THE JOURNAL OF COMPARATIVE NEUROLOGY 295:569-579 (1990)

Glial Fibrillary Acidic Protein and Vimentin Immunohistochemistry in the Developing and Adult Midbrain of the Lizard *Gallotia galloti*

M. MONZON-MAYOR, C. YANES, M.S. GHANDOUR, J. DE BARRY, AND G. GOMBOS
Departamento de Histologia, Colegio Universitario de Las Palmas (Division de Medicina), Universidad de Las Palmas de G.C. 35080 (M.M-M.), Departmento de Biologia Celular, Facultad de Biologia, Universidad de La Laguna, Tenerife (C.Y.), Canary Islands, Spain 38206; Centre de Neurochimie du C.N.R.S. and U 44 INSERM, 67000 Strasbourg, France (M.S.G., J.D.B., G.G.)

ABSTRACT

The distribution of glial fibrillary acidic protein (GFAP)- and vimentin-containing cells was studied by immunohistochemistry in the midbrain of the lizard *Gallotia galloti*. At embryonic stage 32 (E32), vimentin immunoreactivity appeared first in cell bodies located in the ventricular walls, in radial fibers, and subpial end-feet and increased in these structures until E34/E35. Faint GFAP immunoreactivity gradually appeared in the same structures between E34 and E37, and this increased until adulthood, whereas vimentin immunoreactivity decreased after E35, becoming limited to a few end-feet and fibers in the adult, mainly in the tegmentum. Thus, in developing *Gallotia* midbrain a shift from vimentin-containing to GFAP-containing intermediate filaments begins around E36 or E37. At E40, in addition to the cell bodies in the ependymal area, dispersed GFAP-positive cells, possibly immature astrocytes appeared. These cells showed the same shift. In the adult lizard, GFAP-positive radial glia are still present and coexist with GFAP-positive astrocytes, which are prefentially located in the marginal optic tract and the oculomotor nuclei, but are absent in the fasciculus longitudinalis medialis. Optic tectum, pretectum, tegmentum, and isthmic nuclei are the areas richest in GFAP-positive radial fibers: these were much less abundant in the deep mesencephalic nuclei. Thus, in this lizard, GFAP-positive astrocytes display a clear cut regional distribution: they are present in mesencephalon, whereas they are absent in telencephalon (Yanes et al.: *J. Comp. Neurol.*, *'90*:295:559-568.

Key words: glial cells, mesencephalon, reptiles, ontogeny, phylogenesis, intermediate filaments

Radial glia is phylogenetically the most primitive type 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) and is the first to appear during ontogeny (Ramón y Cajal, '11; Levitt and Rakic, '80). In mammals it disappears during development and, thus, is absent in the adult (Choi and Lapham, '78, '80; Schnitzer et al., '81; Franke et al., '82; Choi et al., '83; Pixley and De Vellis, '84), whereas in nonmammalian vertebrates radial glia is still present in the adult (Achucarro, '15; Ebner and Colonnier, '75).

Comparative studies on glial cells in the CNS of different animal species might thus help to clarify some questions, including that concerning the suggested origin of astrocytes from radial glia. But before addressing such questions, a characterization of the glial system in the chosen lower vertebrates is necessary. In order to characterize glial cell development in the lizard *Gallotia galloti*, we used immunohistochemistry with antibodies directed against either glial fibrillary acidic protein (GFAP), a protein specific for gliofibrils (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; Choi et al., '83; Onteniente et al., '83; Bullon et al., '84; Dahl et al., '85; Didier et al., '86),

Accepted December 5, 1989.

Address reprint requests to Dr. M. Monzon-Mayor, Departamento de Histologia, Colegio Universitario de Las Palmas (Division de Medicina). Universidad de Las Palmas de G.C., Canary Islands, Spain, 35080.

or vimentin, a protein present in intermediate filaments of many cells (Bennett et al., '78a,b; 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). We have shown that in the telencephalon of this lizard radial glia is present in the adult. At the same time scattered GFAP-positive astrocytes are absent (Yanes et al., '90, this issue), whereas in other adult reptiles radial glia co-exists with typical astrocytes. During development of the lizard telencephalon, radial glia shifts from vimentin positivity to GFAP positivity.

In the present paper we show that in *Gallotia* mesencephalon GFAP-positive astrocytes are present. Thus we observe two different situations in the central nervous system (CNS) of the same animal, each described, so far, in different species.

MATERIALS AND METHODS

The experimental methods and the material used were as described in Yanes et al ('90, this issue).

RESULTS

Vimentin-like immunoreactivity

A relatively moderate anti-vimentin immunoreactivity is already detectable in the midbrain marginal and ventricular zones (Fig. 1a) at embryonic stage 32 (E32). At E33, a stronger immunoreactivity is present in the anlage of pretectum, optic tectum, and tegmentum (not shown). At E34–E35, the immunoreactivity in the same areas increases: abundant immunofluorescent end-feet are detectable in the marginal zone, whereas an immunofluorescent palisade of radial processes spreads through the intermediate zone from the ventricular zone (Figs. 1a, 2a). Between these immunofluorescent fibers, immunonegative cells (possibly migrating neurons) are stacked up (Fig. 2c). The intensity of immunofluorescence, highest at E35 for the whole midbrain (Fig. 1a), progressively decreases from E36 to E40 except for the subpial end-feet, which remain intensely fluorescent (not shown). At E40, immunofluorescent radial processes and perivascular end-feet are still visible in the optic tectum and in the tegmentum (Fig. 1b). At hatching (Fig. 1c) and early postnatal ages some immunofluorescence is still present in the basal zone of pretectum (Fig. 3a) tegmentum and optic tracts.

In addition to the vimentin-like immunoreactivity in fibers and end-feet described above, vimentin-positive cell bodies were observed in the periventricular, intermediate, and marginal zones from E34 to E40 (Fig. 2b,c).

In the adult midbrain, vimentin-positive cell bodies are absent, but vimentin-positive perivascular end-feet and fibers can be observed mainly in the tegmentum (Fig. 3b).

GFAP-like immunoreactivity

During development. The earliest expression of GFAP was detected at E34 as a weak immunofluorescence in cell somata and end-feet in the ventricular zone (not shown).

At E37, when anti-vimentin immunofluorescence is already decreasing, anti-GFAP immunofluorescence increases and other GFAP-immunoreactive structures such as perivascular processes and additional astrocyte somata appear in the superficial layer of the optic tectum (Fig. 1a').

The immunofluorescence due to the presence of GFAP in periependymal glial cell bodies, radial processes, and end-feet in the optic tectum, tegmentum, and pretectum continues to increase from E38 to E40 (Figs. 1b', 2d, 3c) and hatching (Figs. 1c', 3d), while that of astrocytes remains weak.

At the fifth postnatal day a stronger GFAP immunofluorescence was observed in the periependymal glia at the level of isthmic nuclei (not shown), in subpial end-feet in the tectum, pretectum, and tegmentum (Fig. 3e), and in perivascular end-feet at the level of the oculomotor nuclei (Fig. 3f).

In adult lizards. The most evident GFAP-positive processes in the adult *Gallotia* midbrain are fibers coursing between an intense rim of GFAP immunoreactivity within the ventricular walls (Figs. 5a, c, e, 6a–c) and in the subpial "glia limitans" (Figs. 5a, b, d, 6a) or the blood capillary walls (perivascular end-feet) (Fig. 5c) in all regions. Observations at higher magnification with interference contrast optics suggest that these fibers originate from cell bodies with perinuclear immunoreactivity localized in the ependymal layer (Fig. 5e). These perikarya probably correspond to the "periependymal radial glia" described by Achucarro ('15). These cells together with their fibers constitute the "radial glia" first described by Magini (1888) and more recently defined by Rakic ('71). Neither GFAP-positive somata nor a GFAP-positive plexus were observed in the subependymal layer. The fibers originating from the proliferative zone in the sulcus limitans are particularly interesting, since some

Abbreviations

A	aqueduct (see also below under "V")
bv	blood vessel
EF	end-feet
flm	fasciculus longitudinalis medialis
Ic	n. isthmi, pars magnocellularis caudalis
Ip	n. interpeduncularis
Ir	n. isthmi, pars magnocellularis rostralis
Isp	n. isthmi, pars parvocellularis
IZ	intermediate zone
MZ	marginal zone
nIII	n. oculomotorius
nIV	n. trochlearis
nv	n. nervi trigemini
opt	n. opticus tegmenti
OT	optic tectum or tectum mesencephali (= colliculus anterior)
ot	optic tract
pm	pia mater
pr	n. profundus mesencephali
PT	pretectum
rub	n. ruber
sl	sulcus limitans
TS	torus semicircularis (=colliculus posterior)
TZ	tegmental zone
V	ventricle. Notice that in reptiles the medial ventricular cavity extends more caudally than in mammals. The ventricular space anterior to the commissura posterior corresponds to the III ventricle which thus extends caudally as far as the pretectum. Caudally to the commissura posterior the ventricular cavity becomes Y-shaped and takes the name of "mesencephalic ventricle." At the level of nIV the mesencephalic ventricle narrows down into a short aqueduct which opens caudally into the fourth ventricle.
VZ	ventricular zone

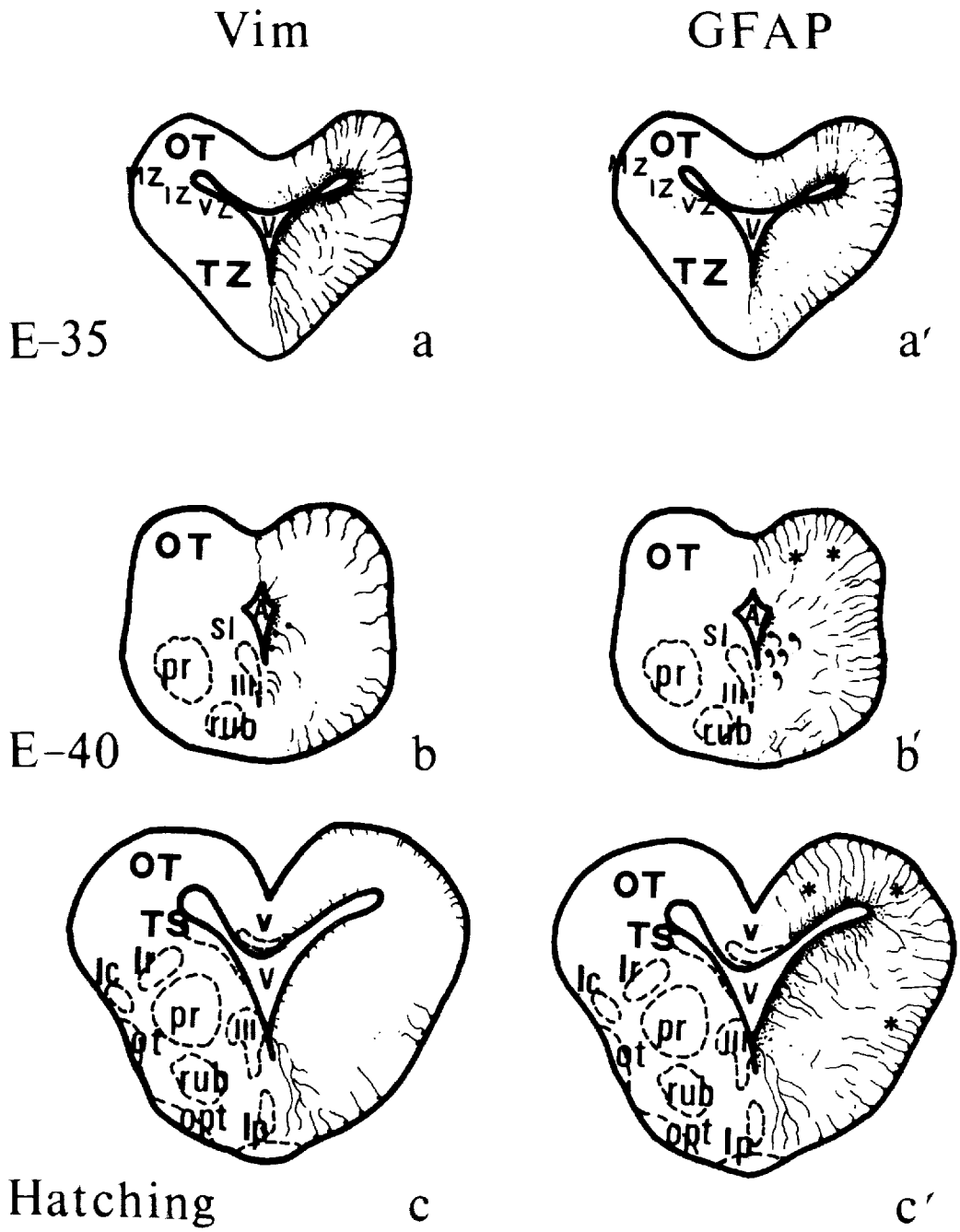

Fig. 1. Schematic drawing of *Gallotia galloti* mesencephalon cross sections at different developmental ages. Drawings a, a', c, and c' represent sections of mid-mesencephalon, while b and b' represent a more caudal mesencephalic section. The different areas, tracts, and nuclei are represented in the **left half** of each section, while in the **right half,** vimentin-immunoreactive structures are drawn in **a, b,** and **c,** and GFAP-immunoreactive structures are drawn in **a', b',** and **c'**: subpial end-feet, radial fibers and periventricular areas are indicated; the asterisks represent GFAP-positive scattered star-shaped cells; and the comma-like marks indicate vimentin-positive or GFAP-positive oval-shaped cells. Bars = 1,000 μm.

of them seem to follow a straight radial course toward the pia mater while others appear to take sinuous pathways (Fig. 6c).

Dispersed GFAP-positive cell bodies are abundant in the tegmentum and present at the level of the oculomotor nuclei (Figs. 4b, 6b–d), of the trochlear nerve nuclei (not shown), and of the optic tract (Fig. 4a,b). They are absent in the fasciculus longitudinalis medialis (flm) (Figs. 4b,c 6b,d), and rare in other areas such as tectum, pretectum, isthmic nuclei (Fig. 6a) and the other tegmental areas (Figs. 4b, 5c, 6b–d).

Identical results were obtained with the two anti-GFAP immune sera. In the midbrain of adult *Gallotia,* GFAP-like

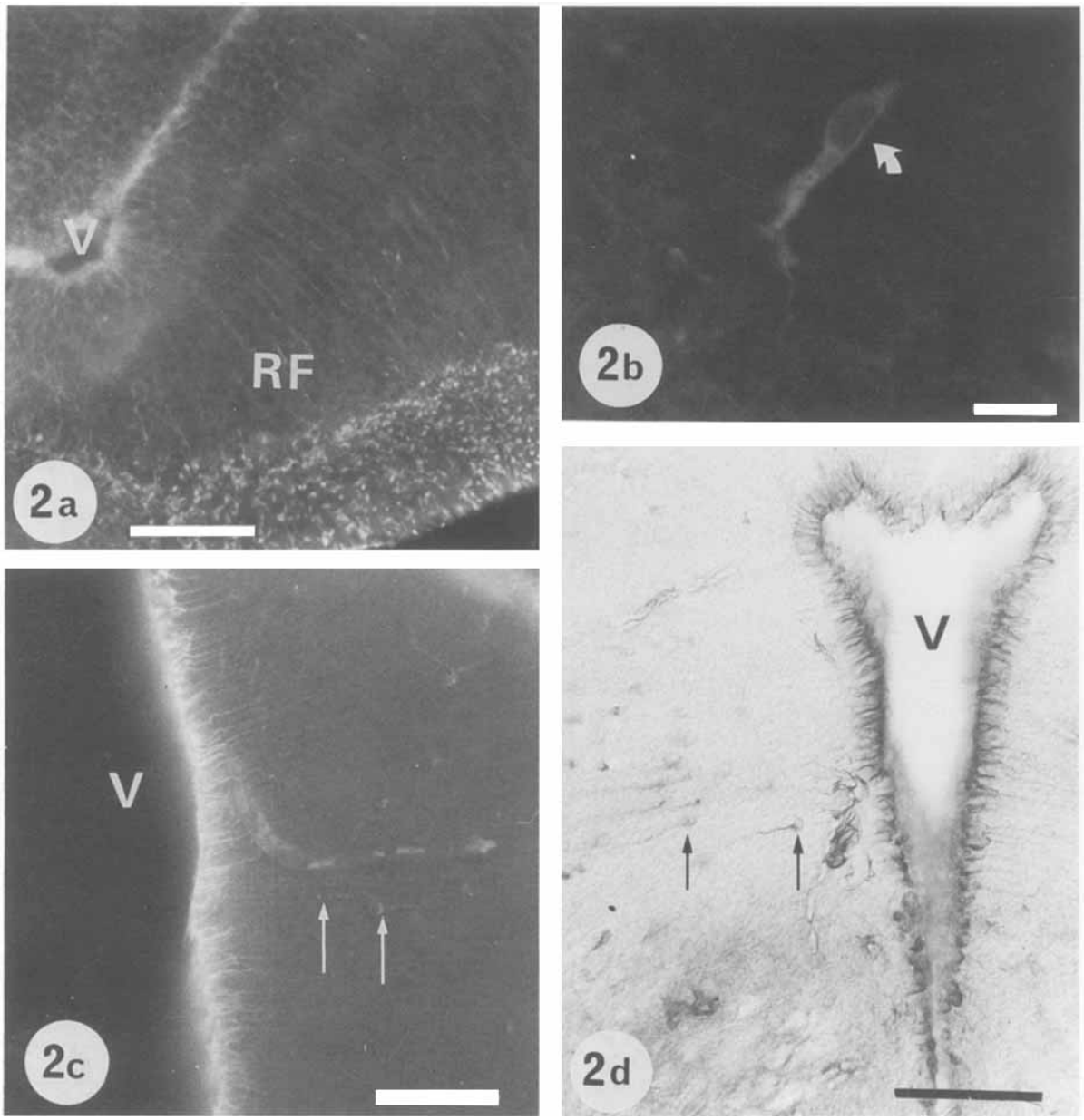

Fig. 2. Indirect immunofluorescence with anti-vimentin antibodies and GFAP immunoreactivity revealed by the PAP technique in transverse sections of embryonic *Gallotia galloti* midbrain. **a:** Embryonic stage E34: in the tegmentum vimentin immunoreactivity is evident in the radial fibers which encompass immunonegative cells. Notice that because of the oblique plane of the section radial fibers near the pial surface are cross-sectioned (**lower right** in the figure). **b:** Embryonic stage E35: notice one of the rare vimentin-containing cells (arrow) in the pretectum extended perpendicularly to the radial fibers. **c:** Embryonic stage E40: notice the strong immunofluorescent vimentin-positive structures at the level of the ventricular walls. The arrows point to vimentin-positive scattered cell bodies. Above these cells a blood vessel is visible because of the vimentin-positive perivascular end-feet. **d:** Embryonic stage E40: GFAP immunoreactivity is shown by the PAP technique. Positive cells along the ventricular wall and scattered oval cells bodies (arrows) indicate the same pattern of labelling as observed with anti-vimentin antibodies. Bars = 100 μm for a,b,d; 20 μm for c.

immunoreactivity was observed in typical astrocytes, radial fibers, glia limitans, perivascular end-feet, and as a periventricular rim.

Such immunoreactivity was never observed in cells of capillary walls or meninges nor in cells which reacted with antibodies specifically recognizing neurons, oligodendrocytes and microglia (manuscript in preparation). Indeed neuronal perikarya appeared as GFAP-negative "holes" (Fig. 6d).

Fig. 3. Indirect immunofluorescence with anti-vimentin and anti-GFAP antibodies in midbrain at late stages of development. *Anti-vimentin* antibodies react with radial fibers in the pretectum at hatching (**a**), and in the adult tegmentum with perivascular end-feet (**b**). *Anti-GFAP*-reactive cell bodies of ependymal glial cells and fibers in transverse sections of pretectum at embryonal stage 40 (**c**), of optic tectum at hatching (**d**), and of tegmentum at postnatal day 5 (**e**). Notice many dispersed cell bodies in the optic tectum (d), the numerous subpial end feet in the tegmentum (e), and the perivascular end feet at the level of the oculomotor nuclei at postnatal day 5 (**f**). Bars = 100 μm.

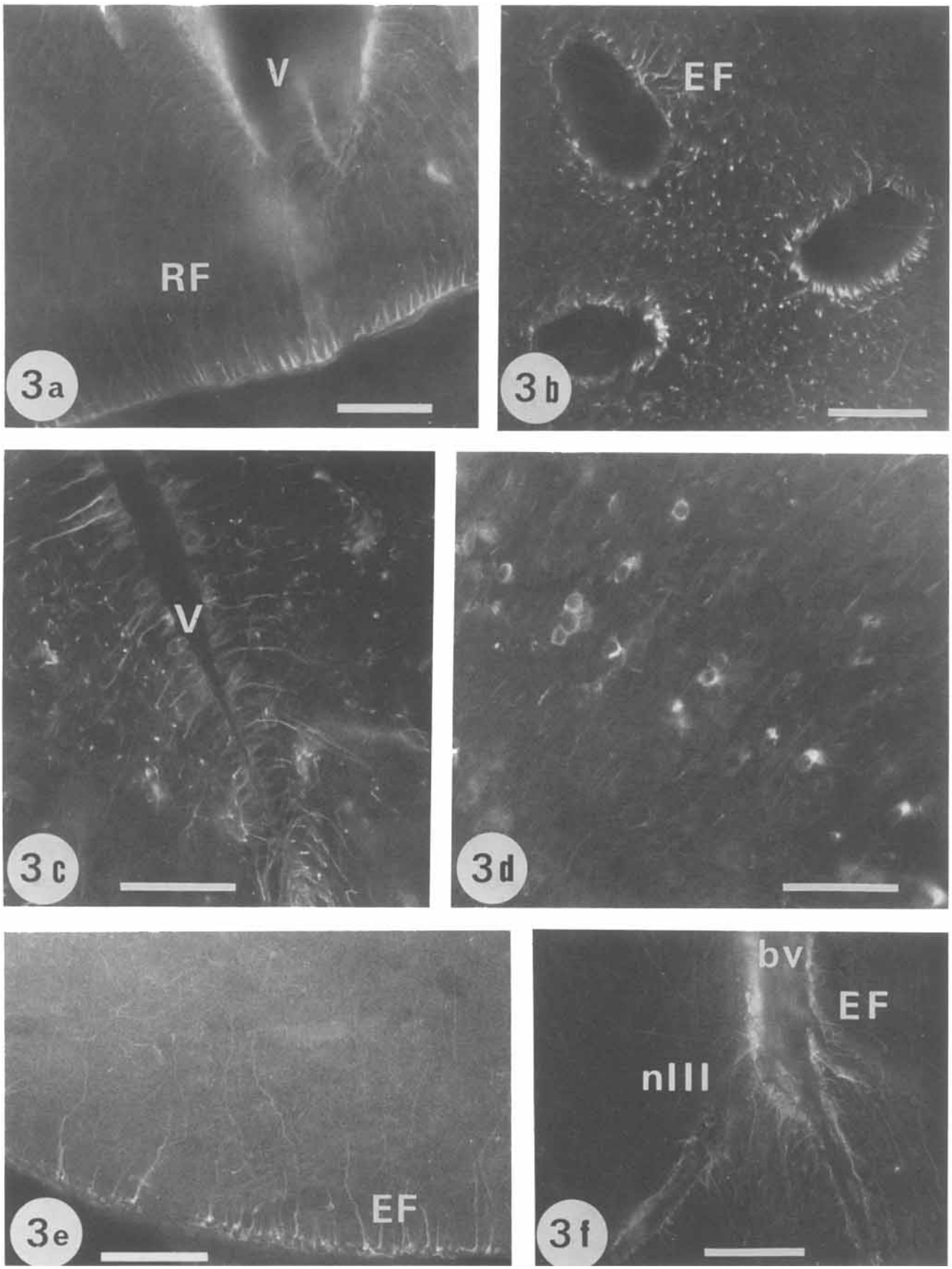

Figure 3

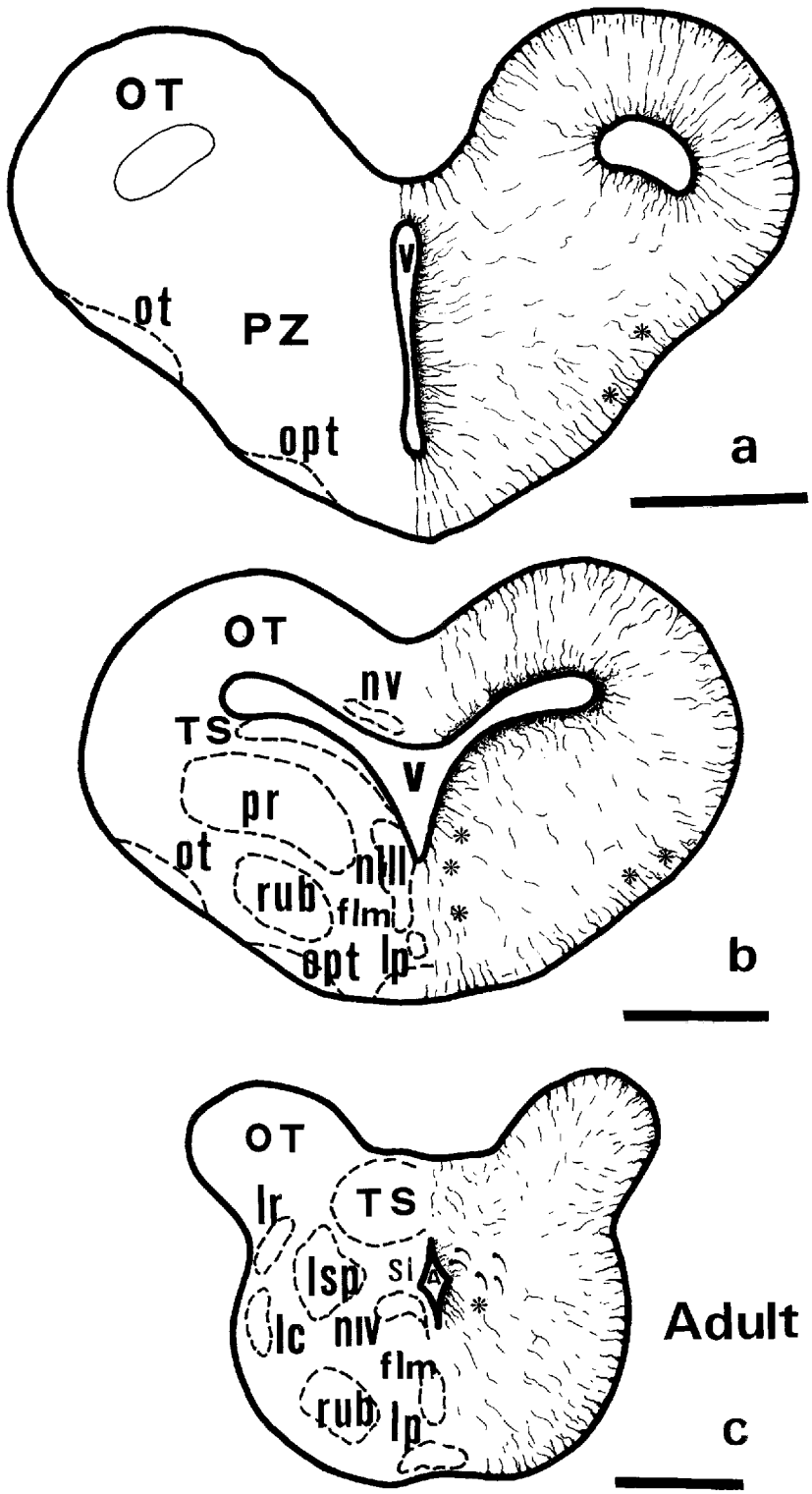

Fig. 4. Schematic drawing of adult *Gallotia galloti* mesencephalon cross sections: drawing **b** represents a sections of mid-mesencephalon, while **a** and **c** represent a more rostral and a more caudal mesencephalic section, respectively. The different areas, tracts, and nuclei are represented in the left half of each section, while in the right half GFAP-immunoreactive structures are drawn: scattered star-shaped cells (asterisks), oval cells (comma-like marks), radial fibers, and periventricular areas. Bars = 1000 μm.

Fig. 5. Immunoreactivity with anti-GFAP immuneserum in transverse and sagittal sections in the adult lizard *Gallotia galloti* midbrain. Notice the immunofluorescent radial and/or sinuous processes in the optic tectum (**a** and **b**), the tegmentum (**c**), and the torus semicircularis (**d**). Bulbous end feet are also observed in the optic tectum (**b**). On the walls of the aqueduct, the outlines of ependymal cells bodies are revealed by the PAP technique observed with Nomarsky optics (**e**). Bars = 100 μm for a,c,d; 20 μm for b,e.

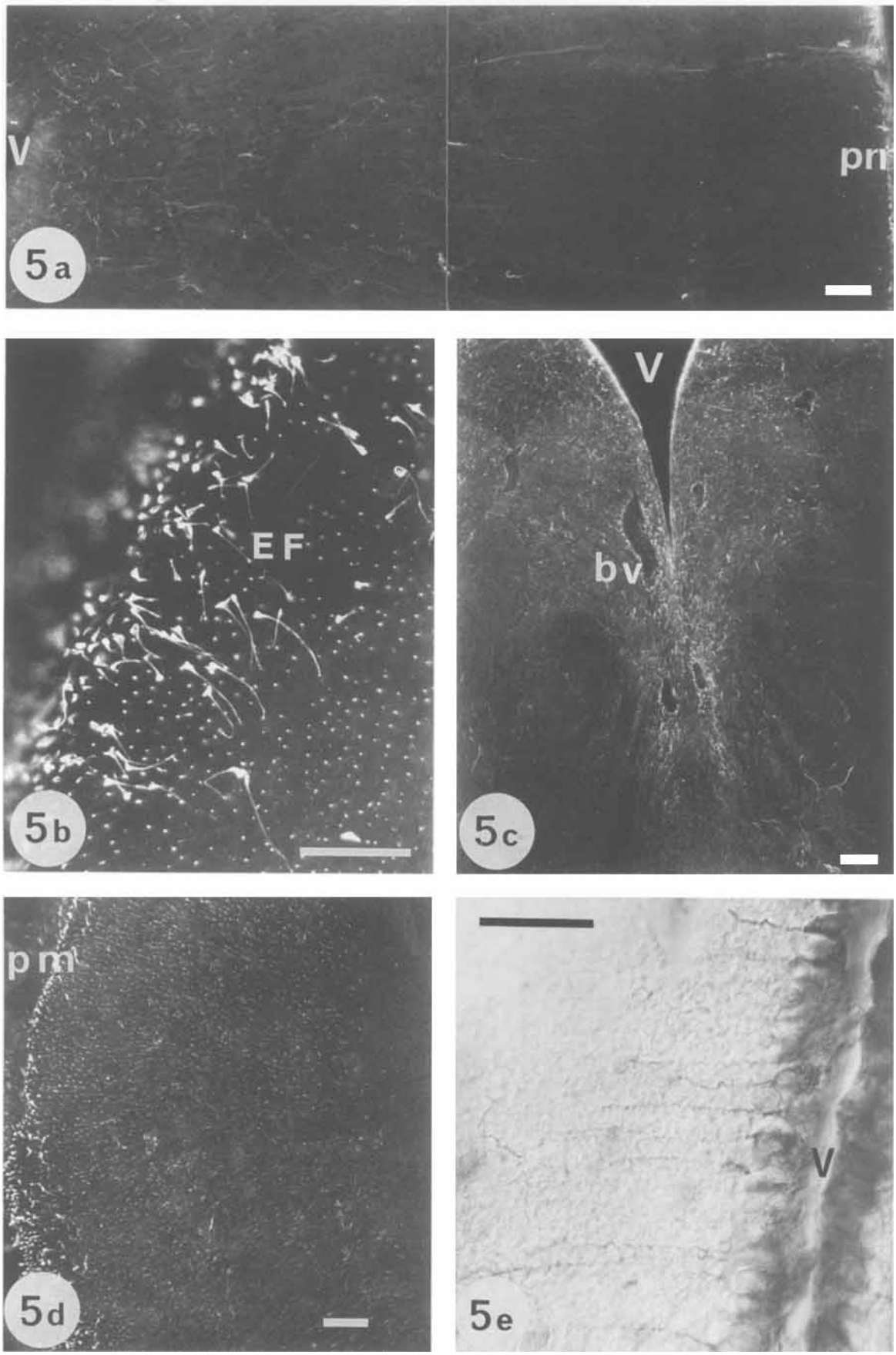

Figure 5

DISCUSSION

Phylogenetic differences in distribution of GFAP-positive astrocytes

Our data are the first to show the astrocyte distribution in a lizard midbrain since the previous studies by Dahl et al. ('85) on the lizard *Anolis carolinensis* were on cerebellum, where GFAP-positive astrocytes and Bergmann glia were shown. Astrocyte distribution in *Gallotia* optic tract is similar to that in the snake, except that typical astrocytes are absent in snake tegmentum (Onteniente et al., '83). These differences probably reflect interspecific differences rather than technical differences in the immunological procedures, since the peroxidase-antiperoxidase (PAP) method used by Onteniente et al. ('83) is more sensitive than our immunofluorescence method.

Interspecies and regional differences in ependymal glia

In *Gallotia*, as in other reptiles (Kirsche, '72; Cruce and Nieuwenhuys, '74) the ependymal layer is multicellular at the level of the "proliferative zones" of the sulci. However, the cell layer in the ventricular wall from which the radial fibers originate shows regional and interspecies differences: the layer can be monocellular or multicellular with different intermingled or stratified cell types. GFAP cellular localization can differ between the animal species or can change with age as the following examples indicate.

In mammals, ependymocytes are GFAP-positive during early development (Levitt and Rakic, '80), but the whole ependymal layer is GFAP-negative in adults (Dahl and Bignami, '73).

In adult birds the ependymal layer is GFAP-positive (Onteniente et al., '83), but no radial fibers are detectable, and in the finch the GFAP-positive cells in the ependymal layer have the morphology of typical astrocytes (Onteniente et al., '83).

In the optic tectum of testudo (turtle) GFAP-positive radial fibers spread out from a thick GFAP-positive multicellular layer around the ventricle (Dahl and Bignami, '73); several cell bodies in these layer are immunonegative.

In teleosts the GFAP-positive layer is the subependymal layer. In goldfish (Stevenson and Yoon, '82) the periventricular wall at the level of the optic tectum is multilayered: ependymocytes are located at the ventricular surface (ependymal layer) and their processes (in contrast to those of ependymocytes in adult mammals) are GFAP-positive and form a local plexus which does not extend into the tectum further than the ependymal layer. Other cells, the so called "periventricular cells," are situated in the subependymal layer. Long radial processes called "periependymal radial glia" originate from these "periventricular cells." These processes do not contain intermediate filaments, are GFAP-negative, but form perivascular and subpial end-feet. These characteristics, however, might be specific to goldfish, which shows several particularities concerning the glial population: although typical astrocytes were also observed, radial glia contains neither GFAP nor gliofibrils, Bergman glia is absent in cerebellum (Dahl and Bignami, '73), and optic nerves do not contain GFAP-positive cells.

Double immunolabeling of the same tissue section with antibodies with different cellular specificity should help us to distinguish between these cell types in the ependymal zone of *Gallotia galloti*.

Developmental changes of mesencephalic glia

At the earliest developmental stages, vimentin-positive cells and fibers did not react either with antibodies directed against neurofilament proteins (unpublished results) or astrocyte markers such as GFAP or glutamine synthetase (Monzon-Mayor et al, 1990). Later on, however, reactivity with anti-GFAP antibodies appeared, indicating that these cells and fibers belong to the astrocyte family: thus, in *Gallotia*, as in other species, (Bignami et al., '82; Dahl et al., '81) vimentin is the major component of the intermediate filament of immature glia.

Dahl et al. ('81, '82), however, observed that in rat encephalon and spinal cord, vimentin decreases and GFAP increases during the second and third postnatal week, while Pixley and De Vellis ('84) have shown that in developing rat brain vimentin disappears with radial glia and GFAP appears with mature astrocytes. In *Gallotia galloti* midbrain we have observed a critical phase at E36 when vimentin begins to decrease and GFAP starts to accumulate. However, some fibers and particularly end-feet are still vimentin-positive in the adult. We do not know if these processes belong to radial glia or to astrocytes. This is not an unique phenomenon; in fact vimentin and GFAP coexist in the same cell in cultured astrocytes and, in some cases, in vivo (Schnitzer et al., '81; Shaw et al., '81; Dahl et al., '82; Lazarides, '82; Schiffer et al., '86; Chiu et al., '88).

Rakic ('72, '82) and Choi et al., ('83) have described in the monkey and in the human, respectively, the disappearance of radial glia and the appearance of cells which look like transitional forms between radial glia and astrocytes (Rakic, '82; Choi et al., '83) or oligodendrocytes (Choi et al., '83). These authors suggested that radial glia is transformed into other adult glial cell types. In *Gallotia* midbrain radial glia does not disappear, but the composition of its intermediate filaments apparently changes. In the adult, star-shaped astrocytes are GFAP-positive, and it is possible that the scattered vimentin-positive cells shown (arrows in Fig. 2c, d) are immature astrocytes, while the GFAP-positive cells shown in Figures 2e and 3d could be the same cells at a more mature stage of development. If this is the case, the vimentin to GFAP shift is common to radial glia and astrocytes and occurs almost simultaneously in the two cell types. This coincidence is unexpected. In fact, the transformation of one cell type into the other appears to be unlikely to occur in lizards or, if present, may involve a small fraction of radial glia.

We must point out, however, that gliogenesis in reptiles is unusual: immature glial cells are present in the multicellular ependymal layer in the "proliferative zone" of the various sulci of the telencephalon, even in the adult (Yanes et al., '89) or in the sulcus limitans of the midbrain (Monzon-Mayor, unpublished). These zones could be a reservoir from which immature glial cells migrate before they mature throughout the lizard life. If this is the case, these immature migrating astrocytes should not be vimentin positive.

The expression of vimentin in birds and mammals coincides with neuronal proliferation and migration (Dahl et al., '81; Schnitzer et al., '81; Pixley and De Vellis, '84; Quitsche et al., '85; Schiffer et al., '86). Similarly the maximal expression of vimentin (e.g., at E34–E35) in *Gallotia* midbrain apparently coincides with neuronal migration as suggested by the presence of the stacks of immunonegative cells piled up in between strongly vimentin positive fibers (Fig. 2b). We cannot at the moment assess whether this is a

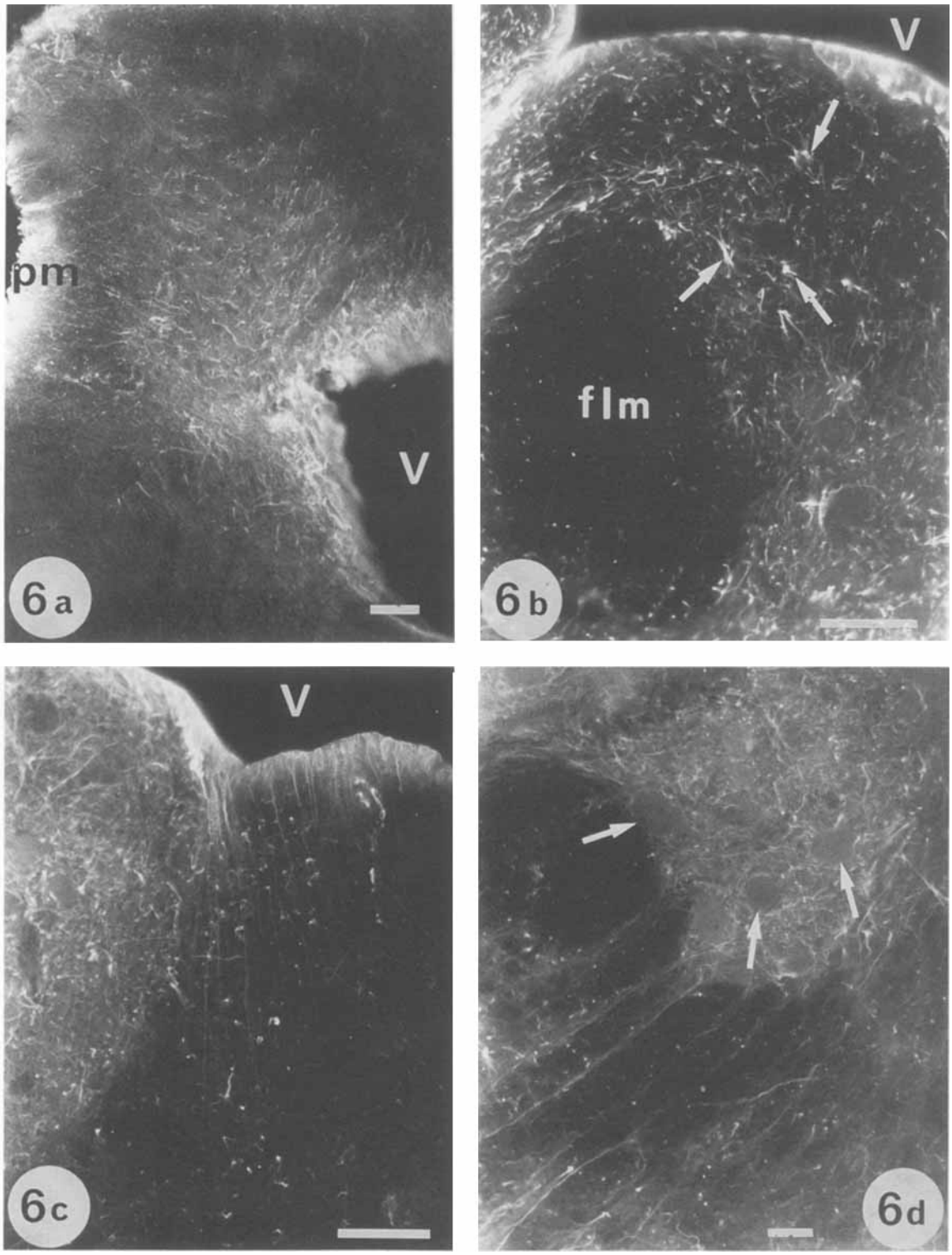

Fig. 6. Indirect immunofluorescence with anti-GFAP immuneserum in adult *Gallotia galloti* midbrain transverse sections at the level of the isthmic (**a**) and the oculomotor (**b–d**) nuclei. Notice the strongly immunofluorescent structures (astrocytes) in the tegmentum (arrows in b). GFAP-positive cells are particularly abundant in the dorsal part of the oculomotor nuclei (arrows in b), which contrasts with the few GFAP-positive astrocytes in the ventral part of the nuclei and the virtual absence of immunofluorescence in fasciculus longitudinalis medialis (flm) (b). Also neurons (arrows in d) are negative. At the level of the sulcus limitans (c) the ventricle wall is also clearly immunofluorescent, and GFAP-positive radial fibers penetrate the tegmentum. Bars = 100 μm.

simple temporal coincidence or whether there is any causal relation.

ACKNOWLEDGMENTS

Drs. M. Monzon-Mayor and C. Yanes Mendez were recipients of travel and research fellowships granted by the Convenio de Colaboration Gobierno de Canarias-Caja General de Ahorros de Canarias (Canary Islands) y Ministerio de Education y Ciencia de Madrid (Spain). This work was partially supported by the research grants 909(2) and PB87-0688-CO2-O2 from CAICYT. G.G. is DR II and M.S.G. is CR I in CNRS, and J.de B. is CR I in INSERM. We are grateful to Mrs. Yolande Schladenhaufen for her technical assistance.

LITERATURE CITED

Achucarro, N. (1915) De l'évolution de la neuroglie, et spécialement de ses relations avec l'appareil vasculaire, Inst. Cajal. Invest. Biol. *13*:169–212.

Bennett, G.S., S.A. Fellini, and H. Holtzer (1978a) Immunofluorescent visualization of 100 A filaments in different cultured chick embryo cell types. Differentiation *12*:71–82.

Bennett, G.S., S.A. Fellini, J.M. Croop, J.J. Otto, J. Bryan, and H. Holtzer (1978b) Differences among 100 A filament subunit from different cell types. Proc. Natl. Acad. Sci. USA *75*:4364–4368.

Bignami, A., L.F. Eng, D. Dahl, and C.T. Uyeda (1972) Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain. Res. *43*:429–435.

Bignami, A., D. Dahl, and D.C. Rueger (1980) Glial fibrillary acidic protein (GFA) in normal neural cells and in pathological conditions. In S. Fedoroff and L. Hertz (eds): Advances in Cellular Neurobiology. New York, Academic Press, pp. 286–313.

Bignami, A., T. Raju, and D. Dahl (1982) Localization of Vimentin, the nonspecific intermediate filament protein, in embryonal glia and early differentiating neurons, Dev. Biol. *91*:286–295.

Bullon, M.M., T. Alvarez-Gago, B. Fernandez-Ruiz, and C. Aguirre (1984) Glial fibrillary acidic (GFA) protein in rat spinal cord. An immunoperoxidase study in semithin sections. Brain. Res. *309*:79–83.

Chiu, F.-C., R.S. Sacchi, L. Claudio, S. Kobayashi, and K. Susuki (1988) Coexpression of glial fibrillary acidic protein and vimentin in the central and peripheral nervous system of the Twitcher mutant. Glia *1*:105–112.

Choi, B.H., and L.W. Lapham (1978) Radial glia in the human foetal cerebrum: A combined Golgi immunofluorescent and electron microscopy study. Brain. Res. *148*:295–311.

Choi, B.H., and L.W. Lapham (1980) Evolution of Bergmann glia in developing human foetal cerebellum: A Golgi electron microscopic and immunofluorescent study. Brain. Res. *190*:369–383.

Choi, B.H., R.C. Kim, and L.W. Lapham (1983) Do radial glia give rise to both astroglial and oligodendroglial cells?, Dev. Brain. Res. *8*:119–130.

Cochard, P., and D. Paulin (1984) Initial expression of neurofilaments and vimentin in the central and peripheral nervous system of the mouse embryo in vivo. J. Neurosci. *4*:2080–2094.

Cruce, W.L.R. and R. Nieuwenhuys (1974) The cell masses in the brain stem of the turtle Testudo hermanni; a topographical and topological analysis. J. Comp. Neurol. *156*:277–306.

Dahl, D., and A. Bignami (1973) Immunochemical and immunofluorescence studies of the glial fibrillary acidic protein in vertebrates. Brain. Res. *61*:279–293.

Dahl, D., D.C. Rueger, A. Bignami, K. Weber, and M. Osborn (1981) Vimentin, the 57,000 dalton protein of fibroblast filaments, is the major cytoskeletal component in immature glia. Eur. J. Cell Biol. *24*:191–196.

Dahl, D., P. Strocchi, and A. Bignami (1982) Vimentin in the central nervous system. Differentiation *22*:189–190.

Dahl, D., C.J. Crosby, A. Sethi, and A. Bignami (1985) Glial fibrillary acidic (GFA) protein in vertebrates: Immunofluorescence and immunoblotting study with monoclonal and polyclonal antibodies. J. Comp. Neurol. *239*:75–88.

Didier, M., M. Harandi, M. Aguera, B. Bancel, M. Tardy, C. Fages, A. Calas, M. Stagaard, K. Mollgard, and M.F. Belin (1986) Differential immunocytochemical staining for glial fibrillary acidic (GFA) protein, S-100 protein and glutamine synthetase in the rat subcomissural organ, non specialized ventricular ependyma and adjacent neuropil. Cell. Tissue Res. *245*:343–351.

Ebner, F.F., and M. Colonnier (1975) Synaptic patterns in the visual cortex of turtle: an electron microscopic study. J. Comp. Neurol. *160*:51–80.

Eng, L.F., J.J. Vanderhaeghen, A. Bignami, and B. Gerstl (1971) An acidic protein isolated from fibrous astrocytes. Brain. Res. *28*:351–354.

Eng, L.F., and J.W. Bigbee (1978) Immunochemistry of nervous system specific proteins. In B.W. Agranoff and M.H. Aprison (eds): Advances in Neurochemistry. New York: Plenum Publishing Corp., pp. 43–98.

Eng, L.F. (1980) The glial fibrillary acidic (GFA) protein. In R.A. Bradshaw and D.M. Schneider (eds): Proteins of the Nervous System. New York: Raven Press, pp. 85–117.

Franke, W.W., E. Schmid, M. Osborn, and K. Weber (1978) Different intermediate sized filaments distinguished by immunofluoresce microscopy. Proc. Nat. Acad. Sci. USA *75*:5034–5038.

Franke, W.W., E. Schmid, S. Winter, M. Osborn, and K. Weber (1979) Widespread occurrence of intermediate-sized filaments of the vimentin-type in the culture cells from diverse vertebrates. Exp. Cell. Res. *123*:25–46.

Franke, W.W., C. Grund, C. Kuhn, B.W. Jackson, and K. Illmensee (1982) Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin filaments. Differentiation *23*:43–59.

Friede, R.L., K.M. Hu, and M.T. Stone (1969) Glial footplates in the bowfin. I. Fine structure and chemistry. J. Neuropathol. Exp. Neurol. *28*:513–539.

Gabbiani, G., E. Schmid, C. Winter, C. Chaponnier, C. De Chastonay, J. Vandekerckhove, and K. Weber (1981) Vascular smooth muscle cells differ from other muscle cells: predominance of vimentin filaments and a specific alpha-type actin. Proc. Natl. Acad. Sci. USA *78*:298–302.

Ghandour, M.S., O.K. Langley, and J. Clos (1983) Immunohistochemical and biochemical approaches to the development of neuroglia in the CNS, with special reference to cerebellum. Int. J. Dev. Neurosci. *1*:411–425.

Ghandour, M.S., O.K. Langley, G. Vincendon, and G. Gombos (1979) Double labeling immunohistochemical technique provides evidence of the specificity of glial cell markers. J. Histochem. Cytochem. *27*:1634–1637.

Kirsche, W. (1972) Die Entwicklung des Telencephalons der Reptilien und deren Beziehung zur Hirn-Bauplanlehre. Nova Acta Leopoldina. *37*:1–78.

Kruger, L., and D.S. Maxwell (1966) The fine structure of ependymal processes in the teleost optic tectum. Am. J. Anat. *119*:479–498.

Lazarides, E. (1980) Intermediate filaments as mechanical integrators of cellular space. Nature (Lond.) *283*:249–256.

Lazarides, E. (1982) Intermediate filaments: A chemically heterogeneous, developmentally regulated class of proteins. Annu. Rev. Biochem. *51*:219–250.

Levitt, P., M.L. Cooper, and P. Rakic (1981) Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: an unstructural immunoperoxidase analysis. J. Neurosci. *1*:27–39.

Levitt, P., and P. Rakic (1980) Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing Rhesus Monkey brain. J. Comp. Neurol. *193*:815–840.

Magini, G. (1888) Sur la neuroglie et les cellules nerveuses cerebrales chez le foetus. Arch. Ital. Biol. *9*:59–60.

Miller, R.H., and F.J. Liuzzi (1986) Regional specialization of the radial glial cells of the adult frog spinal cord. J. Neurocytol. *15*:187–196.

Monzon-Mayor M., Yanes C., Tholey G, de Barry J. and Gombos G. (1990) Immunohistochemical localization of glutamine synthetase in mesencephalon and telencephalon of the lizard Gallotin galloti during ontogeny, Glia, in press.

Onteniente, B., H. Kimura, and T. Maeda (1983) Comparative study of the glial fibrillary acidic protein in vertebrates by PAP immunohistochemistry. J. Comp. Neurol. *215*:427–436.

Paulin, D., H. Jakob, F. Jacob, K. Weber, and M. Osborn (1982) In vitro differentiation of mouse teratocarcinoma cells monitored by intermediate filament expression. Differentiation *22*:90–99.

Pixley, S.K.R., and J. De Vellis (1984) Transition between immature radial glia and mature astrocytes studied with a monoclonal antibody to Vimentin. Dev. Brain. Res. *15*:201–209.

Polak, M., W. Haymaker, J.E. Johnson, and F. D'Amelio (1982) Neuroglial and their reactions. In W. Haymaker and R. Adams (eds): Histology and Histopathology of the Nervous System. Springfield, Illinois: C.C. Thomas Pub., pp. 363–445.

Quitschke, W., P.S. Jones, and N. Schechter (1985) Survey of intermediate filament protein in optic nerve and spinal cord: Evidence for differential expression. J. Neurochem. *44*:1465–1476.

Rakic, P. (1971) Guidance of neurons migrating to the fetal monkey neocortex. Brain. Res. *33*:471–476.

Rakic, P. (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. J. Comp. Neurol. *145*:61–84.

Rakic, P. (1982) The role of neuronal-glial cell interaction during brain development. In T.A. Sears (ed): Neuronal-Glial Cell Interaction. New York: Springer Verlag pp. 25–38.

Ramaekers, F.C.S., M. Osborn, E. Schmid, K. Weber, H. Bloemendal, and W.W. Franke (1980) Identification of the cytoskeletal proteins in lens forming cells, a special epithelioid cell type. Exp. Cell. Res. *127*:309–327.

Ramón y Cajal, S. (1911) Histologie du Systeme Nerveux de l'Homme et des Vertébrés. Tome 2. Paris Maloine. Reprinted by Consejo Superior de Investigaciones Cientificas, Madrid, 1955.

Schachner, M., E.T. Hedley-White, D.W. Hsu, R.G. Shoonmaker, and A. Bignami (1977) Ultrastructural localization of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labeling. J. Cell. Biol. *75*:67–73.

Schachner, M., I. Sommer, C. Lagenaur, and J. Schnitzer (1982) Developmental expression of antigenic markers in glial subclasses. In T.A. Sears (ed): Neuronal-Glial Cell Interactions. New York: Springer-Verlag, pp. 321–336.

Shaw, G., M. Osborn, and K. Weber (1981) An immunofluorescence microscopical study of the neurofilament triplet proteins, vimentin and glial fibrillary acid protein within the adult rat brain. Eur. J. Cell. Biol. *26*:68–82.

Schiffer, D., M.T. Giordana, A. Migheli, G. Giaccone, S. Pezzota, and A. Mauro (1986) Glial fibrillary acid protein and vimentin in the experimental glial reaction of the rat brain. Brain. Res. *374*:110–118.

Schnitzer, J., W.W. Franke, and M. Schachner (1981) Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. J. Cell. Biol. *90*:435–447.

Starger, J.M., W.E. Brown, A.E. Goldman, and R.D. Goldman (1978) Biochemical and immunological analysis of rapidly purified 10 nm filaments from baby hamster kidney (BHK-21) cells. J. Cell. Biol. *78*:93–109.

Stevenson, J., and M. Yoon (1982) Morphology of radial glia, ependymal cells, and periventricular neurons in the optic tectum of goldfish. J. Comp. Neurol. *205*:128–138.

Yanes, C.M., M.A. Perez Batista, J.M. Martin Trujillo, M. Monzon and A. Rodriguez (1989) Development of the ventral striatum in the lizard Gallotia galloti. J. Anatomy *164*:92–100.

Yanes, C.M., M. Monzon-Mayor, M.S. Ghandour, J. de Barry and G. Gombos (1990) 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. J. Comp. Neurol. *295*:559–568.