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Radial Glia and Astrocytes in Developing and Adult Telencephalon of the Lizard *Gallotia galloti* as Revealed by Immunohistochemistry With Anti-GFAP and Anti-Vimentin Antibodies

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ABSTRACT

The development of radial glia and astrocytes in the telencephalon of the lizard *Gallotia galloti* was studied by immunohistochemistry with anti-vimentin and anti-GFAP antibodies.

Vimentin appears at embryonic stage 32 (E32) in the proliferative zone of the lateral ventricle and subpial end-feet in the marginal zone. At E34–35 the staining intensity for vimentin in all radial glia is maximal. It then decreases and disappears in most structures in adult animals.

GFAP appears at E35 in the end-feet in the marginal zone and its intensity increases until adulthood, particularly in radial and sinuous fibers and in fibers that originate from the sulci and invade the ventral striatum and the septum. In contrast, the reaction is weak in the cortex, in the anterior dorso-ventricular ridge, and in the amygdala nuclei.

Radial glia is still present in the adult, and the composition of its intermediate filaments changes during development from vimentin to GFAP.

No GFA-positive cell bodies except those of ependymal glia were detected in telencephalon.

Key words: reptiles, cortex, basal nuclei, glial cells, intermediate filaments, ontogeny, phylogenesis

Radial glia is a phylogenetically primitive and ontogenetically immature form of glia (Kruger and Maxwell, '66; Friede et al., '69; Schachner et al., '77; Polak et al., '82; Onteniente et al., '83; Miller and Liuzzi '86). Radial glia perikarya are localized in the periependymal region (Ebner and Colonnier, '75; Rakic, '82), and its radial glial processes are considered to act as guides for neuronal migration. Because of the palisade-like organization of its processes and the possible role in neuronal migration, Bergmann glia in the cerebellum is considered to be a variant of radial glia (Rakic, '71, '72). Bergmann glial cell bodies are localized far from the ependyma and are present in the adult. In mammals, radial glia disappears when central nervous system (CNS) development ends (Choi and Lapham, '78, '80; Schnitzer et al., '81; Franke et al., '82; Choi et al., '83; Pixley and De Vellis, '84), while in teleosts, lizards, and other reptiles, radial glia is still present in the adult (Achucarro, '15; Ebner and Colonnier, '75).

Studies on reptilian glial cells are scarce and have been carried out mainly with traditional morphological methods, but there are recent reports of the use of immunohistochemical techniques with antibodies recognizing the "glial fibrillary acidic protein" (GFAP) (Dahl and Bignami, '73; Onteniente et al., '83; Dahl et al., '85). GFAP is a protein specific for gliofibrils, the intermediate filaments present in astrocytes of all types [typical star-shaped fibrous or protoplasmic astrocytes, Bergmann glia, (peri)ependymal radial glia or tanycytes] in mammals or in other vertebrates (Eng et al., '71; Bignami et al., '72, '80; Dahl and Bignami, '73; Schachner et al., '77; Eng and Bigbee, '78; Ghandour et al., '79, '83; Eng, '80; Levitt and Rakic, '80; Levitt et al., '81; Shaw et al., '81;

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Choi et al., '83; Onteniente et al., '83; Bullon et al., '84; Dahl et al., '85; Didier et al., '86). GFAP is clearly detected in all the vertebrates studied except cyclostomes such as the lamprey in which "glial" cells contain GFAP-negative intermediate filaments (Onteniente et al., '83). Amphibians contain low levels of GFAP (Dahl et al., '85; Miller and Liuzzi, '86). Most of the immunological characteristics and the cellular specificity of GFAP are conserved during phylogenesis (Dahl and Bignami, '73; Onteniente et al., '83; Dahl et al., '85), but ontogenetic and phylogenetic differences exist with regard to GFAP distribution in the different cell types. Also the proportion of the astrocyte subtypes and their regional distribution in different species is variable.

Astrocyte maturation is accompanied by modification of its intermediate filament composition. In the immature astrocyte, intermediate filaments consist of vimentin (Bennett et al., '78a; Franke et al., '78, '79; Starger et al., '78; Lazarides, '80, '82; Ramaekers et al., '80; Dahl et al., '81; Gabbiani et al., '81; Bignami et al., '82; Paulin et al., '82; Schachner et al., '82; Cochard and Paulin, '84; Pixley and De Vellis, '84). Vimentin is also present in mesenchymal cells and in some neural cells during development (Lazarides, '82; Langley et al., '84). It is highly conserved, as shown by the cross reaction of antibodies raised against mammalian vimentin with bird and amphibian cells (Bennett et al., '78a,b; Franke et al., '78, '79). As maturation proceeds, vimentin disappears from astrocytes and intermediate filaments consisting of GFAP appear (Eng and Bigbee, '78; Bignami et al., '80; Levitt and Rakic, '80; Onteniente et al., '83). In the adult mammalian nervous system, anti-vimentin antibodies react with meninges, smooth muscle cells, endothelial cells, and red blood cells in blood vessels (Lazarides, '82; Langley et al., '84). No data on vimentin in astrocytes during reptile development are available.

The present data, obtained by using immunohistochemistry with anti-vimentin and anti-GFAP antibodies, concern glial cells in the developing and adult *Gallotia* telencephalon.

MATERIALS AND METHODS

Chemicals and reagents

Sheep anti-rabbit Ig conjugated with fluorescein isothiocyanate (FITC) or with horseradish peroxidase and FITC-conjugated goat anti-mouse IgG were all purchased from Institut Pasteur Production (Paris, France). Rabbit anti-bovine GFAP immune serum were from Dako Immunoglobulins Ltd. (Copenhagen, Denmark), goat anti-rabbit antibody from Nordic immunological Laboratories (Tilburg, The Netherlands), rabbit PAP complex from Sternberger-Meyer (Jarrettsville, MD USA), and mouse Ig were purchased from Sigma (Saint Louis, MO USA). The anti-vimentin monoclonal antibody produced in mice (Goetschy et al., 1986) and the rabbit anti-human GFAP immune-serum were kind gifts from Drs. J. Ciesielski-Treska and J.P. Delaunoy, respectively. Other reagents were of analytical grade.

Animals and tissue fixation

We used a total of 50 embryos (from E32 to hatching), 50 young animals (from the 5th to the 15th postnatal day), and 60 adults of *Gallotia galloti* (family Lacertidae, order Squamata) a lizard indigenous to the island of Tenerife (Bischoff, '82). The stages of the embryo development were defined according to the tables of equivalence between the development of the *Gallotia galloti* (Ramos, '80) and of *Lacerta vivipara* (Dufaure and Hubert, '61).

In ether-anesthetized adults, the central nervous system was fixed "in situ" by intracardiac perfusion with 2% paraformaldehyde and 0.25% glutaraldehyde in PBS (0.15 M Nacl in 0.1 M phosphate buffer, pH 7.2) or TBS (0.15 M NaCl in 0.1 M Tris-HCl, pH 7.2). The skull was opened and telencephalon and brainstem were excised, cleaned from meninges, and then immersed for 3–4 hours in the fixative. Embryos and young lizards were decapitated and their heads immersed for about 2 days in the fixative. Handling of the embryo sections was very difficult because of their small size; hence, the encephalon and brainstem of embryos and young animals were embedded in 4% agarose before slicing. Sections, 50–100 μm thick, were obtained with a Vibratome (Oxford Instrument, Foster City, USA) and then kept at +4°C until processed as described below.

Immunohistochemistry

All incubations were performed at room temperature unless otherwise specified. Antibodies were diluted in PBS for the immunofluorescence technique, and in TBS for the PAP (peroxidase-antiperoxidase) procedure. After each step, the sections were washed three times (10–15 minutes each time) either in PBS or in TBS depending on the procedure.

For immunofluorescence, free-floating sections were first preincubated (1 hour) in 2% BSA, then incubated (2 hours) with the primary antibody (e.g., rabbit anti-GFA immune-serum, or preimmune rabbit serum for controls, both diluted 1:100 while the anti-vimentin monoclonal antibody and the mouse Ig used for control were diluted 1:20). After washing, tissue sections were incubated for 2 hours with the secondary antibody (either FITC-conjugated anti-rabbit Ig or anti-mouse IgG raised in goat diluted 1:100).

For the PAP technique, free-floating sections permeabilized with 20% acetic acid in ethanol (at −20°C for 10 minutes) were preincubated in 4% BSA and then incubated overnight with the primary antibody (e.g., anti GFAP immuneserum diluted 1:100 and normal sheep serum diluted 1:100 together in TBS containing 0.02% NaN_3). The next day the sections were incubated for 30 minutes with goat anti-rabbit Ig (diluted 1:20 in TBS) and then with the PAP complex (diluted 100-fold) for another 30 minutes.

Abbreviations

ADVR	anterior dorsal ventricular ridge
DC	dorsal cortex
DMC	dorsomedial cortex
IZ	intermediate zone
LC	lateral cortex
MC	medial cortex
MZ	marginal zone
Nsa	nucleus septalis anterior
Nsd	nucleus septalis dorsal
Nsl	nucleus septalis lateral
pm	pia mater
SL	sulcus lateralis
SSM	sulcus septomedialis
ST	sulcus terminalis
StV	striatum ventralis
SV	sulcus ventralis
V	ventricle
VZ	ventricular zone

Peroxidase activity was revealed with diaminobenzidine (DAB) according to the method of Graham and Karnovsky ('66). Microscopic observations were carried out as previously described (Ghandour et al., '83).

RESULTS

Vimentin-like immunoreactivity

The anti-vimentin antibody stained different zones of the developing Gallotia galloti telencephalon (Fig. 1). Changes in staining intensity during the lizard development were observed.

At embryonic stage 32 (E32), every zone of the lateral ventricle wall, namely, the sulci lateralis (SL), terminalis (ST), ventralis (SV), and septomedialis (SSM), and the zones in between the different sulci, are vimentin-positive (Fig. 2a). At this stage the staining is weak, though the labelling in some of the cells of the ventricular wall is clear-cut. Vimentin-positive fibers originating from these cells run radially in the cortex, in the ventral striatum, and through the basal nuclei (anterior dorsal ventricular ridge, or ADVR, amygdala nuclei, and septum). In these areas the positive fibers are not straight but follow undulating pathways (not shown). In the marginal zone (MZ), end-feet appear intensely stained (Fig. 2a).

At E33 and E34, a slight increase of the staining intensity was found in all the above-cited zones. At E34 an immunoreactive perinuclear ring was observed in the cells of the ventricle wall (Fig. 2d). In the ADVR a network of interwoven, undulating and straight vimentin-positive fibers was observed (Fig. 2d and insert).

At E35, the immunoreactivity is still present in all areas and is particularly intense in the sulcus lateralis and in the end-feet in the marginal zone bordering the striatum ventralis (Fig. 2f,g). The fibers in the sulcus septomedialis and those crossing the striatum ventralis are straight (Fig. 2b). A striking feature at this stage is the presence of blood vessels covered with numerous immunopositive end-feet (Fig. 2c).

At E36, the immunoreactivity begins to decrease in the sulcus ventralis. Then, from E37, the intensity decreases more rapidly in all areas until vimentin reactivity disappears in the adult except for some subpial end feet in the dorsal cortex (not shown).

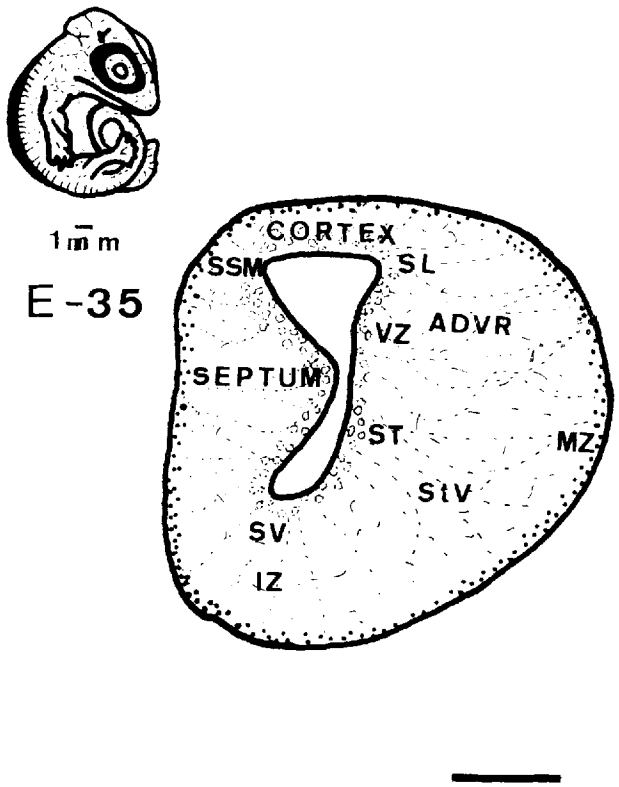

Fig. 1. Diagram of a transverse section of the telencephalon of G. galloti at embryonic stage E35: vimentin immunoreactivity in the different zones around the lateral ventricle is shown. Scale bar: 100 μm.

GFAP immunolabeling

GFAP immunolabelling appears at E35 in the end-feet of the marginal zone then progressively increases in intensity and appears in the other zones (Fig. 3). At E40, immunoreactive ependymal glia cell bodies and radial process are detected around the lateral ventricles (Fig. 4a,b). Furthermore, at this stage, the first GFAP-positive end-feet surrounding blood vessels become detectable. In the cortex, the ependymal glial cell processes maintain their radial distribution. From early post-hatching stages (Fig. 4c,e) to the adult, the staining intensity continues to increase in perivascular end-feet (Fig. 4d). A weak GFAP staining appears in cell processes in the ventral striatum and in the septum (Fig. 4d). GFAP-positive perinuclear rings in ependymal glial cells become clearly detectable (not shown).

In the adult, the different basal nuclei, the cortex, and the different zones in the walls of the lateral ventricles show immunostaining of different intensity. Cell bodies and processes in the sulci in the lateral ventricles are the most intensely stained. In the sulcus lateralis, fibers take sinuous paths and cross over each other, and many of them terminate with subpial end-feet, while in the sulci terminalis and ventralis, the ependymal glial cell processes are straight, spread radially and end either on a blood vessel (Fig. 5a,b) or subpially. A special feature of these end-feet is their bulbous or button-like appearance (Fig. 6a). Cell processes originating from the sulcus septomedial are also straight and spread radially (Fig. 6b), but in the medial and dorsomedial cortex some of these processes pursue radial paths while others run parallel to the ventricular wall (Figs. 5c, 6b). Ependymal glial cells in the ventricular wall in the zones in between sulci (which are usually defined as the layer of "ependymal glial cell") also show GFAP-positive perinuclear rings; furthermore, their processes are mostly undulatory.

GFA-positive cell processes are numerous in the neuropil of dorsal cortex, septal nuclei, and the ventral striatum (Figs. 5c, 6c–e), less abundant in the amygdala nuclei and virtually absent in the ADVR and in the lateral cortex.

DISCUSSION

Ramón y Cajal ('28) and Akers ('77) have shown that during the development of mammalian cerebral cortex the radial glial fibers disappear postnatally. In the telencephalon of *G. galloti*, radial glial processes persist in the adult, but the composition of their intermediate filaments changes during development.

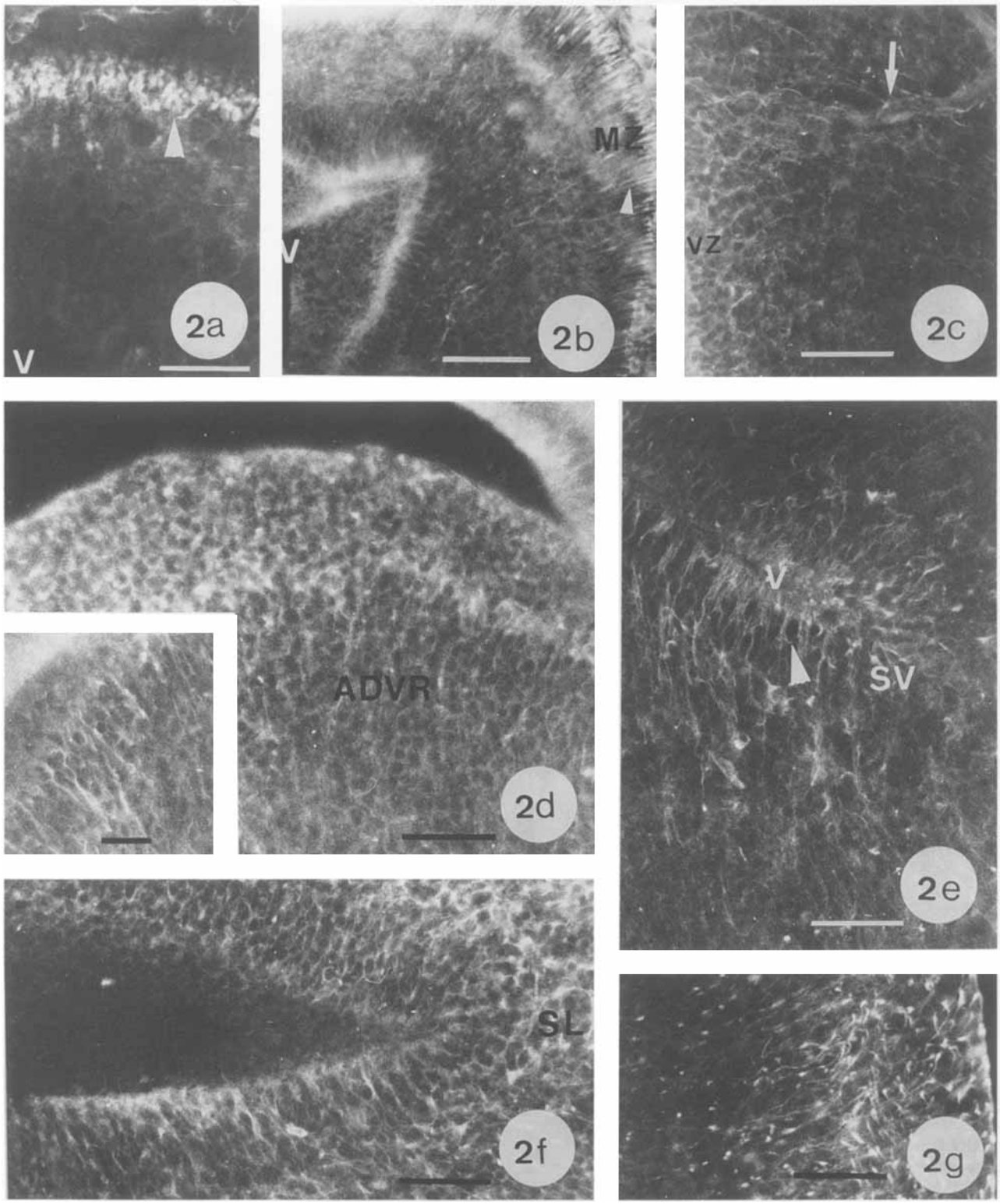

Fig. 2. Vimentin localization at different embryonic stages of telencephalon as revealed by immunofluorescence: **a**) stage E32—subpial end-feet (arrowhead) in the MZ; **b**) stage E35—end-feet in the marginal zone at the level of the SSM. Note the positive perinuclear ring of the ependymal cells; **c**) stage E35—radial glial fibers in the VZ; some of them surround a blood vessel (arrow); **d**) stage E34—radial glial cell fibers in the ADVR. **Insert:** Cell bodies and processes at higher magnification. **e:** Stage E35—positive cell bodies (arrowhead) and radial fibers are immunofluorescent in the SV. **f:** Stage E35 in the anterior part of the SL. **g:** Stage E35—end-feet in the MZ at level of the SV. Scale bars: 100 μm; insert, 50 μm.

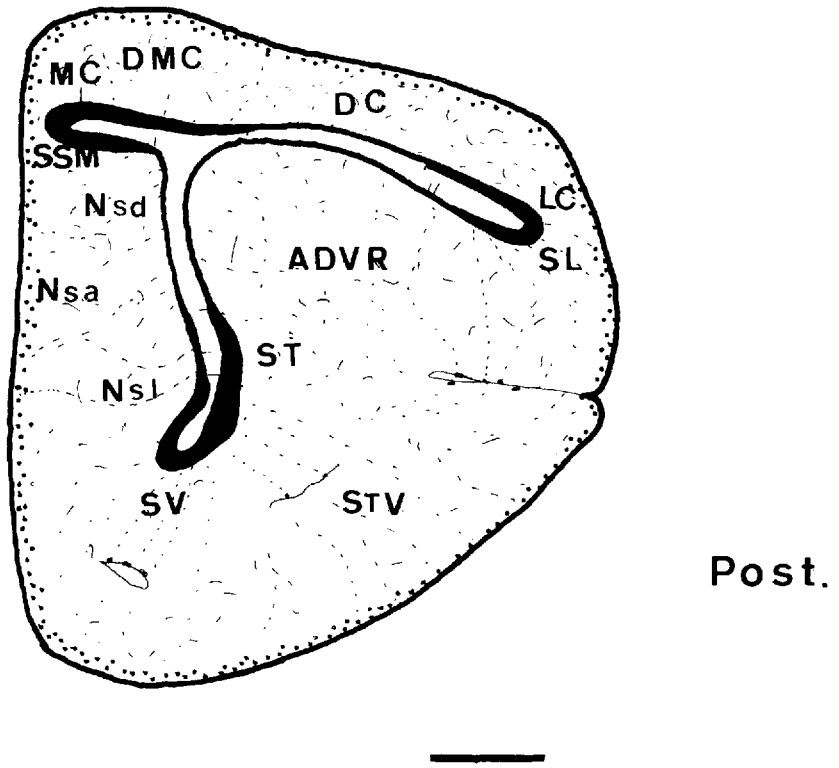

Fig. 3. Diagram of a transverse section of the postnatal *G. galloti* telencephalon, showing anti-GFAP immunoreactivity in the wall of the lateral ventricle, in the cortex and in the basal nuclei. Scale bar: 100 μm.

Vimentin-positive structures

In the *G. galloti* embryo the major component of intermediate filaments of radial glial fibers is vimentin. Its cellular localization corresponds to that described in mammals by Lazarides ('80, '82), Schnitzer ('81), Bignami et al. ('82), and Pixley and De Vellis ('84). Also, the vimentin-positive perinuclear rings in ependymal glial cell bodies are similar to those reported by Schnitzer ('81) in the mouse. In this respect vimentin-containing radial glia in the lizard is very similar to that in mammals. However vimentin-positive scattered cells, which correspond to immature astrocytes in developing mammalian nervous system, are absent in *G. galloti* telencephalon.

Vimentin-GFAP transition

In rodents, vimentin appears during the first embryonic stages and disappears during early postnatal development; vimentin immunoreactivity is also present in immature astrocytes, then disappears with maturation and is replaced by GFAP immunoreactivity (Pixley and De Vellis, '84). In *G. galloti* telencephalon, vimentin immunoreactivity decreases starting from E37 and disappears almost completely in the adult except for a few end-feet. The disappearance of vimentin is not associated with the disappearance of radial glial fibers but with the appearance of GFAP in these fibers. At E35 a weak GFAP immunoreactivity appears in the end-feet of the marginal zone. Between E37 and E40 both vimentin and GFA are present either in the same or in contiguous fibers. Schnizter et al. ('81) have suggested that two systems of intermediate filaments (one made of GFAP and the other of vimentin) could coexist at certain stages in the same mouse cells.

The simplicity of the radial fibers system in *Gallotia* telencephalon allows us to establish a temporal (but not necessarily a causal) correlation between the presence of vimentin in these fibers and neuronal cell body and growth-cone migration. Rakic ('71, '72) and Levitt and Rakic ('80) have suggested that radial glia fibers are the support for neuroblast migration and Dupouey et al. ('85) have suggested that radial glia also guides the migration of axonal and dendritic growth cones. We have observed that in the telencephalon of *G. galloti* the first wave of neuroblast migration occurs between embryonic stages E32 and E35 (Yanes, '85; Yanes et al., '87, '89), and that axonal and dendritic growth also occurs between E32 and E37 (Yanes, unpublished results), e.g., when the fibers are vimentin-positive.

Another role for vimentin-containing fibers was proposed by Pixley and De Vellis ('84), who suggested their involvement in transport processes between "the extracellular fluid compartment" (i.e., extracellular space, cerebrospinal fluid, blood) and the adjacent tissue parenchyma. This hypothesis was based on the following considerations: all cultured cells express vimentin; all the cells which express vimentin in the adult in vivo are cells in contact with extracellular fluids; and, above all, in the CNS vimentin-positive radial fibers disappear at the time of the dramatic reduction of extracellular space. In *Gallotia*, however, our data do not support this hypothesis since some end-feet remain vimentin-positive even in the adult, and the developmental change of

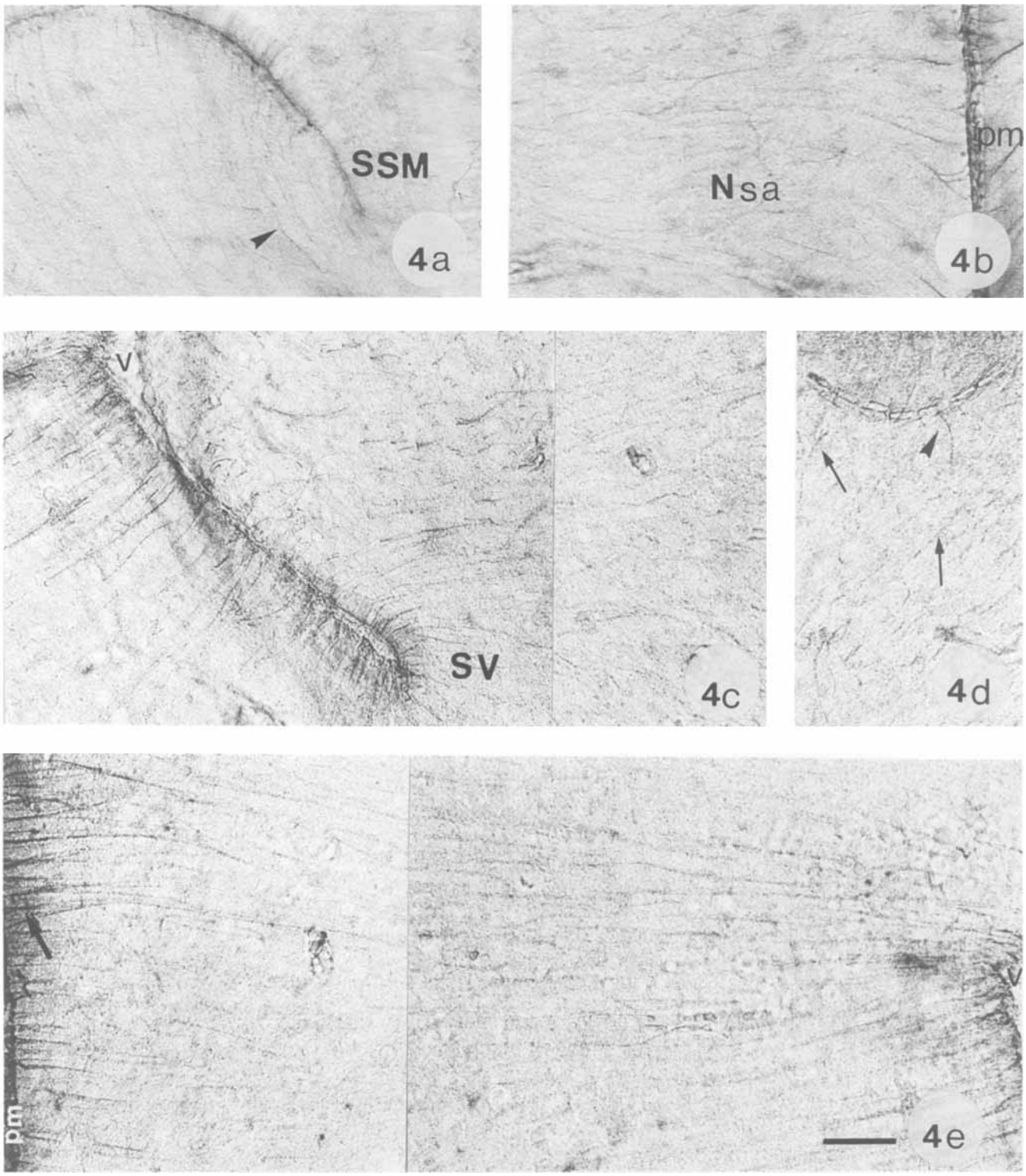

Fig. 4. Immunohistochemical detection of GFAP in transverse sections of telencephalon at E40 and at the 15th post-hatching day (PAP method). **a:** E40 embryo—weak staining of radial fibers (arrowhead) in the SSM. **b:** E40 embryo—radial fibers and subpial end-feet in the Nsa. **c:** Ependymal glial cells and radial fibers in the SV. **d:** Astrocytic processes (arrows) and perivascular end-feet (arrowhead) over a ramification of the archistriatal artery in the StV. **e:** Radial fibers in the ST and their subpial end-feet (arrow). Scale bar: 200 μm.

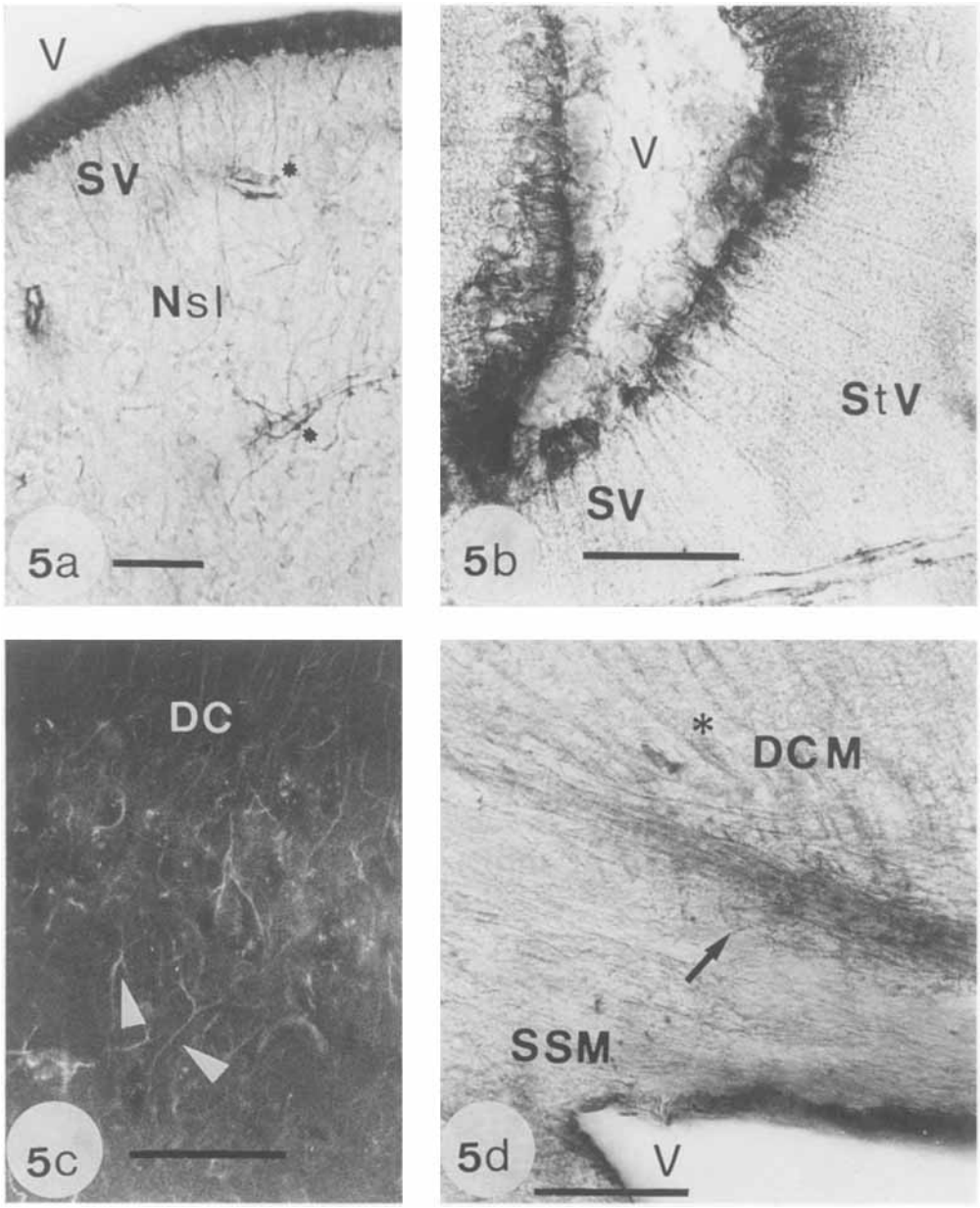

Fig. 5. Immunohistochemical detection of GFAP in adult telencephalon. **a:** Intense staining of ependymal glial cells in the SV, sinuous and straight processes in neuropil of the Nsl and perivascular end-feet (asterisks). **b:** Positive cell bodies in the sulcus ventralis; notice also the radial orientation of the stained fibers in StV. **c:** Astrocytic processes (arrow-heads) in the DC. **d:** Positive ependymal cells in the SSM; two systems of fibers are also stained, e.g., fibers parallel to the ventricular wall (arrow) and radial fibers (asterisk). Scale bar: a, 250 μm; b–d, 100 μm.

extracellular space is not associated with the disappearance of radial glial fibers. The vimentin-GFAP shift must thus be associated with other developmentally regulated changes of glial function.

GFAP immunoreactivity

In adult telencephalon, GFAP-positive radial fibers and the ependymal glial cell bodies from which they originate are present. GFAP-positive processes are observed in the cortex, in the striatum ventralis, and in other telencephalic areas, but GFAP-positive typical astrocyte cell bodies are absent. The same pattern of GFAP immunoreactivity was observed in the turtle by Kriegstein et al. ('86). In contrast GFA-positive astrocytes are present in snake hippocampus as shown by Onteniente et al. ('83); thus, in this respect, *Lacertidae* represent a phylogenetic intermediate between *Ofidia* and *Testudo*.

The absence of GFAP-positive astrocytes is not a general feature of the entire *G. galloti* CNS. In fact, in the optic tectum, GFAP-positive astrocytes are present together with radial glia (Monzon-Mayor et al., '90). This might reflect the fact that in reptiles the telencephalon is more primitive than

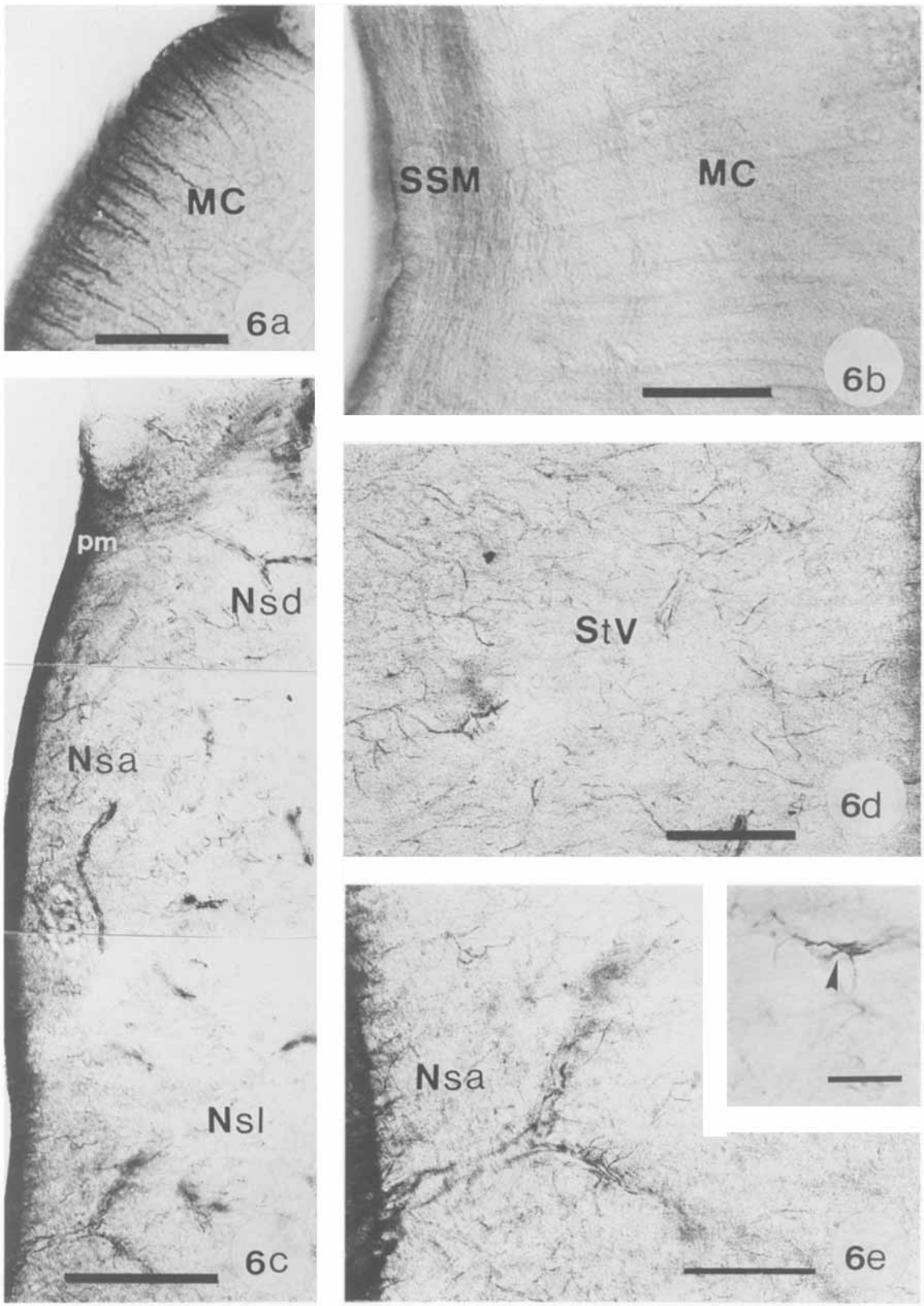

Fig. 6. Immunohistochemical determination of GFAP in the adult. **a:** Intensely stained end-feet in the MC. **b:** Adult—positive fibers radial and parallel to the ventricular wall in the SSM. **c:** Transverse section at the anterior level of the septum: numerous astrocytic processes terminate on blood vessels. **d:** Astrocytic processes in the StV. **e:** Perivascular processes in the Nsa and intense staining near the pia mater; the insert shows perivascular end-feet (arrowhead) at higher magnification. Scale bars a–e 100 μm; inset, 20 μm.

the optic tectum, which in reptiles attains the highest phylogenetic development.

In mammals, the ventricular walls are GFAP-negative, since ependymocytes loose their GFAP immunoreactivity (Bignami and Dahl, '74, Levitt et al., '81) and radial glia disappears.

GFAP-positive fibers in *G. galloti* telencephalon do not have a homogeneous distribution and orientation but are similar to those observed in other inferior vertebrates (Ramón y Cajal, '11; Achucarro, '15; Nakajima, '65); such fibers are more abundant in areas rich in myelinated fibers (Yanes et al., unpublished results) and sometimes are oriented along the bundles of myelinated nerve fibers present in the same area (Yanes et al., unpublished results) like the GFAP-positive fibers in the medial cortex which run parallel to the ventricular wall (Fig. 5d, 6b). In frog CNS, Miller and Liuzzi ('86) reported that astrocytic processes in the neighbourhood of myelinated fibers were particularly rich in intermediate filaments. This preferential localization of filaments could reflect a functional specialization of astrocyte processes.

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