Hutchins & Casagrande, 1989). Therefore, in this study anti-vimentin has been used to investigate the arrangement and characteristic features of radial glial fibres and cells in various areas of the prosencephalon during the second half of gestation. The time span, 6th until 8th gestational month, has been chosen to provide a basis for neuropathological investigations in preterm delivery. Haemorrhage in the proliferative zones is a frequent lesion in the brain of premature infants (Volpe, 1995). Such haemorrhage would also destroy cell bodies of the radial glia. So far nothing is known about the consequences that can result from such damage with regard to the functional roles of the radial glia.

MATERIALS AND METHODS

A total of 23 fetal brains obtained from legal elective abortions were used for this study. Fetuses ranged in age from the 22nd to the 32nd week of gestation. Routine neuropathological examination showed no evidence of pathological alterations in these brains. Brains were fixed in paraformaldehyde (4% PFA), cryoprotected [in 30% sucrose in TBS (TRIS buffered saline, 0.05 M, pH 7.4)], and cut into 3 frontal slices. The slices were cut into 120 μm thick sections on a cryostat. Free floating sections were rinsed in TBS buffer after each step of the incubation procedure.

To block nonspecific immunostaining, 2 preincubation steps were performed: (1) 10% methanol and 7% hydrogen peroxide in TBS (30 min); (2) 1.5% lysine, 0.25% triton X-100 and 10% bovine serum albumin (BSA) (all from Sigma–Aldrich, Deisenhofen, Germany) in TBS (2 h). Sections were then transferred to the primary antibody (anti-vimentin, Sigma–Aldrich) at 4 °C on a rotator for ~48 h (antibody dilution 1:200 in TBS containing 2% BSA). Subsequently, sections were incubated in the secondary antibody for 2 h (biotinylated antimouse IgG [Vector Laboratories, Burlingame, USA], diluted 1:200 in TBS containing 2% BSA). The avidin–biotin–peroxidase complex (Vector Laboratories), diluted 1:25 in TBS containing 2% BSA, was then allowed to react for 2 h. The immunocomplex was visualised with the aid of 0.07% diaminobenzidine (Sigma–Aldrich) in TBS and 0.003% hydrogen peroxide. The sections were mounted on gelatin-coated slides, dehydrated in a graded series of alcohol, cleared in xylene and coverslipped with DePeX (Boehringer Ingelheim, Heidelberg, Germany). For details concerning the immunohistochemical procedure see Ulfig et al. (1998*a*).

RESULTS

Distribution of vimentin-immunoreactive structures in the 6th gestational month

The diencephalic nuclei only contain few vimentin-immunoreactive structures. Details of these structures are not illustrative of ontogenetic events at the developmental stages under investigation here. These nuclei are therefore not considered in the following descriptions.

In accordance with data of the literature (Virgintino et al. 1993) vascular cells (endothelial cells and pericytes) are occasionally immunolabelled with anti-vimentin.

An area-specific immunostaining was seen in all coronal sections investigated. In coronal sections through the anterior thalamic nucleus a particularly high density of vimentin-positive fibres was visible within the ganglionic eminence and in the laterally adjacent white matter (Fig. 1*a*). Within the ventricular

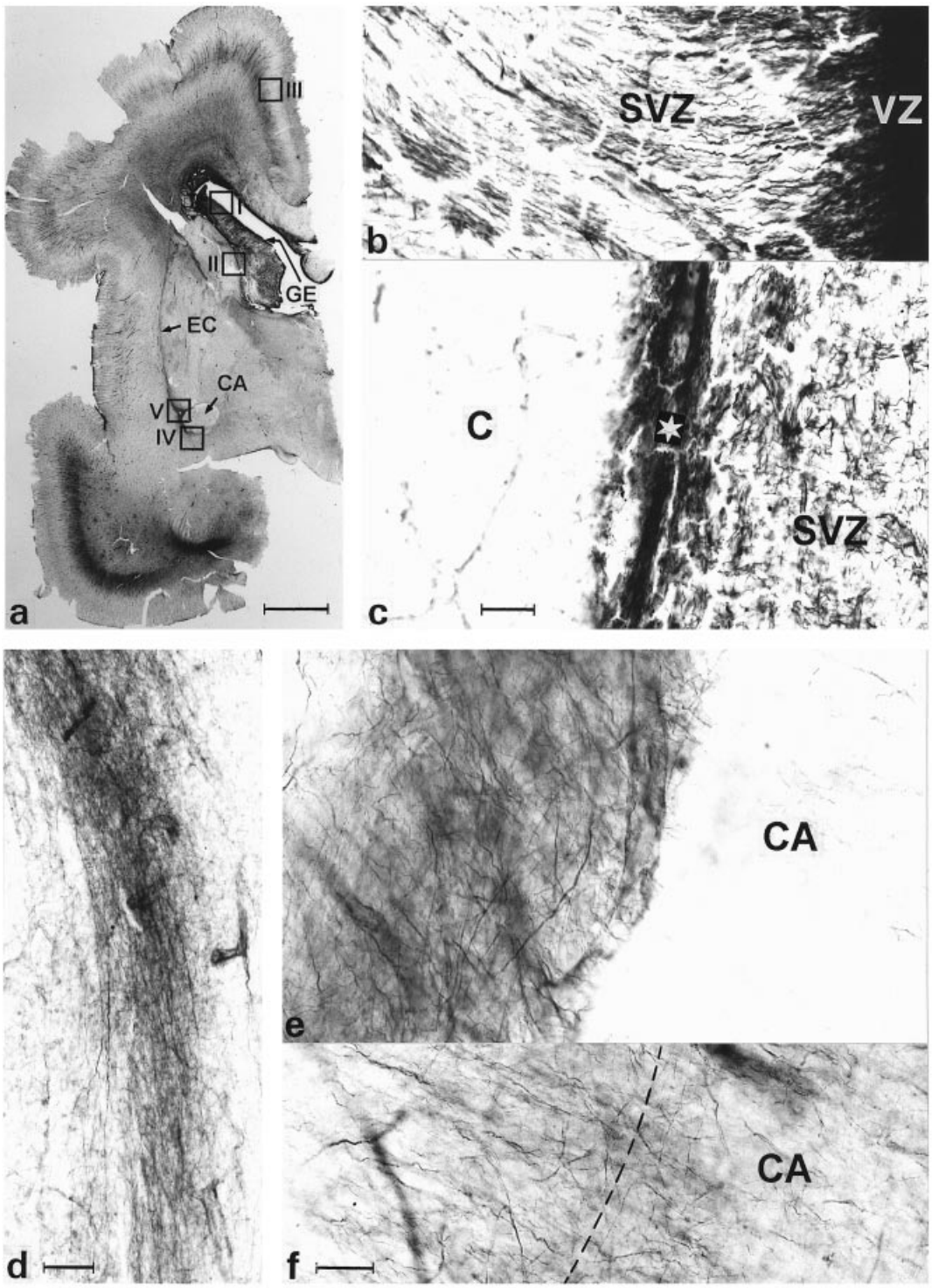

Fig. 1. (*a*) Coronal vimentin-immunostained section through the anterior thalamic nuclei of the 6th gestational month displaying the differential distribution of vimentin-positive fibres in the various prosencephalic areas. The marked areas (frames, I–V) are enlarged in Figs 1*b–f* and 2. (*b*) Ventricular (VZ) and subventricular (SVZ) zone of the ganglionic eminence, taken from I in Fig. 1*a*. (*c*) Subventricular zone (SVZ) of the ganglionic eminence (GE) bordering upon the caudate nucleus (C). Note the intense vimentin-immunolabelling in the border area (marked by an asterisk), taken from II in Fig. 1*a*. (*d*) Densely packed vimentin-positive fibres in the external capsule (EC) in Fig. 1*a* seen in enlargements taken from IV (*e*) and V (*f*) in Fig. 1*a*. Vimentin-positive fibres are diffusely oriented next to the lateral superior (*e*) and lateral inferior (*f*) margins of the anterior commissure (CA). The latter contains a few fibres in its inferior portion. Interrupted line marks the lateral border of the anterior commissure. (*a*) Bar, 5 mm; (*b*, *c*) bar, 50 μm; (*d–f*) bar, 20 μm.

zone of the ganglionic eminence there were a large number of vimentin-positive cells with a high packing density. These cells sent fibres into the subventricular zone of the ganglionic eminence (Fig. 1*b*). The adjacent caudate nucleus was devoid of immunoreactive structures. Numerous fibres were seen at the

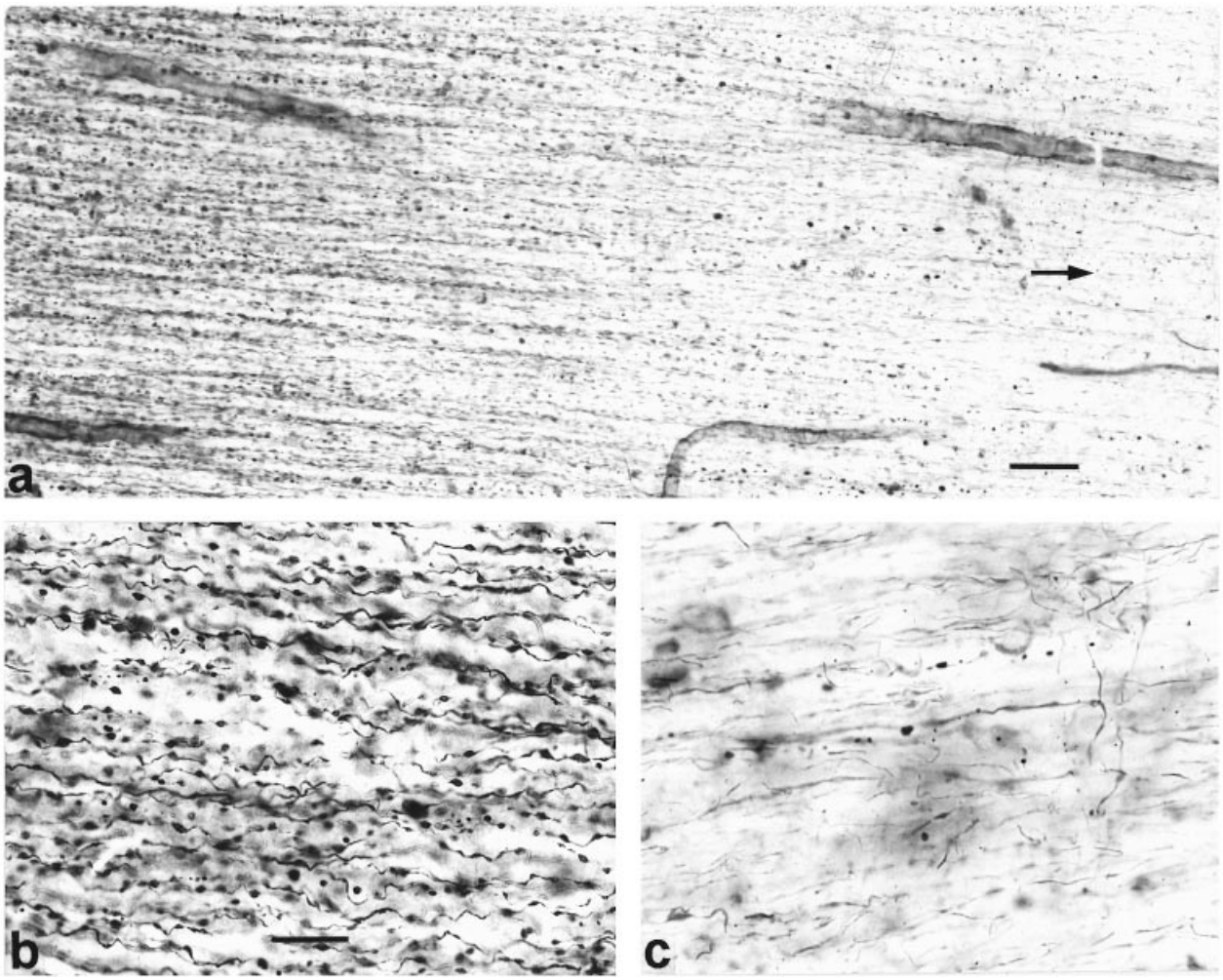

Fig. 2. (*a*) Zone of thick vimentin-positive fibres (left side) merges with zone of slender fibres in the upper white matter (right side), taken from III in Fig. 1*a*. Arrow points towards the pial surface. Enlargements taken from the left side (*b*) and right side (*c*) of *a*. Numerous thick vimentin-positive fibres displaying varicosities are aligned radially. (*a*) Bar, 50 μm; (*b*, *c*) bar 20 μm.

margin of the ganglionic eminence which borders upon the caudate nucleus (Fig. 1*c*).

A large number of fibres were present within the internal capsule next to the dorsal thalamic nuclei. Densely packed vimentin-positive fibres were also encountered in the external capsule (Fig. 1*d*) and next to the lateral margin of the anterior commissure (Figs. 1*e*, *f*). These fibres appeared to be diffusely oriented. The anterior commissure contained few smooth fibres also showing no preferential orientation.

In the white matter of the upper half of the cerebral wall a broad band-like zone of intense immunolabelling was visible. This zone harboured radially aligned fibres which appeared slightly undulated and displayed conspicuous varicosities. When analysed at different levels of focus the fibres often appeared discontinuous (Fig. 2). Intermingled between the thick fibres slender fibres were visible. Above this zone of intense immunolabelling thin parallel fibres with small varicosities were seen. There was a gradual change from the zone of thick to that of thin fibres (Fig. 2*a*, *b*). The slender fibres ascended through the cortical plate.

In coronal sections of the frontal lobe thick fascicles of vimentin-positive fibres were found next to the subventricular zone (Fig. 3*a*). Ascending towards the cortical plate the fibres of the fascicles first diverged and then converged to form less thick bundles (Fig. 3*b–d*). A great number of the fibres of these fascicles appeared to terminate in a clear-cut line which was located approximately in the middle of the cerebral wall (Fig. 3*c*). This line, which was ~ 40 μm in thickness contained blood vessels. At higher magnification vimentin-positive fibres were seen to terminate on these vessels (Fig. 3*e*). Beyond this line a small number of radially aligned slender fibres with varicosities ascended towards the pial surface (Fig. 3*f*).

In coronal sections through the crus of the fornix

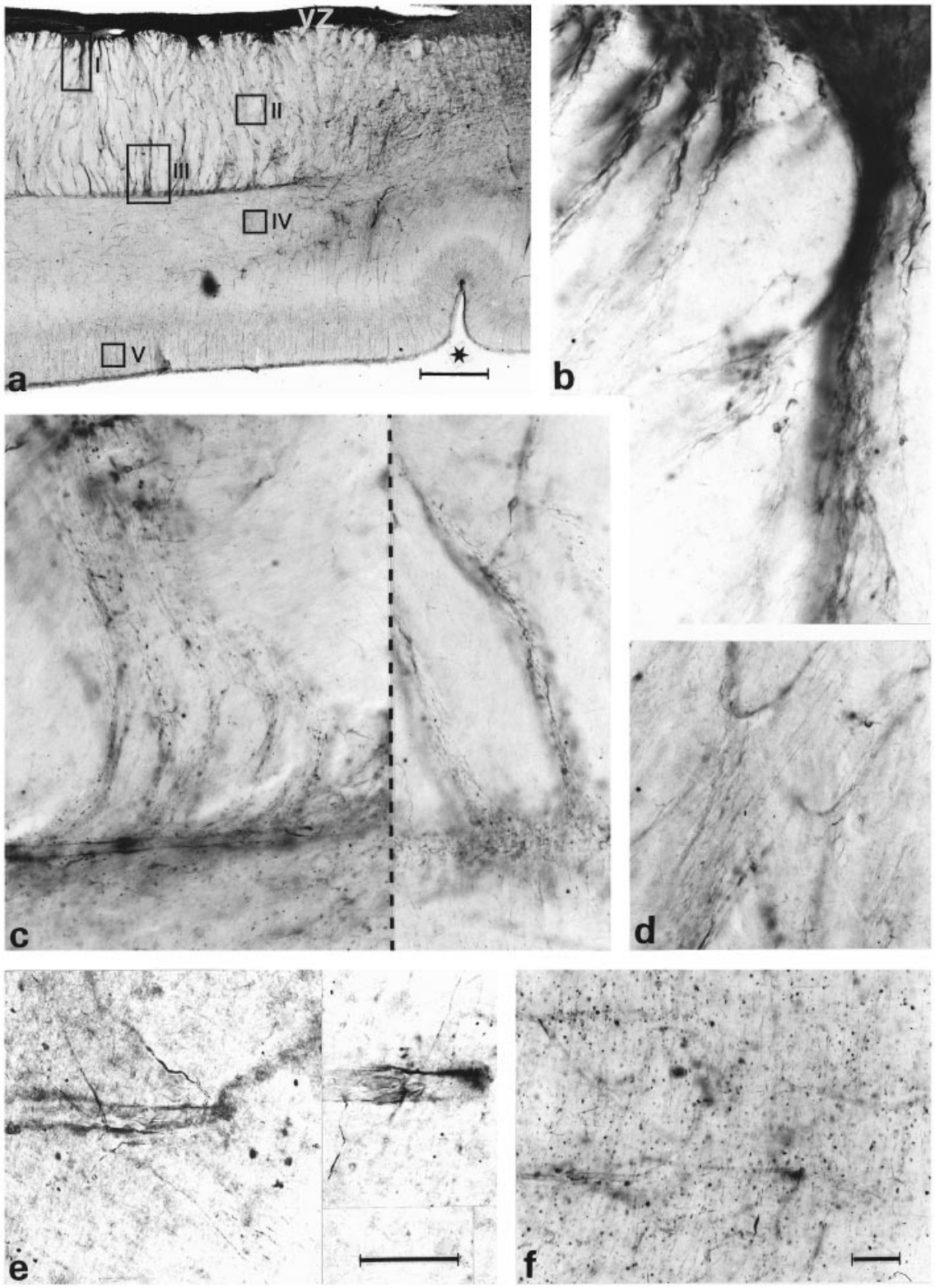

Fig. 3. (*a*) Part of a coronal vimentin-immunostained section through the frontal lobe from the 6th gestational month. Note the more intense vimentin-immunolabelling in the lower half of the cerebral wall and the intensely labelled stripe in the middle of the cerebral wall. (*b*) Thick fascicle of vimentin-positive fibres next to the ventricular zone (VZ), taken from I in Fig. 5*a*. (*c*) Fibre bundles bordering upon the intensely labelled stripe in the middle of the cerebral wall, taken from III in Fig. 5*a*. (*d*) Nonfasciculated fibres, taken from II in Fig. 5*a*. (*e*) Single vimentin-positive fibres terminate upon vessels, taken from IV in Fig. 5*a*. (*f*) Radially aligned slender fibres, densely packed, taken from V in Fig. 5*a*. The above described immunolabelling patterns are not visible below the cingulate sulcus (asterisk). (*a*) Bar, 1 cm; (*b–d*) bar, 50 μm; (*e*) bar, 20 μm; (*f*) bar, 50 μm.

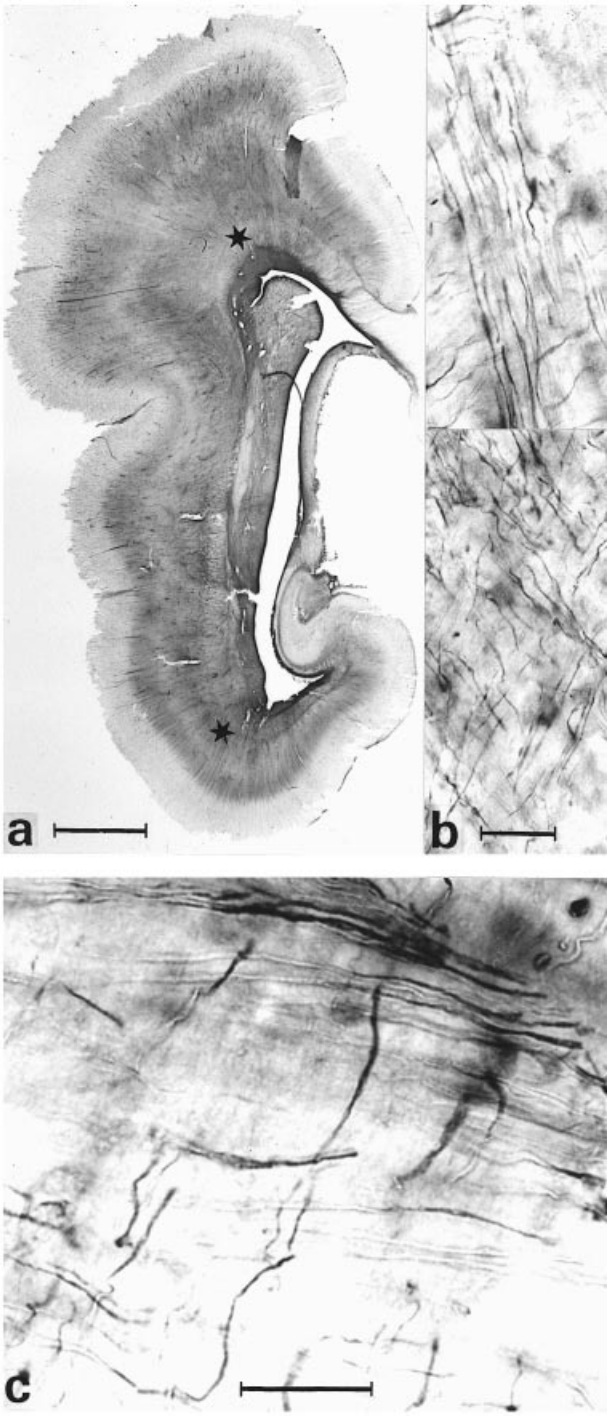

Fig. 4. (*a*) Coronal vimentin-immunostained section through the crus of the fornix of the 6th gestational month. The proliferative zones and adjacent areas are densely immunolabelled. (*b*, *c*) Vimentin-immunostained fibres taken from the white matter areas adjacent to the proliferative areas (asterisks in *a*). Note the crossing of vimentin-positive fibres. (*a*) Bar, 5 mm; (*b*) bar, 20 μm; (*c*) bar, 100 μm.

the white matter harboured numerous vimentin-positive fibres with a homogeneous packing density (Fig. 4*a*). Next to proliferative areas fibres often ran at right angles to each other (Fig. 4*b*, *c*). Such crossing fibres were also seen in the internal capsule next to the ganglionic eminence.

In the occipital lobe where the calcarine sulcus is an outstanding feature various patterns of vimentin-immunoreactive structure were observed (Fig. 5*a*). These different patterns were encountered in band-like zones which were oriented parallel to the ventricular and pial surfaces. Fascicles of vimentin-positive fibres displaying no varicosities were seen adjacent to the proliferative zone. The number of fibres forming a fascicle varied considerably (Figs 5*b*, *c*, 6). Irregularly oriented fibres occasionally revealing varicosities were encountered in the adjacent outer zone. The density of fibres was moderate or high and they sometimes appeared discontinuous or undulated. Above this zone vimentin-positive fibres with a moderate number of varicosities were aligned radially (Fig. 5*d*). The number of varicosities increased when ascending towards the cortical plate (Fig. 5*e*). A small number of very thin fibres with small varicosities traversed the cortical plate in a straight line perpendicular to the pial surface (Fig. 5*f*). Below the depth of the calcarine sulcus 2 different patterns of immunolabelling were seen. Next to the ventricular zone a dense network of immunoreactive fibres was visible. Obliquely oriented fibres all running parallel were discernible above this zone (Fig. 5*g*, *h*).

Distribution of vimentin-immunoreactive structures in the 8th gestational month

Comparing the immunopreparations of the 6th and 8th gestational months no principal differences in immunolabelling patterns were seen. Characteristic features, such as crossing, fasciculation and curvature of fibres, different zones of various immunostaining patterns across the cerebral wall were more pronounced. In order to avoid redundancy, sections presented here of the 6th and 8th gestational month were taken from different levels.

In coronal sections through the posterior part of the thalamus the bulk of vimentin-immunoreactive structures was found next to the remnants of the ganglionic eminence (Fig. 7*a*). In these locations fibres running at right angles were frequently observed. Parallel fibres displaying considerable curvature were found with a high packing density next to the isocortical gyri (Fig. 7*b*). In regions of the white matter that were not beneath gyri the number of vimentin-positive fibres was distinctly lower. Within the allocortical hippocampal formation an intense immunolabelling is seen in the dentate gyrus whereas Ammon's horn only harboured a few diffusely oriented fibres (Fig. 7*c*, *d*).

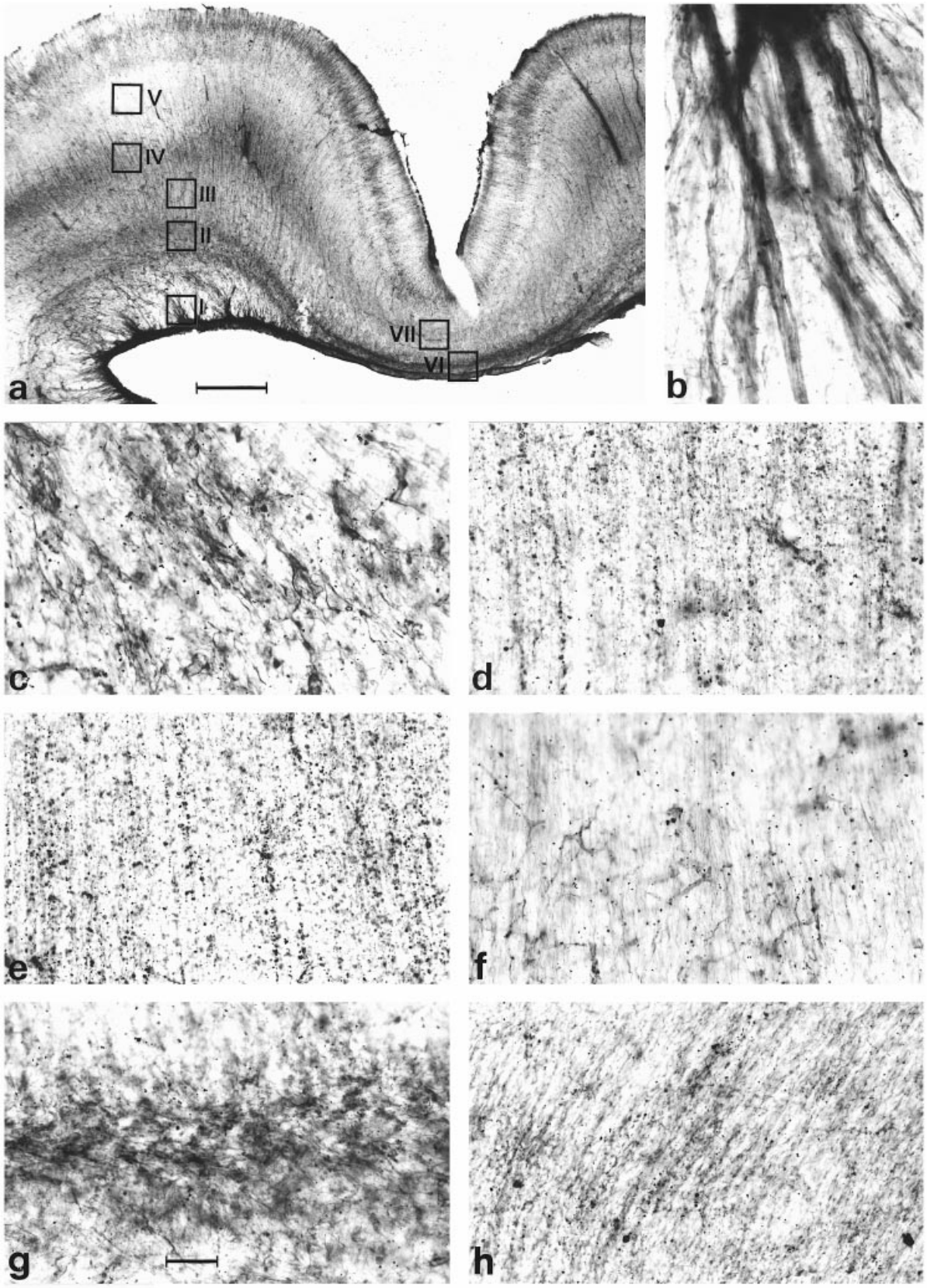

Fig. 5. (a) Part of a coronal vimentin-immunostained section through the calcarine sulcus of the 6th gestational month. Different patterns of immunolabelling are present in band-like zones which are oriented parallel to the ventricular and pial surfaces. (*b–f*), Enlargements taken from I–V in *a*. Below the depth of the calcarine sulcus a dense network of immunoreactive fibres is seen (*g*), taken fromVI in *a*. Parallel aligned fibres are observed above this zone (*h*), taken from VII in *a*. (*a*) Bar, 1 cm; (*b–h*) bar, 50 μm.

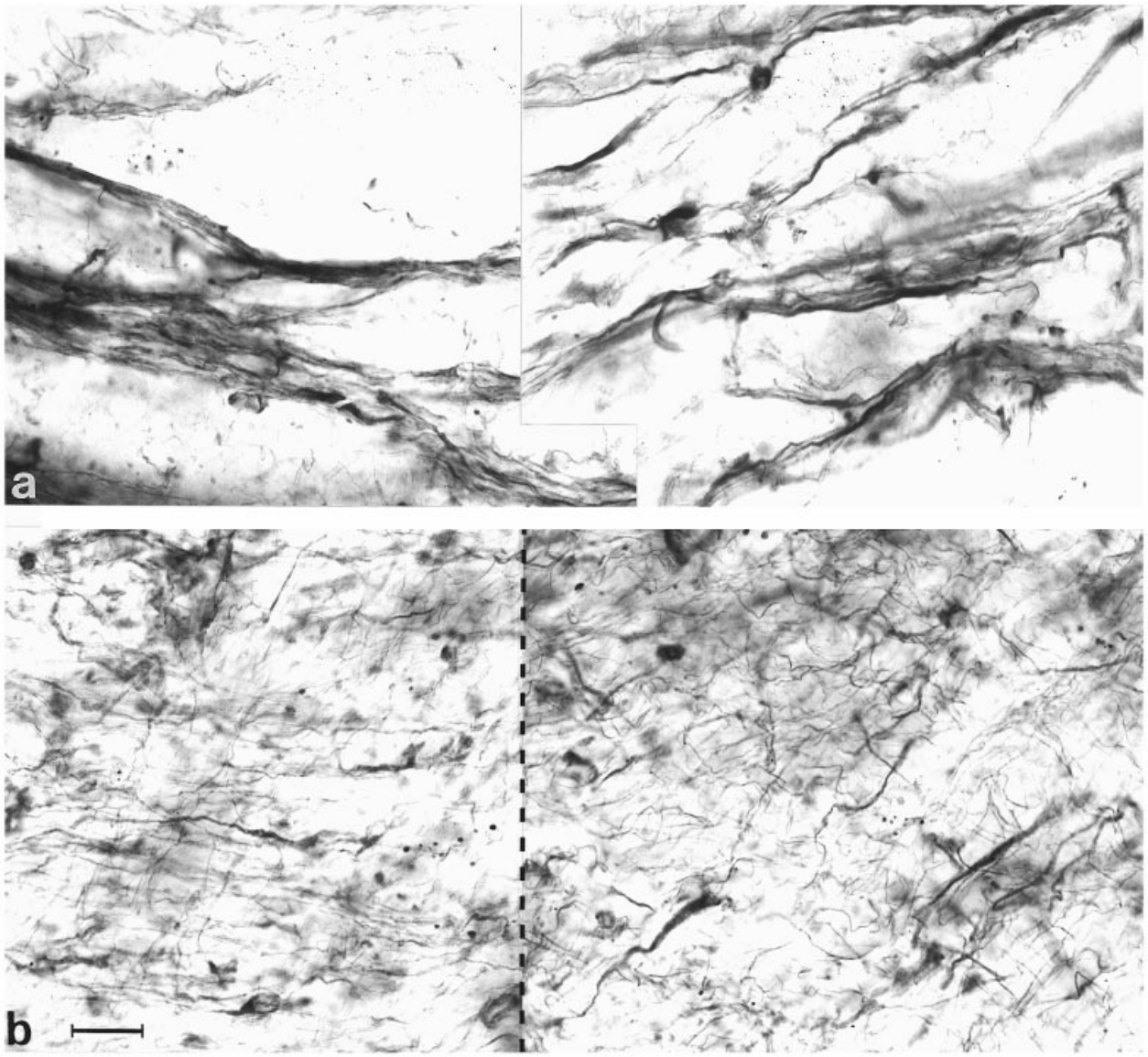

Fig. 6. (*a*) Varying numbers of vimentin–positive fibres forming a fascicle, taken from I in Fig. 5*a*. (*b*) Intersecting fibres, taken from II in Fig. 5*a*. (*a*, *b*) Bar, 20 μm.

A larger number of vimentin-positive astrocytes showing a typical stellate shape were found in the telencephalic white matter (Fig. 8*a*). Numerous cell somata often giving rise to processes were detectable in the ventricular zones (Fig. 8*b*) and bipolar cells regularly oriented perpendicular to the ventricular surface were seen in the white matter in the vicinity to the subventricular zone (Fig. 8*c*). The ascending process of these radial glial cells was directed towards the pial surface and the descending process could be traced towards the ventricular zone. Apart from these bipolar radial cells a moderate number of monopolar cells which only gave rise to an ascending process were located among the bipolar cells (Fig. 8*d*). Frequently, numerous beaded vimentin-immunoreactive fibres running parallel to each other were present (Fig. 8*e*).

DISCUSSION

The radial glial cells which are present transiently in the developing central nervous system represent a specialised class of the astroglial cell lineage. The radial glial cells have been implicated in the guidance of young neuroblasts. They display a bipolar form and their processes span the width of the cerebral wall. The radial glial soma is mainly situated in the ventricular or subventricular zone. Their short descending processes are anchored at the ventricular surface, whereas their long ascending processes reach the pial surface (Rakic, 1995*b*). So far, only investigations on the distribution on vimentin-positive cells or on vimentin-positive structures (including fibres) of the first half of gestation are found in the literature

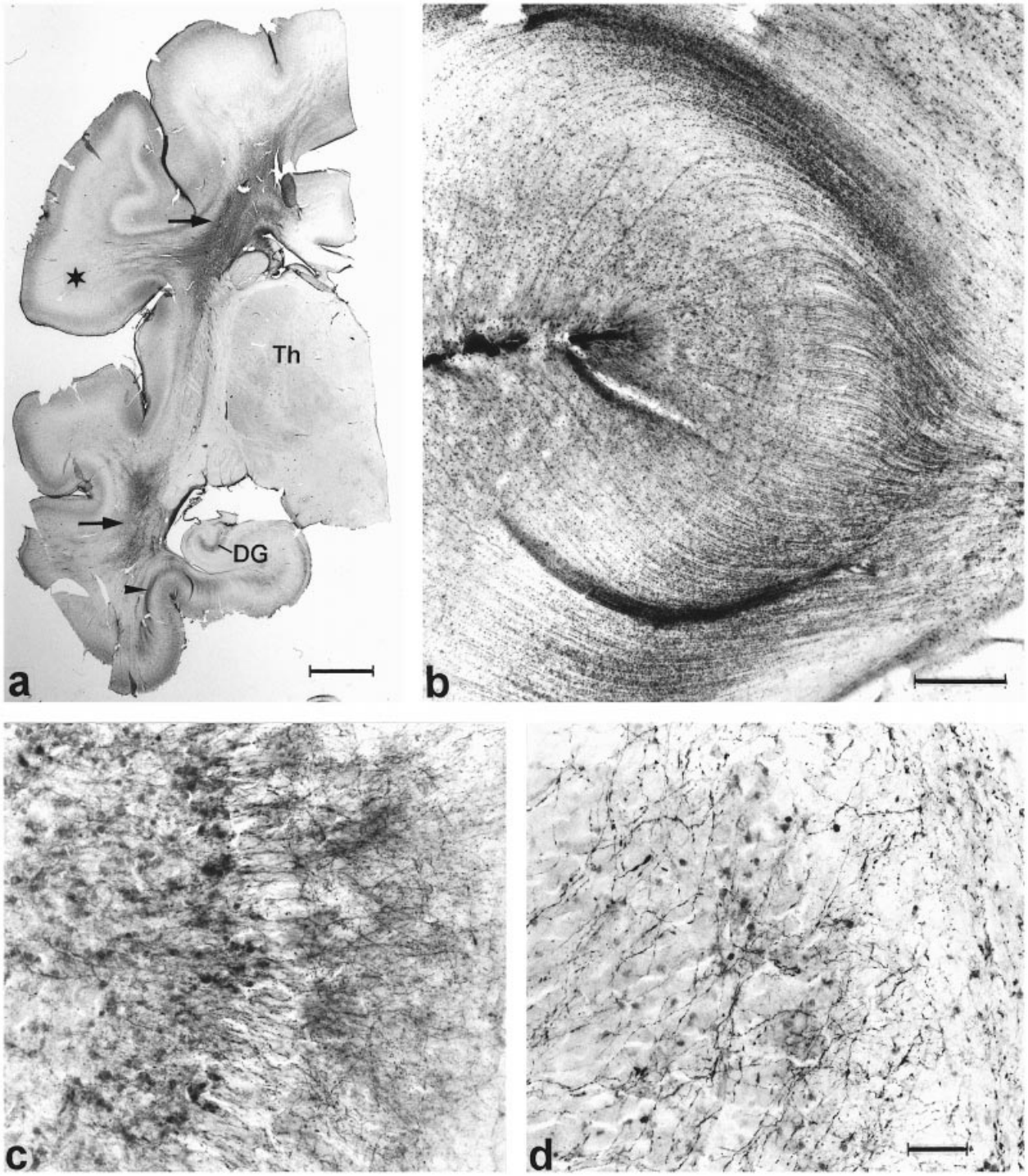

Fig. 7. (*a*) Coronal vimentin-immunostained section through the posterior part of the thalamus (Th) in the 8th gestational month. Note the intense vimentin-immunolabelled fibres next to the remnants of the ganglionic eminence marked by arrows (7*b*). Parallel thick fibres in a curved arrangement next to the collateral sulcus (marked by an arrowhead in *a*). (*c*) Numerous intensely vimentin-immunolabelled fibres in the dentate gyrus (DG in *a*). (*d*) Sector CA2 of the Ammon's horn only harbours a few diffusely oriented fibres. (*a*) Bar, 5 mm; (*b*) bar, 0.5 mm; (*c*, *d*) bar, 50 µm.

(Sasaki et al. 1988; Stagaard & Møllgård, 1989; Stagaard Janas et al. 1991).

Characteristic features concerning the arrangement and packing density of vimentin-positive radial fibres can be demonstrated in relatively thick sections as used for this study.

Area and strata-specific vimentin-immunostaining

The characteristics of packing-density, arrangement or calibre of vimentin-positive radial glial fibres display distinct differences in the various prosencephalic areas. These differences may be interpreted

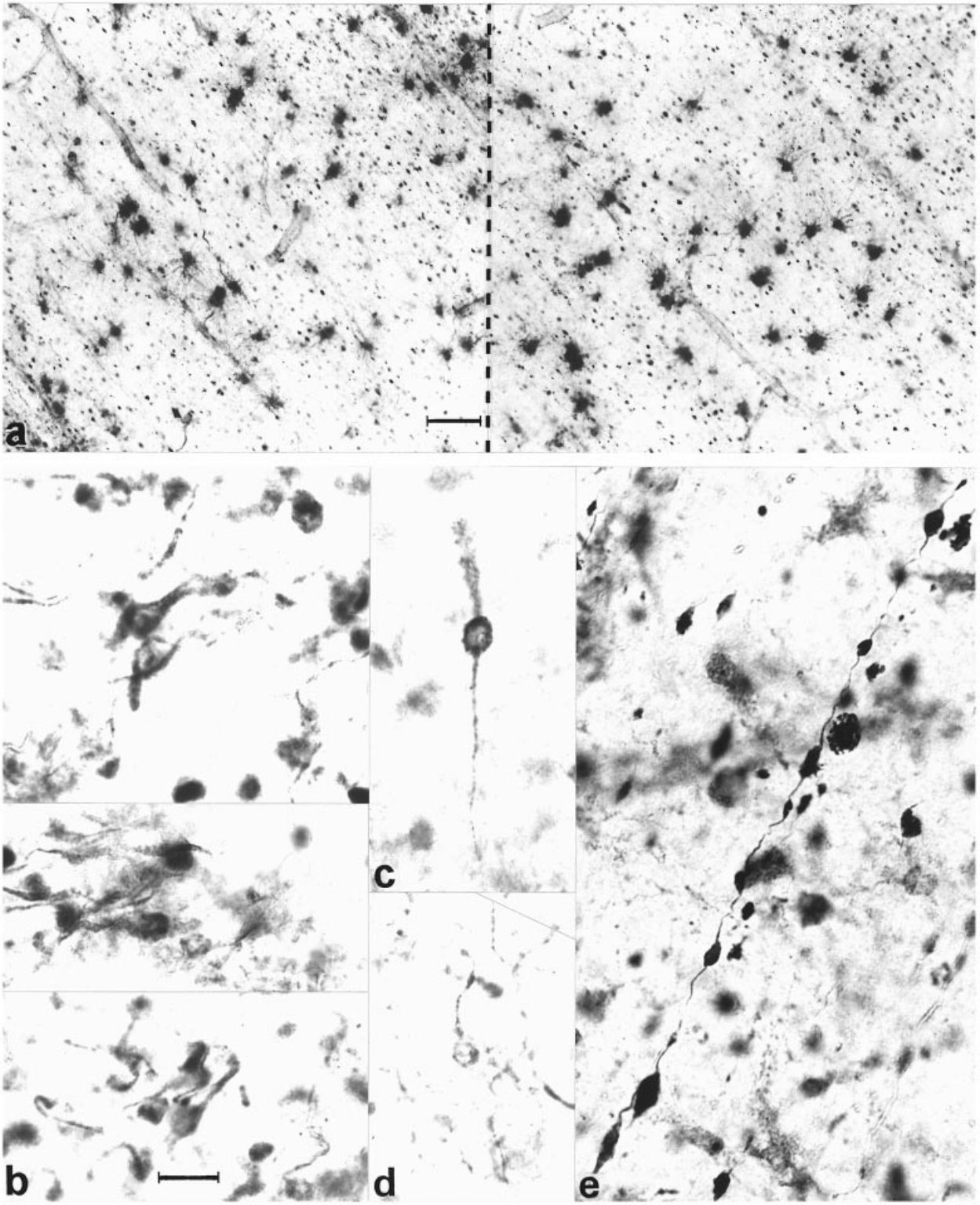

Fig. 8. (*a*) A large number of vimentin-positive stellate cells with numerous processes are present in the white matter (asterisk in Fig. 7*a*). (*b*) Vimentin-positive cells in the ventricular zone of the ganglionic eminence. Bipolar (*c*) and monopolar (*d*) vimentin-positive radial glial cells in the white matter close to the ganglionic eminence. (*e*) Beaded and fragmented radial glial fibres. (*a*) Bar, 50 μm; (*b–e*) bar, 20 μm.

with regard to the functional significance of the fibres or the process of their regression. In particular, various patterns of vimentin-immunolabelling can be differentiated within the white matter.

During early stages of development radial glial fibres running from the ventricular to the pial surface are uniformly radial in alignment (Schmechel & Rakic, 1979). In brains from the second half of gestation investigated here the lower half of the cerebral wall regularly displays fasciculation, crossing

or diffuse orientation of vimentin-positive fibres. In the upper half, however, vimentin-positive fibres no longer intersect; they are radially aligned or reveal curvature with a strictly parallel course next to cerebral sulci. Tangential fibres running at right angles to radially aligned fibres have so far not been considered with regard to their functional role.

The majority of cortical neurons migrate along the radially aligned fibres. In this way neurons originating from a given part of the proliferative zone form a ontogenetic column which represents a definite cyto-architectonic area (radial unit hypothesis; Rakic, 1988). A substantial tangential dispersion of clonally related cortical neurons, however, has been demonstrated (Hatten, 1993; Rakic, 1995*b*). Tangential migration of postmitotic neurons has been shown to contribute to this dispersion (O'Rourke et al. 1995). Although tangential migration is generally believed to take place independently of radial glia (Pearlman et al. 1998) it cannot be excluded that tangential migration in the intermediate zone below the subplate is guided by fibres running parallel to the pial and ventricular surface. This aspect has so far not been considered due to obvious interspecies differences in the arrangements of vimentin-positive tangential fibres (Cardone & Roots, 1990).

In rodent brain a tangential orientation of radial glia has been described for few circumscribed areas (Gadisseux et al. 1989; Oudega & Marani, 1991). In nonhuman primates radial glial is more prominent and obviously its arrangements are more complex than in subprimate species (Schmechel & Rakic, 1979). When comparing our results with those described for the monkey brain, deviation from the radial arrangement appears to be even more pronounced, in particular the phenomenon of intersecting, providing a high number of tangential fibres, is frequently detectable.

Within the hippocampal formation an area-specific immunostaining with anti-vimentin can be observed in the 8th gestational month. The packing density of vimentin-positive structures in the dentate gyrus is conspicuously higher than in the sectors of Ammon's horn. This differential distribution is consistent with studies demonstrating that nerve cells in the dentate gyrus are born much later than those of Ammon's horn (Angevine, 1965). Experimental studies (Rakic & Nowakowski, 1981; Rickmann et al. 1987) have provided evidence for a delayed and prolonged development of the dentate gyrus.

The high number of vimentin-immunoreactive fibres which was also encountered next to isocortical gyri could indicate a late migration of neurons. It should be stressed, however, that the presence of vimentin-positive fibres is not a reliable indicator of migrating neurons. Radial glial fibres can persist after neuronal migration is completed and fulfil other roles. However, the presence of a vimentin-positive scaffold is a prerequisite for migrating neurons. In animal brains it has been shown that cells from adult proliferative zones are only capable of reaching their target when vimentin-positive structures still exist (Alvarez-Buylla et al. 1987; Gates et al. 1995; Peretto et al. 1997).

The ganglionic eminence appearing as a large bulge of the subventricular zone contains precursor cells of, among others, the caudate nucleus and the putamen. In vimentin-immunopreparations of the 6th gestational month a sharp border is seen between the ganglionic eminence and the adjacent caudate nucleus. Thus it can be assumed that migration is complete in this area.

An arrangement of fibres in thick fascicles is seen next to proliferative areas. These vimentin-positive fascicles of radial glia show elongation of processes within fascicles. Such a mechanism of fibre growth based on differential adhesion is comparable to the outgrowth of axons within fascicles (Van Vector, 1998). Ascending towards the cortical plate, fibres of the fascicles regularly diverge. A similar observation has been described for the murine brain (Gadisseux et al. 1989). These authors postulated that migrating cells themselves may dissect their way among fibres of the fascicles as they ascend from the intermediate zone towards the subplate. Another mechanism underlying the process of defasciculation may involve strata-localised molecules which alter adhesion between glial fibres (Keilhauer et al. 1985; Ruoslathi & Pierschbacher, 1987).

Functional roles of vimentin-positive structures in axonal guidance

As late as in the 8th gestational month when migration of young neurons has mainly been completed a high number of vimentin-positive fibres is still encountered in the prosencephalon. It may thus be assumed that during this developmental period radial glia is likely to fulfil other functions in addition to its role in the guidance of young neurons. The orientation and position of radial glial fibres appear appropriate to provide a preformed pathway for outgrowing axons.

The establishment of complex networks of neurons requires directed outgrowth of axons towards their targets. Experimental studies have demonstrated preferential axonal growth on glial surfaces (Joosten

& Gribnau, 1989). Evidence has been provided that radial glial fibres play important roles in the guidance of axonal growth cones during the formation of neuronal circuits (Silver et al. 1982; Norris & Kalil, 1991; Gary et al. 1995).

The concentration of radial glial fibres within the superior part of the internal capsule next to the ganglionic eminence demonstrated in this study is in accordance with the concept that radial glia is involved in axon guidance. It is known from experimental studies that the ganglionic eminence serves as an intermediate target area of growing axons, i.e. corticothalamic or thalamocortical fibres (Metin & Godement, 1996). The radial glial fibres could thus act as guiding cues directing axons towards the ganglionic eminence. Vimentin-positive fibres are also observable in the inferior parts of the internal capsule. In this location another intermediate target region of corticothalamic fibres is found, i.e. the perireticular nucleus (Ulfig et al. 1998*b*). Thus, direction of axons towards the perireticular nucleus also might be accomplished by radial glial fibres.

Another hint at the involvement of radial glia in guiding outgrowing axons is the intense vimentin-immunolabelling next to the lateral margin of the anterior commissure and within the external capsule, as shown in this study.

Transformation of radial glial cells into astrocytes

The process of transformation of radial glial cells encompasses (1) the detachment from the ventricular surface, (2) the outward displacement of the radial cell soma, (3) the loss of radial orientation, and (4) the growth of new stellate processes. These stellate cells corresponding to immature astrocytes still express vimentin. This vimentin expression gradually ceases and is replaced by GFAP (glial fibrillary acidic protein) expression in the mature astrocytes (Oudega & Marani, 1991).

Recent studies have demonstrated that the process of radial glia transformation is under control of diffusible signals (Sotelo et al. 1994; Hunter & Hatten, 1995). In the absence of these diffusible signals radial glia would be transformed into mature astrocytes (Soriano et al. 1997). Reelin which is secreted by Cajal-Retzius cells in cortical layer I may facilitate the action of soluble factors. Thus, Cajal-Retzius cells may regulate the maintenance of the radial glia cells and their processes (Hunter & Hatten, 1995; Soriano et al. 1997). It can be speculated that the distinctly low packing density of the vimentin-positive fibres in the zone bordering upon layer I presented here may be due to decreases in reelin. The lack of reelin could result in a retraction of fibres.

In this study somata of bipolar as well as monopolar radial glial cells are found at variable levels of the white matter. Vimentin-positive cells revealing the distinctive stellate morphology (with several processes) of protoplasmic astrocytes are encountered more superficially in the white matter.

So far it is not known why astroglial cells express 2 kinds of intermediate filament protein during their lifetime. As concerns the expression of vimentin and/or GFAP considerable interspecies differences have been described (Lukáš et al. 1989). Both proteins are expressed during a variable period. The change in cellular morphology, i.e. the occurrence of the multipolar shape, does not occur in parallel with a definable expression pattern of the 2 proteins.

Transformations in the radial fibre system

In the 7th gestational month the peripheral segments of radial glial fibres in all telencephalic areas reveal signs indicative of degeneration. Fibres appear fragmented and display conspicuous varicosities. The latter have been shown to contain large numbers of autophagic vacuoles and lysosomes (Kadhim et al. 1988).

In the frontal lobe the cortical anlage and an adjacent thick stripe of the white matter contain only few radial glial fibres. At the inferior margin of this stripe radial fibres are regularly observed to terminate on vessels, an observation that also points to degeneration of radial fibres. Lukáš et al. (1989) and Zerlin et al. (1995) suggested that blood vessel contact plays a role in the differentiation of radial glial cells into astrocytes. After detachment and displacement of cell somata, single radial fibres of these cells have been described as terminating on blood vessels. In our material single fibres also terminate on vessels (Schmechel & Rakic, 1979; Virgintino et al. 1993). Cells contacting blood vessels did not show the characteristic multibranched morphology as seen in astrocytes. Thus blood vessel attachment precedes the full elaboration of a complex pattern of elaborated astrocytic processes.

The oval somata of bipolar radial glial cells are translocated from the proliferative zone towards the intermediate zone, i.e. the white matter. A transformation of radial glia into immature astrocytes then takes place. A transitional cell type between the bipolar radial cells and multipolar astrocytes has been described. This monopolar cell type develops from the bipolar radial cell through the loss of the descending

process. At first, the ascending process of the monopolar cell is slender and radially aligned. It may reach the pial surface and is indistinguishable from ascending processes of bipolar radial cells. Later on, the ascending process of monopolar cells becomes thicker and may emit 2 or 3 apical branches. Moreover, this process no longer reaches the cortical plate and is often observed to be in contact with vessels (see above). The mechanism underlying the transformation of bi to monopolar radial cells is likely to include interactions between nerve and glial cells (Takahashi et al. 1990).

The characteristics of monopolar cells and their processes as described above are in accordance with the results of the present study. A gradual change in the density and diameter of vimentin-positive fibres is clearly visible within the white matter at some distance from the cortical plate. In the upper part of the cerebral wall few thin fibres are seen whereas in the lower part thick and thin fibres with high packing densities are observed. The latter pattern may reflect the presence of ascending (thin) processes of bipolar and ascending (thick) processes of monopolar cells. The upper part only contains thin processes of bipolar radial cells.

It should be stressed that during the period under investigation here no apparent decline in radial fibre concentration is seen. The most obvious difference between the 6th and 8th gestational months is an increase in morphological signs of a transformation in the radial fibre system. Such degenerative changes in radial fibre morphology are associated with transformation events of radial glial somata.

Significance for developmental neuropathology

The results of this study demonstrating a complex radial fibre architectonic provide a basis for further investigations on possible alterations in the radial glial scaffold in dysplasias of the cerebral cortex which are due to migrational disturbances. Moreover, the results are needed to examine CNS complications in preterm infants. The incidence of preterm delivery is relatively high and unchanging. The survival rates for the smallest premature infants continuously increase. Thus haemorrhage in proliferative zones represents a major problem as its incidence is directly correlated with the degree of prematurity (Volpe, 1995). Such haemorrhage has so far mainly been linked to the destruction of glial precursor cells. However, it would also damage somata of radial glia and thus impair the network of vimentin-positive fibres. Furthermore, preterm infants have a predilection for white matter injury as a result of hypoxic-ischaemic insults (periventricular leukomalacia). The most common localisation of the lesions is the white matter in close vicinity to the lateral angles of the ventricle (Kinney & Armstrong, 1997). At this location a concentration of vimentin-positive fibres crossing each other is seen. So far, it is not known whether periventricular leukomalacia also affects the arrangement and the number of vimentin-positive fibres.

Recently, neuronal necrosis in the fascia dentata of the hippocampus has been observed in 60 % of cases with periventricular leukomalacia (Torvik et al. 1992). The results presented here demonstrate a dense network of vimentin-positive fibres in this location. It would be interesting to investigate whether an impairment of the radial glia can also be observed in such cases.

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