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The Embryonic Development of the Cortical Plate in Reptiles: A Comparative Study in *Emys orbicularis* and *Lacerta agilis*

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ABSTRACT

From the earliest stage of its ontogenesis, the mammalian cerebral cortex displays a remarkable cytoarchitectonic organization, with its neurons oriented radially within the cortical plate (CP).

It is not known whether this radial organization of cortical neurons is characteristic of every cerebral cortex or whether it reflects a progressive phylogenetic acquisition. In order to study this question, the embryonic development of the cortex has been examined in reptiles, where it is the most primitive. Two species, *Emys orbicularis* and *Lacerta agilis*, representative of the two principal reptilian orders (chelonians and squamates), have been studied with histological methods, Golgi impregnation, and electron microscopy. Very similar patterns of cell proliferation, migration, maturation, and synaptogenesis have been observed. However, important species differences are present in the cellular organization of the cortical plate. Whereas in *Emys* the structure of the cortical plate is rudimentary, in *Lacerta* it appears well developed and quite reminiscent of its mammalian counterpart. Preliminary comparisons with embryological preparations of *Sphenodon* and *Crocodilus niloticus* show that the organization of the cortical plate displays significant variations among the different reptilian groups.

The present results suggest that the radial organization of cortical neurons is not an all or nothing phenomenon but has been acquired independently and is thus a case of homoplasy, probably due to convergence (Northcutt, '81). Several possible implications of these findings are discussed and a working hypothesis based on the role of radial glial cells in the formation of cytoarchitectonic patterns (Rakic, '80) is presented.

Key words: nervous system, cortical plate, development, reptiles, embryos

The cerebral cortex is the product of a long evolutionary process, culminating in the development of the multilaminar mammalian pallium. In all mammals studied so far, the cerebral cortex develops according to a common sequence (reviewed by Caviness and Rakic, '78). Briefly, neuronal precursors proliferate into germinative zones lining the ventricles; postmitotic elements migrate outward to finally settle into the cortical plate, where they assume a remarkable radial differentiation (Shoukimas and Hinds, '78; Goffinet and Lyon, '79). Marin Padilla ('78) suggested that the developing mammalian cortex proceeds through a transient stage, which he named the "primordial cortical organization" because in appearance it was reminiscent of the reptilian cortex. It would be interesting to know whether the basic features of cortical development thus defined are general and, more specifically, whether the radial organization of cortical plate cells is inherent to every cortex or rather results from progressive phyletic acquisitions. Information on these questions could presumably be gained by examining the development of the cerebral cortex in reptiles, where it is the most primitive.

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These considerations prompted me to study cortical histogenesis in embryos from a turtle (*Emys orbicularis*) and a lizard (*Lacerta agilis*), two reptiles representative of widely separated orders. In order to correlate the observations with available data on mammalian and reptilian development, as well as on paleontological filiations, the development of the cortical plate has also been examined in collections of embryos from *Sphenodon punctatus*, *Crocodilus niloticus*, and some squamates. A preliminary account of the present work has been presented elsewhere (Goffinet, '81).

MATERIALS AND METHODS

Incubation of the eggs

Eggs from *Lacerta agilis* were provided by B. Langerwerf and incubated at 30°C as described by Raynaud ('67). Eggs from *Emys orbicularis* were given by C. Pieau and incubated at 30°C, 95% humidity, as previously described (Pieau, '74).

Staging of embryonic material

Within any given species and at a given temperature, the age of the egg is a good indication of development. It was found useful to estimate the approximate size of the embryo by transillumination before sacrifice. Once the embryo was taken out of the egg, embryonic weight and external morphology were used as developmental criteria. For *Lacerta*, the tables of development established by Dufaure and Hubert ('61) for *L. vivipara* were followed. For *Emys orbicularis* a table for staging is provided by Pieau and Dorizzi ('81), after Yntema ('68). Interspecies comparison is difficult, and the final test was the histological maturity of the central nervous system. The appearance of the cortical plate (except in snakes) coincides with the appearance of individual digits of the extremities. The present report concentrates on two stages of development of the cortical plate. The first stage (stage 1) corresponds to the early differentiation of the cortical plate [± 150 mg in *Lacerta*, corresponding to stage 34 of Dufaure and Hubert ('61); ± 400 mg in *Emys*, corresponding to stage 20 of Yntema ('68)]. At the second stage (stage 2), the architectonic differentiation of the cortical plate is already well established [± 300–350 mg in *Lacerta*, stage 36 of Dufaure and Hubert ('61); 1,000–1,400 mg in *Emys*, stage 22 of Yntema ('68)].

Light microscopy

Specimens were immersed in Bouin's fluid, embedded in paraplast, cut in the sagittal or frontal plane at 7 or 10 μm, and stained with hematoxylin-eosin; some material was also processed with neurofibrillar impregnations of Bodian or Rogers (cited in Gabe, '68), but the latter did not prove useful with the embryonic cortex.

Golgi method

Embryos were fixed by injection at low pressure (± 60 cm H_2O) of 0.8–1% glutaraldehyde plus 3.7% formaldehyde in phosphate buffer (0.1 M, pH 7.5) into the cerebral ventricles and around the brain. After overnight postfixation and three rinses of 30 minutes in phosphate buffer, the blocks were immersed into chromation solution ($K_2Cr_2O_7$, 3%; OsO_4, 0.25%) for 3–4 days at 4°C, transferred to 0.75% silver nitrate for 1–2 days, dehydrated, embedded into celloidin, and then serially cut at 100–150 μm, in the frontal plane.

Electron microscopy

The fixative was injected at low pressure into the ventricles or through the tectum and around the brain; this technique yielded good results with stage 1 embryos and acceptable histology at stage 2. The preservation was less satisfactory at later developmental stages. The fixative was 2.5% glutaraldehyde (EM grade, Serva) in 0.1 or 0.12 M cacodylate buffer pH 7.4, with $CaCl_2$ 0.03%. After overnight postfixation, the blocks were osmicated (OsO_4, 2% in cacodylate buffer for 90 minutes), dehydrated, and embedded in Epon®. For screening and orientation, semithin sections were stained with toluidin blue in borax. Thin sections were cut on a Reichert OM2 microtome and stained with uranyl acetate and lead citrate. Sections were routinely mounted on square grids (200 or 300 mesh). For orientation purposes, it was often useful to process them according to Galey and Nilson ('66), that is, by collecting and staining the floating sections and then transferring them to single-slot (1 × 2 mm), Pioloform®-coated grids.

Other reptilian embryos were available embedded in wax. Embryonic collections of *Sphenodon punctatus* were studied in King's College (courtesy of Prof. C.B. Cox). Embryos from *Angis fragilis*, *Lacerta viridis*, *Viper aspis*, and *Natrix tesselata* were provided by Prof. A. Raynaud (Laboratoire de Biologie, Université de Toulouse). Embryonic collections of *Crocodilus niloticus* were given by Prof. S. Haumont (Dept. of Histology, University of Louvain).

RESULTS

Lacerta: stage 1

In *Lacerta*, the earliest evidence of a cortical plate is found in the dorsolateral field of the hemispheres (Fig. 1a,b). At this early stage, the telencephalic wall is approximately 80–90 μm thick and composed of four concentric strata, namely: the internal prominent ventricular zone (VZ; ± 15–20 μm); a cell-poor intermediate zone (IZ; ± 15–30 μm); the very thin cortical plate (CP; 20 μm); and the incipient marginal zone (MZ; 20 μm). In all layers the extracellular space is prominent; given the good ultrastructure, this probably reflects the physiological state of the tissue (Fig. 1c).

The ventricular zone (VZ) is densely populated with neuroepithelial cells actively engaged in proliferation. Mitotic profiles are abundant, lining the ventricular surface, and have their spindles oriented preferentially parallel to the ventricle. Neuroepithelial cells (Fig. 2a) are arranged in a palisade, with ellipsoidal, radially elongated nuclei (± 5 μm) containing chromatin at various stages of condensation. They are bipolar and send an external extension toward the pial surface. The ventricular surface of these cells is heavily ciliated and the ventricular cytoplasm contains basal bodies as well as abundant and large fatty droplets, mitochondria, and free ribosomes. Cells are attached together near the ventricle with embryonic junctional complexes, primarily made of adhaerens junctions; tight junctions appear poorly developed and gap junctions have not been identified (Fig. 2b). In the external part of the VZ and within the IZ, cells are seen, which have a larger nucleus than cells in the VZ, often with an immature nucleolus. Their morphology is thus intermediate between ventricular cells and cells in the CP. They have a stellate shape and are literally floating among a large extracellular space. The cells in the IZ with intermediate morphological features probably correspond to migrating elements;

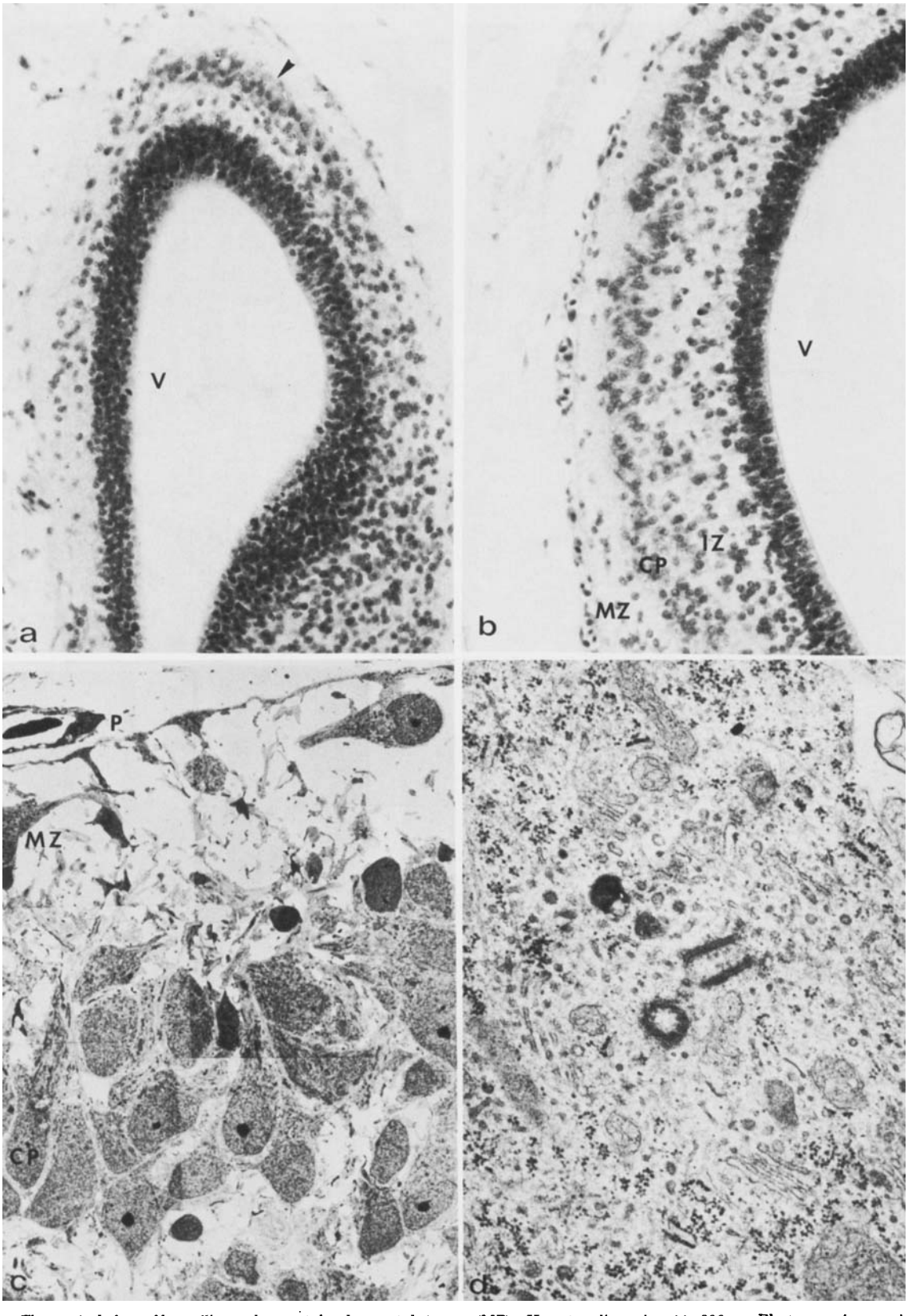


Fig. 1. The cortical plate of lacertilian embryos at developmental stage 1. a. Frontal section through the telencephalic vesicle, showing the lateral ventricle (V) and the very immature cortical plate (arrowhead). Hematoxylin-eosin, × 225. b. At a slightly more advanced stage than in a, the four layers of the telencephalon are well individualized: The ventricular zone, the intermediate zone (IZ), the cortical plate (CP), and the marginal zone (MZ). Hematoxylin-eosin, × 300. c. Electron microscopic montage showing the radial organization of the early cortical neurons in the cortical plate (CP): The marginal zone (MZ) is sparsely cellular, traversed by radial components. P, pial surface. × 1,900. d. An electron microscopic picture of the dendritic pole of a neuron located in the cortical plate. × 30,000.

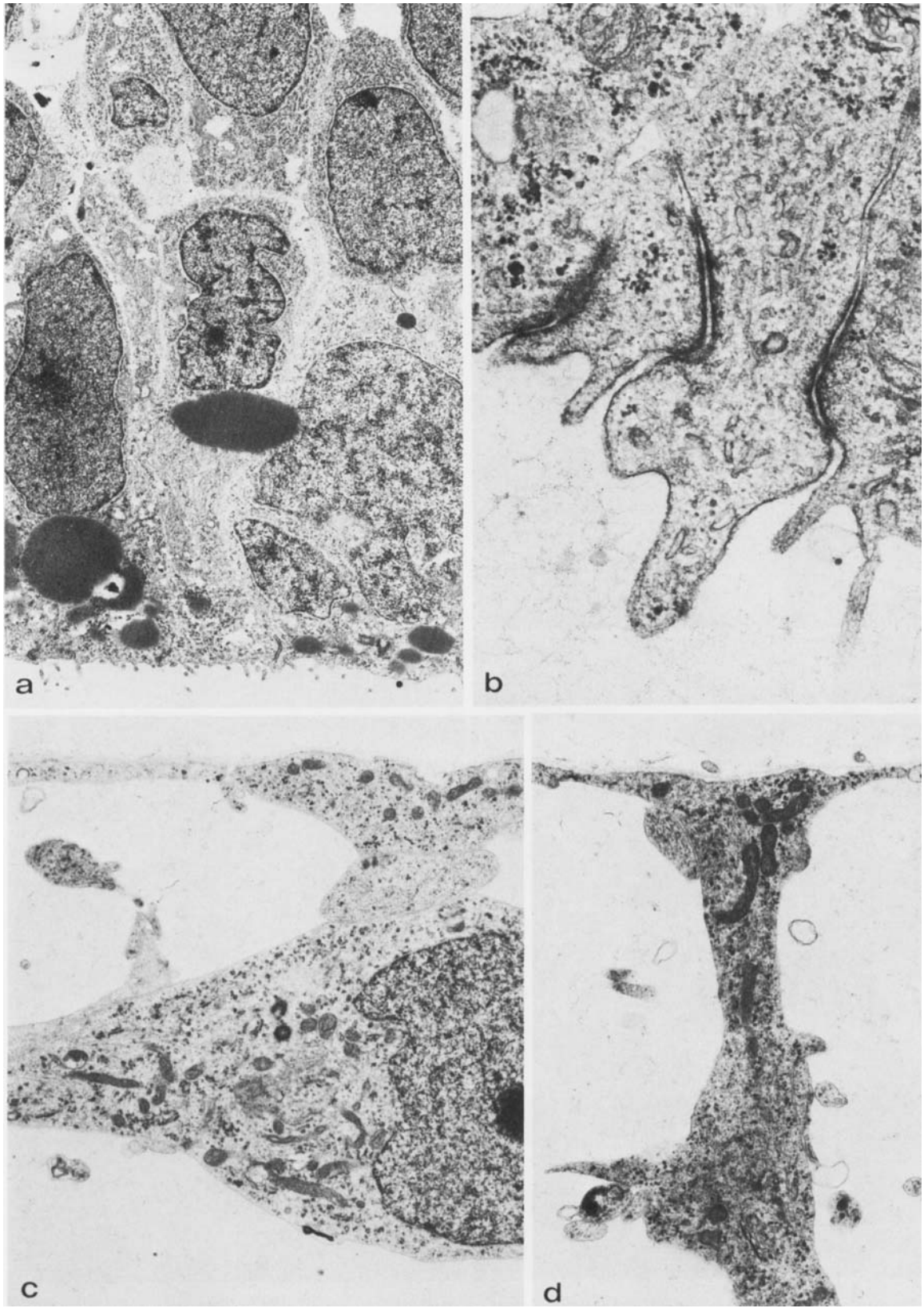


Fig. 2. Electron microscopic pictures of telencephalic components of *Lacerta* at developmental stage 1. a. The cells in the ventricular zone are heavily ciliated and contain large lipid droplets (× 9,000); their ventricular pole, and particularly the intercellular junctions, are clearly visible in b (× 40,000). c. Photomicrograph of horizontal neuron in the marginal zone (× 12,000). d. Photomicrograph of a radial glial end foot abutting the basement membrane (× 15,000).

some of them, however, could already have ended their migration.

Numerous neurites and cell extensions are seen in the IZ and correspond to immature axons, dendrites, and radially elongated processes from ventricular cells. However, their precise identity has not been determined. Radial fibers sometimes come into close contact with immature neurons.

In contrast to *Emys* (see below), the cortical plate in *Lacerta* is, from its earliest developmental stage, remarkably well defined (Fig. 1c). It is best described as a condensation of young, bipolar neurons. The cells in the CP have a prominent nucleus (7–8 μm in diameter) with evenly dispersed reticulate chromatin and well-formed nucleolar structures. The cells are radially oriented, and their asymmetrically distributed cytoplasm is more abundant at the external pole. Ultrastructurally, the outer cell pole corresponding to the early "apical" dendritic tree contains the centrioles, abundant but nonfasciculated microtubules, profuse Golgi membranes, smooth and coated vesicles, mitochondria, free ribosomes, smooth reticulum, and a few rough reticular cisternae (Fig. 1d). The inner cell pole contains a sparse cytoplasm, with a few organelles, but it can sometimes be identified as the site of emergence of a primitive axon. The cells in the CP frequently come into contact with each other and with adjacent neurites and cell extensions. Punctae adhaerentes diminutae are usually observed at the contact site.

The marginal zone (MZ) extends between the CP and the pial surface. Its external boundaries are defined by the limiting membrane – formed by end feet of radial neuroepithelial cells, abutting the basement membrane (Fig. 2d). The end feet contain free ribosomes, a few membranous profiles, some mitochondria, and occasional multivesicular bodies. Cells in the MZ are rare and, in sharp contrast to the neurons in the CP, they tend to be horizontally oriented (Fig. 2c). Apart from their different orientation, they share with CP cells all the features of immature neurons. Two principal types of fibers are seen in the MZ: first, radial profiles, which are considered to correspond to the external processes stemming from ventricular cells; second, oblique dendrites which can occasionally be traced to CP cells or to intrinsic neurons in the MZ. Axons and synapses appear in the MZ at stage 1, with the earliest synapses being of the asymmetric type.

Lacerta: Developmental stage 2

At this stage in *Lacerta*, the cerebral cortex is no longer composed of a homogeneous plate. Three cytoarchitectonic fields are clearly distinguishable: the medial (hippocampal), dorsal (general), and lateral (pyriform) cortices. These areas are well defined and separated from each other. Two regions of superposition, corresponding to the lateral and medial superpositions of De Lange ('11), are found at their borders.

Radial neuronal orientation is better defined in the hippocampal area than in the dorsal CP – which is, in turn, better organized than the pyriform cortex. In our material, it is also clear that at least two further subdivisions must be considered. First, in the medial cortex (Fig. 3d), there is an obvious difference in neuronal size. Neurons are smaller in the interhemispheric field and larger in the dorsal area of the hippocampus. The transition between the small- and large-celled segments of the medial cortex is, however, progressive. Second, the dorsal pallium (Fig. 3c) is subdivided into a medial band, adjacent to the medial cortex, with radially dispersed neurons and a "loose" CP, and a lateral band, densely populated with radially oriented neurons, and in which the CP is thinner and sharply defined. These cytoarchitectonic features, not present in the cortex of *Emys*, make it appear rudimentary when compared to *Lacerta* (see below).

At the level of the dorsal cortex (Fig. 3c), the telencephalon of *Lacerta* is approximately 200 μm thick and the relative thicknesses of its four strata are, for the VZ, 12–15 μm, the IZ, 50 μm, the CP, 50 μm, and the MZ, 80–85 μm. Therefore, the growth of the neural wall between stages 1 and 2 primarily concerns the MZ and CP.

The ventricular zone is one to two cells thick and is populated with radial neuroepithelial and/or glial elements. The nuclei are more elongated vertically than at stage 1, measuring approximately 3–4 μm to 8–10 μm. The density of mitotic figures is lower than in younger embryos. The ultrastructural features of ventricular cells are identical to those described at stage 1. The external pole of radial cells gives rise to the radial "glial" fibers which run across the whole thickness of the tube until they reach the pial surface. With Golgi impregnation (Fig. 5a,b), the radial process appears long, slender, and heavily invested with fine, delicate extensions of various shapes and dimensions. Many of the radial fibers divide into two or more branches which run separately toward the pial surface. The point of branching is preferentially located near the external border of the cortical plate with tributaries rarely divergent by more than 60°. In the external part of the VZ some cells have a rounded nucleus (approximate diameter: 5–6 μm). They are similar to young cells in the IZ and might be early postmitotic neurons entering their migration.

The intermediate zone is larger at stage 2 than at stage 1, due to a large increase in its content of fibers. Golgi impregnations demonstrate that these fibers correspond to axons stemming from cortical plate or IZ cells, and to afferent fibers (sometimes capped with growth cones), coming from undetermined adjacent areas (Fig. 5a). In addition to various fibers, the IZ also contains rare cells. Some of these are reminiscent of rounded cells in the VZ and correspond to migrating neurons. Others have a morphology suggestive of a greater differentiation (a large nucleus with well-differentiated nucleoli, rough endoplasmic reticulum) and may have ended their migration in the IZ. Golgi stain shows that these cells have a polymorphic shape and send their dendrites locally or through the adjacent cortical plate.

As described above, the cortical plate in *Lacerta* at stage 2 is composed of three cytoarchitectonic parts. As architectonic differentiation is more complete at these levels, the cytological description of cortical neurons will be focused primarily on the dorsal and, secondarily, the medial cortex.

The cortical plate is densely populated with young neurons, which contain rounded or ellipsoidal nuclei, with diameters of 7–9 μm. The chromatin is reticulated and contains a prominent nucleolus. With the exception of the medial band in the dorsal pallium, neurons in the lacertilian CP are parallel to each other, arranged radially in a sharply defined plate. The neurons in the CP are bipolar but asymmetric. The cytoplasm is more abundant at the external than at the internal cell pole, although a minor proportion of the cells are inverted. The "apical dendrite" is characterized by a well-developed vacuolar apparatus composed

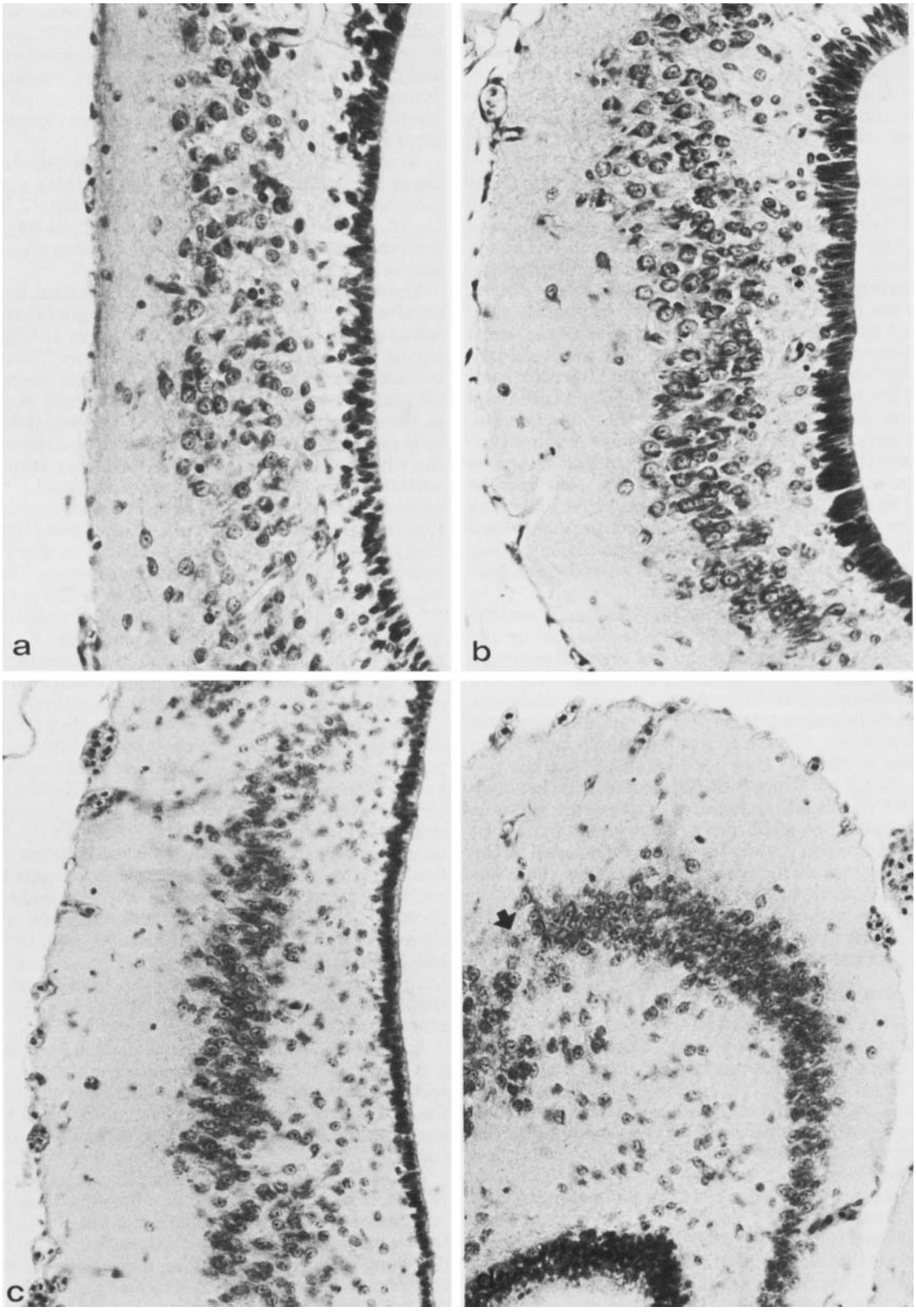


Fig. 3. Photomicrograph of the principal areas of the cerebral cortex in *Emys* and *Lacerta* at developmental stage 2. a. The dorsal cortex in *Emys* contains neurons loosely arranged in a poorly defined plate. Hematoxylin-eosin, × 275. b. The medial (hippocampal) cortex in *Emys:* The cortical plate is better defined than in the dorsal pallium, yet it remains poorly organized. Hematoxylin-eosin, × 275. c. The dorsal pallium in *Lacerta* contains a radially organized plate. Hematoxylin-eosin, × 290. d. The hippocampus in *Lacerta* has a sharply defined cortical plate; on the left of d the area of superposition between the medial and the dorsal pallium is seen (the superposition of de Lange; arrow). Hematoxylin-eosin, × 290.

of Golgi membranes, smooth reticulum, and also a large amount of rough reticulum (which was characteristically sparse at earlier developmental stages). It extends toward and within the MZ, where it divides into several branches. The inner cell pole contains only a few organelles and is the site of origin of the axon. In Golgi-impregnated material, the principal cell type is a prepyramidal neuron (Fig. 5). The cell body is usually strictly radially oriented and both the apical dendrite and the initial segment of the axon align along the same axis. The apical dendrite divides into several tributaries which ramify through the MZ into a symmetrical "bouquet" rarely diverging by more than 120°. Spines are seen on dendrites at this stage, but they are not very abundant. A second type of neuron with polymorphic features, identical to the cells in the IZ described above, is encountered at the inner part of the CP.

Compared to stage 1, the lacertilian MZ at stage 2 is characterized by a large decrease in extracellular space and by an enormous increase in its content in fibers. Synapses are abundant and the great majority of them are asymmetric. Symmetric synapses have not been clearly identified. The nature and origin of the dendritic and axonal fibers cannot be ascertained by the methods employed here but, from Golgi preparations, some of them have been shown to originate from the neurons in the CP and from intrinsic elements in the MZ, while others derive from extrinsic afferents of unknown origin. Some cells located within the MZ have distinctive neuronal features, although the glial nature of others cannot be excluded. A few of these cells have been impregnated by the Golgi method and reveal a morphology reminiscent of stellate neurons.

Emys: Developmental stage 1

At this early stage, the telencephalic wall is very thin and immature (Fig. 4a). Its thickness at the dorsolateral level is approximately 130 μm, with its internal portion occupied by the ventricular zone, and the external field populated by young, postmitotic neurons and neuropil. Extracellular space is prominent in all cortical strata.

The VZ contains a dense population of neuroepithelial cells actively engaged in the proliferative cycle. Many mitotic shapes are seen lining the ventricular surface, with spindles predominantly oriented tangential to this surface. The nuclei of neuroepithelial cells are small (4-6 μm), contain a dense chromatin at various stages of clumping, and usually have a radially elongated shape. The cells themselves are bipolar with a short internal and an elongated external process. The ventricular cell surface is covered with cilia which protrude into the ventricular lumen. The adjacent cytoplasm contains basal bodies, mitochondria, and free ribosomes. The cells are in close contact near the ventricle where they are sealed by junctional complexes composed of an adhaerens junction and immature tight junctions.

Externally to the VZ lies a sparsely cellular layer, the intermediate zone (IZ) (Fig. 4b). It contains a few cells with a morphology intermediate between the cells in the MZ and the young neurons in the CP: The nuclei range 6-8 μm in diameter and contain a lighter chromatin than those in the VZ; the cytoplasm is sparse and the cell body assumes a stellate shape, with no preferential orientation. Many of these cells probably correspond to young, postmitotic neurons engaged in cell migration. However, the most mature of them could already have ended their migration.

The IZ contains two types of processes: First, oblique or horizontal immature axons; second, cytoplasmic extensions which run at various angles through the IZ and belong to local cells or to the cells from the VZ. These can sometimes be identified since they have a predominantly radial course, and contain many microtubules and mitochrondria and some rough reticular profiles. They occasionally come into close contact with the cell bodies of migrating, immature neurons.

Externally to the IZ, occupying the external half of the telencephalic wall, a population of postmitotic, young neurons is found. Separated by abundant extracellular space, they appear as an obliquely arranged, loose network. In contract to *Lacerta*, there is no clear condensation of neuronal cells into a "plate" (compare Figs. 1a,b, 4a).

The early neuronal cells in this region have a large nucleus (7-9 μm), with evenly dispersed chromatin and well-individualized nucleoli. The cytoplasm is asymmetrically distributed and usually more abundant on the pole of the cell which is directed toward the pial surface. Ultrastrucurally, these cells have the typical morphology of immature bipolar neurons – the dendritic pole contains the centrosome, smooth vesicles and cisternae, Golgi membranes, mitochondria, free ribosomes, dispersed microtubules, and some profiles of rough reticulum. The other cell pole contains sparse organelles and is the site of origin of the axon, as occasionally revealed by the fasciculation of microtubules into a narrow neurite. Various neurites and cell extensions extend within the cortical plate, most of which have the morphology of immature dendrites, rich in free ribosomes and mitochondria; some axons are also present and run through the cortex toward the MZ.

The marginal zone (MZ) is well defined externally by the limiting membrane. It is less clearly delimited internally, where it becomes progressively more cellular and merges with the "cortical plate." The MZ contains rare cells, which have a morphology very similar to cells in the CP. However, they are predominantly horizontally spread, are smaller and have a slightly denser nucleus. This early stage of development is characterized by the appearance of axons and synapses in the MZ. In some embryos the MZ is virtually devoid of axonal profiles, whereas axons are quite abundant in other specimens which are at comparable developmental stages (judged from their external morphology). The early axons run obliquely through the MZ and form asymmetrical synapses with unidentified postsynaptic dendrites (Fig. 4c). Some synapses en passant are also encountered (Fig. 4d). The origin of the majority of the axons in the early MZ has not been identified.

Emys: stage II

Although not as clearly defined as in the adult brain, three major cytoarchitectonic subdivisions are present in the pallium of *Emys* at this stage: The medial (hippocampal), dorsal (general), and lateral (pyriform) cortex. The orientation and distribution of the cells differ in these three areas, with neurons being most densely packed, and the cortical plate best defined in the medial and least packed and most poorly defined in the lateral pallium (Fig. 3a,b).

When compared to the lacertilian telencephalon, the architectonic areas in chelonians are less clearly delimited. The superpositions of De Lange are not obvious and the finer architectonic subdivisions made in *Lacerta* are not apparent.

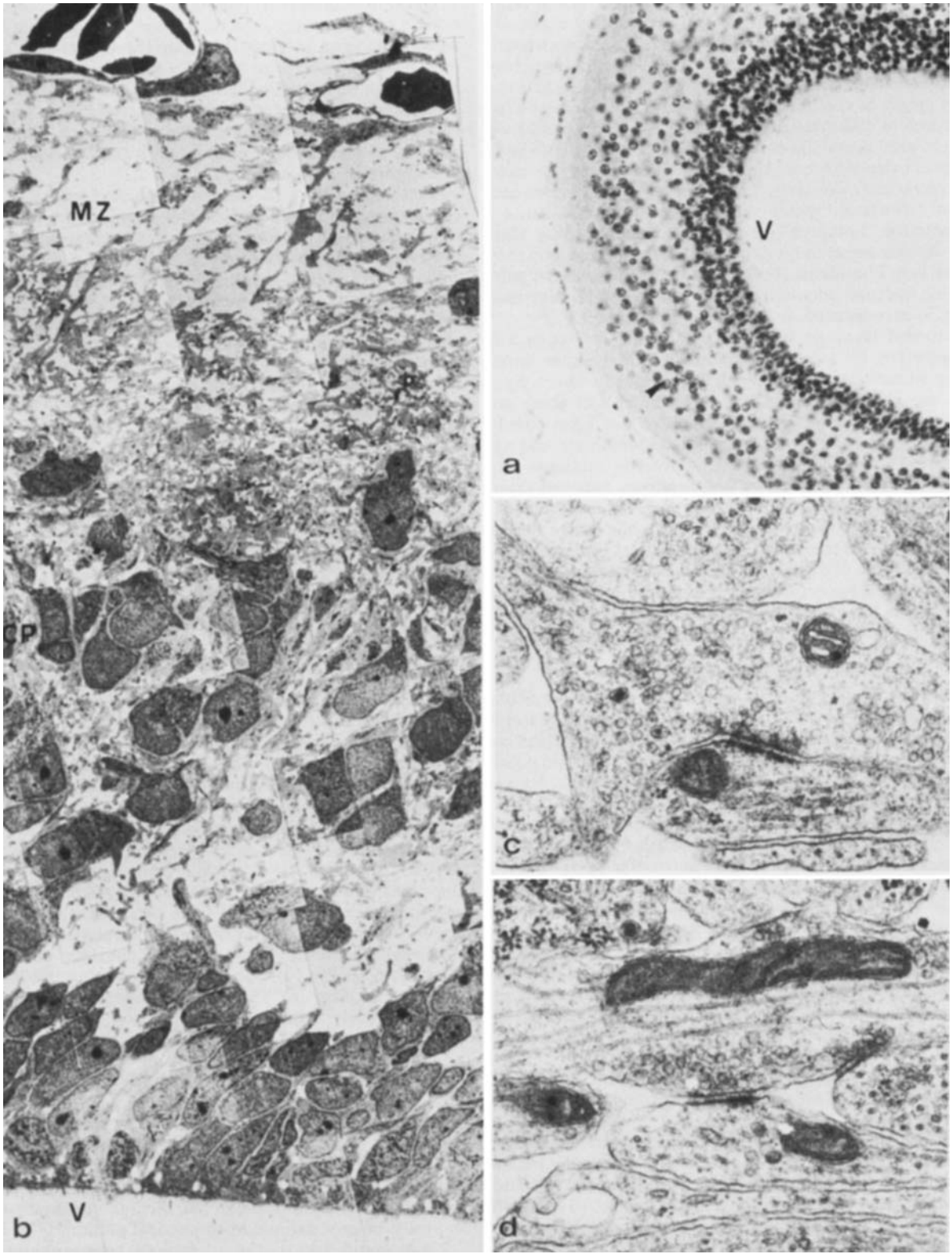


Fig. 4. The cerebral cortex of embryonic *Emys orbicularis* at developmental stage 1. a. Light photomicrograph of the early cortex: Near the ventricle (V), the ventricular zone is well defined, but the cortical plate (arrowhead) is extremely loose (compare with Fig. 1a and b). Hematoxylin-eosin, × 210. b. Electron microscopic montage of the telencephalon: From the ventricle (V) to the pial surface, the ventricular zone, the cortical plate (CP), and the marginal zone (MZ) are well visible, but the CP is poorly defined (compare with Fig. 1c). × 1,800. c. Electron photomicrograph of an axon terminal forming an asymmetrical synapse in the marginal zone. × 40,000. d. Electron photomicrograph of an axon making a synapse en passant in the marginal zone. × 40,000.

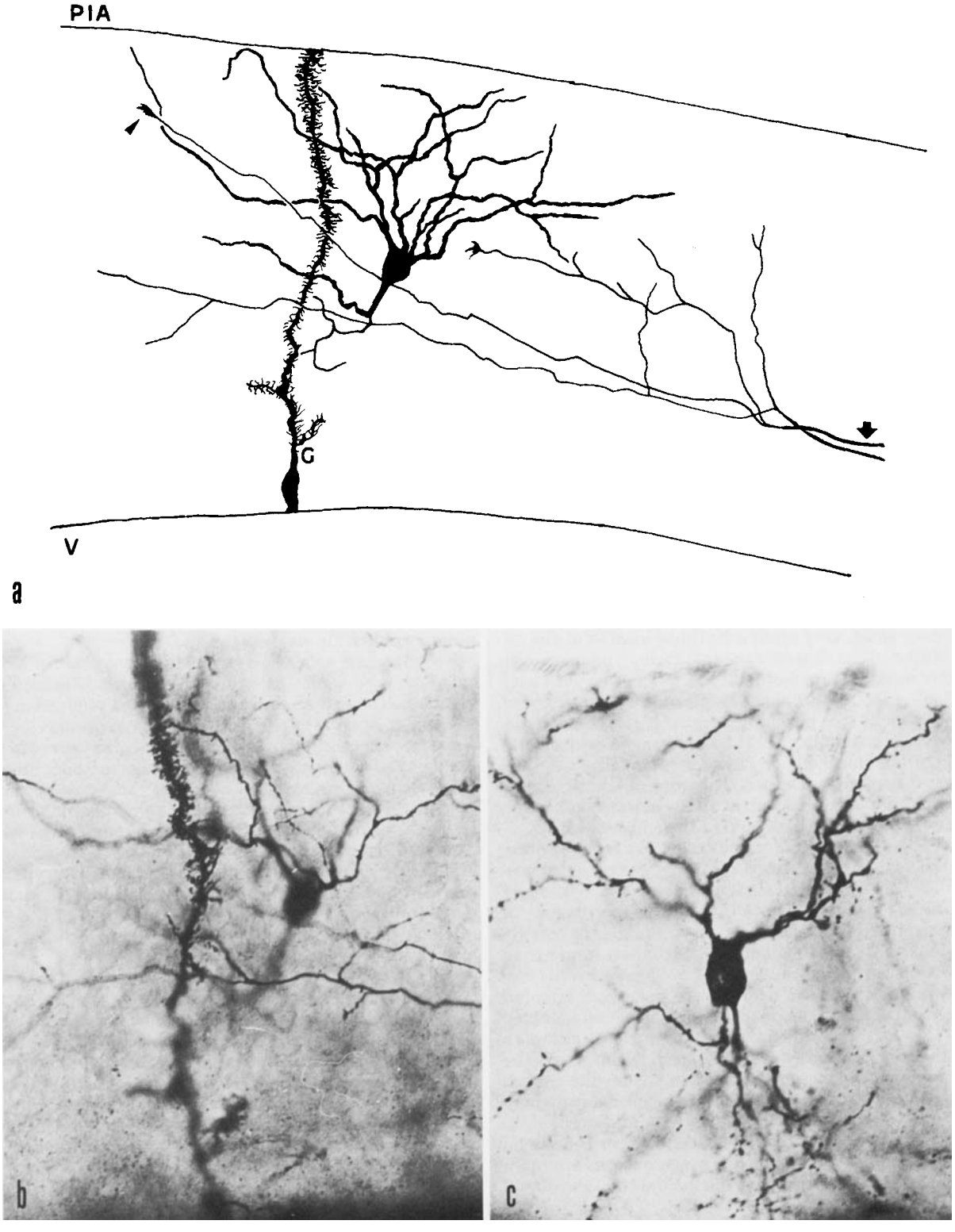


Fig. 5. Golgi impregnation of the cortical plate of lacertilian embryos at developmental stage 2. a. A representative field in the dorsal pallium, with a radial neuron, a radial glial fiber (G), and afferent axons (arrow), capped with growth cones (arrowhead); camera lucida drawing. b. Photomicrograph of the same field as a. × 400. c. Photomicrograph of a typical radial neuron in the dorsal cortex. × 540.

At the level of the dorsal cortex (Fig. 4a), the thickness of the telencephalic wall is approximately 150 μm, and it is composed of the four strata already described, namely, the VZ (±25 μm), IZ (±25 μm), CP (±50 μm), and MZ (±50 μm). It is thus clear that the growth of the cortical wall between stages 1 and 2 occurs primarily by an increase in the thickness of the CP and of the MZ.

The ventricular zone is populated with elongated cells with elliptical nuclei, variable in shape but, as a rule, more radially elongated than at earlier stages (±3-5 μm wide and 8-10 μm long). A few mitoses are still present within the VZ at this stage.

At the external border of the VZ and to a lesser extent within the VZ itself, cells with a dense and rounded nucleus (±7 μm) are seen. These probably correspond to early postmitotic elements starting their migration, for identical cells are seen within the intermediate zone. The Golgi impregnation of neuroepithelial (or "radial glial") (Fig 6b,c) cells demonstrates the presence of their long external extension. This process runs through the entire thickness of the neural tube and ends against the pial surface. These radial fibers are covered with cytoplasmic appendages, variable in length and shape. They take an undulating course through the cerebral wall, and although predominantly radial, they sometimes cross each other at an angle of up to 45°. In addition, they usually branch into a few tributaries (up to four in our specimens), sometimes as deep as midcortex. The branches then run obliquely toward the pial surface.

The intermediate zone contains rare cells belonging to two types: First, immature, presumably migrating cells with dense nuclei, very similar to those located at the external border of the ventricular zone; second, neurons with distinctly more mature features (a large nucleus with well-individualized nucleoli, and cytoplasmic rough reticular profiles) which are probably postmigratory elements. Axons are quite abundant in the IZ, some of which clearly stem from intrinsic cells or from the cortical plate. Dendritic extensions as well as radial neuroepithelial processes are also individualized; the latter often contain some rough reticular membranes which help in their identification.

The cortical plate of *Emys* at stage 2 is more densely populated and better defined than in younger embryos, particularly in the dorsal and medial cortex. The most abundant and typical cell in the CP has the morphology of a young neuron: It contains a rounded, globular or polygonal nucleus, with a 9-10 μm diameter (slightly larger than in *Lacerta*), with one or two nucleoli and a pale, evenly dispersed chromatin. These neurons are polarized, oriented obliquely or radially. The dendritic pole is usually located on the side of the cell facing the pial surface, although some "inverted" neurons are also seen. Neurons in the dorsal pallium are larger than those in the medial cortex. In addition, the latter are more densely packed and display a better radial orientation. The difference in size could reflect a more advanced stage of maturation in the dorsal than in the medial cortex. At this stage mitoses are more abundant in the VZ underlying the medial than the dorsal cortex.

Another feature of interest, best shown with light microscopy, is that in the dorsal pallium, neurons located in the external portion of the CP are generally slightly more mature than those which settle at an inner level. This morphological characteristic is also present in the CP of the medial cortex when it becomes more developed.

The cells in the CP have ultrastructural characteristics of immature neurons. The apical dendritic pole, usually externally located, contains a well-developed vacuolar system among which rough reticular profiles become abundant. It extends into the MZ, where it ramifies. The other (usually inner) cell pole contains few organelles and is the site of origin of the axon. In Golgi-impregnated material (Fig. 6a), the main neuronal type in the cortical plate is a polarized neuron with an orientation which can assume any angle between radial and horizontal, the former being the most frequent. The axon usually buds from the inferior cell pole or from a lateral face of the cell body and runs within the IZ. Collaterals, some of which run back toward the MZ, are sometimes seen. Thick dendrites originate at the outer pole of the cell body and run obliquely through the CP and the MZ, until they reach the vicinity of the pial surface. Thinner dendrites also stem from the lateral or basal portion of the cell body. As a whole, the dendritic arborization does not have a well-defined geometry but is widely spread and covers a large cortical field. Sometimes the ramifications can diverge to an angle of nearly 180°. Spines begin to form on all dendritic branches but will become profuse only at later developmental stages. A different neuronal type is frequently impregnated within the deep part of the plate near the inner cortical border. It is smaller than the CP neuron and has a polymorphic shape – its polygonal cell body sending dendrites both to the IZ and through the overlying CP. Although a few of these cells might correspond to young cells destined for the CP, the majority of them are postmigratory neurons, for Golgi impregnation at a later stage still reveals the presence of polymorphic neurons near the IZ.

The marginal zone of *Emys* at stage 2 appears as a fiber-rich, cell-poor zone, the neuropil of which is exceedingly complex. The extracellular space is much reduced in comparison to stage 1. Cells in the MZ have distinctive neuronal features, although the presence of glial elements may not be distinguishable. With the Golgi method, some of these neurons are stellate, nonspiny, and may represent true intrinsic elements. Others are spiny neurons with all the features of displaced cortical plate cells – often with a distorted dendritic arborization. Synapses are abundant and nearly all of them are of the asymmetrical type. The origin of the various neurites has not been identified. However, from Golgi data, it is clear that some of them are intrinsic to the cortex, whereas others are extrinsic afferents.

DISCUSSION

Several studies have dealt with the development of the central nervous system, and particularly the telencephalon, in various reptilian species. Telencephalic development has been examined in turtles by Johnston ('16), and recently by Kirsche ('72); in *Sphenodon punctatus* by Hines ('23); in the American alligator by Reese ('10); in several lizards by Faul ('26) and Hetzel ('74); in the water snake (*Natrix sipedon* or *Nerodia*) by Warner ('46); certain interspecies comparisons have been made by Källen ('51) and Bergquist ('54). These works largely predate the elaboration of present views on mammalian cortical development and are primarily focused upon questions of neuroanatomical development. They are only anecdotally concerned with cell differentiation and cortical histogenesis. Many morphological analyses have also been done on the cerebral cortex of different adult reptiles. They have yielded a

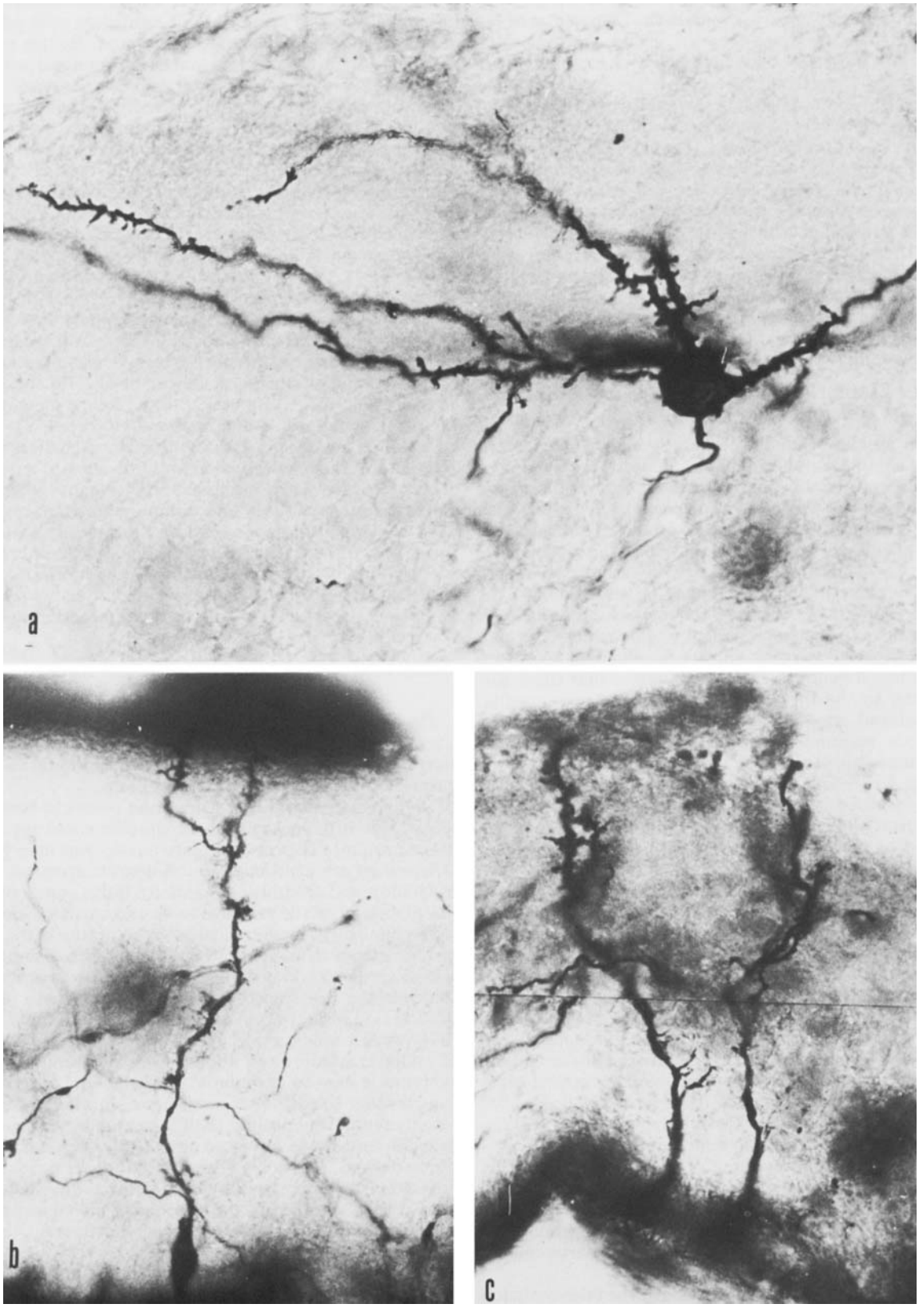


Fig. 6. Golgi impregnation of the cerebral cortex in *Emys orbicularis* at developmental stage 2. a. Photomicrograph of a typical neuron in the dorsal pallium. × 675. b. Photomicrograph of a radial glial fiber traversing the dorsal cortex. × 550. c. Photomicrograph of two radial glial fibers running through the hippocampal cortex. × 520. The path of radial fibers in *Emys* is not as straight as in *Lacerta* (compare with Fig. 5a,b).

large volume of information on the connections and histology of the reptilian cortex and on the homologies of telencephalic structures both between reptiles and in relation to mammals and birds. This field has been extensively reviewed by Northcutt ('81).

The results reported here are, as far as we know, the first comparative analysis of cortical development in reptiles belonging to widely separated taxons. They show that, among this complex taxonomic group, the development of the telencephalic cortical plate not only proceeds according to common general patterns but also shows peculiar interspecies variations. The first part of this discussion will be concerned with the common developmental features observed in *Emys* and *Lacerta*, regarding cell proliferation, migration, maturation, and synaptogenesis; the second part will be focused on the differences observed at the level of the developing cortical plate and on their possible biological meaning.

Cell proliferation, migration, maturation, and synaptogenesis

In both species studied, the development of the cortex proceeds along a common scheme. Neurons are generated from neuroepithelial cells in ventricular zones lining the cerebral ventricles. Mitoses occur near the ventricle, with spindles generally oriented parallel to it. This suggests that the phenomenon of cell generation in the reptilian VZ are basically similar to their extensively studied mammalian counterparts (Sauer, '35; Angevine and Sidman, '61; Hinds and Ruffett, '71; Derer, '74; Caviness and Rakic, '78). No equivalent to the subventricular zone which is prominent at the level of the presumptive mammalian neocortex has been found in our material. The ventricular zones corresponding to the three reptilian cortical areas (medial, dorsal, lateral) are thus reminiscent of the proliferative zones in the mammalian hippocampus, where no subventricular zone is present (Nowakowski and Rakic, '81).

Both in *Emys* and *Lacerta*, immature postmitotic cells leave the ventricular zone and migrate through the IZ to settle within the cortical plate. A minority of these elements go to the MZ or stay beneath the cortical plate. Some young cells in the IZ, presumably migrating cells, are stellate – reminiscent of migrating cells in the mammalian hippocampus (Nowakowski and Rakic, '79) and in the neocortex at an early stage (Derer, '74; Goffinet and Lyon, '79). The presence of abundant and well-developed radial neuroepithelial fibers, seen in both EM and Golgi preparations, and the observation that these radial fibers are often found in contact with cell bodies within the IZ, suggest that they assist in the radial guidance of migrating cells. A similar role for glial fibers in neuronal migration toward and within the mammalian cortical plate has been amply demonstrated (Caviness and Rakic, '78; Rakic, '80; Evrard et al. '80). Radial fibers in *Emys* and *Lacerta* usually divide at levels which approximately correspond to the local external limit of the cortical plate. This morphological feature is also present in mammals, particularly in mice (Goffinet, unpublished). This raises the question whether the branching point could be a morphological marker of differentiation, possibly related to nerve cell migration, along the axis of the fiber.

The sequence of maturation of cells in the telencephalon is also quite similar in the two reptiles studied. As maturation proceeds from the stage of the ventricular cell to the neuron of the cortical plate, nuclei become larger, their chromatin more reticulate, and nucleoli increase in size and organization. The cytoplasm of immature cells primarily contains a smooth vacuolar apparatus. Rough reticular cisternae begin to appear in young neurons and become increasingly prominent as they enlarge. During the last third of incubation, patching of rough reticulum into immature Nissl bodies can be seen. In proliferating cells, centrioles are located at the ventricular pole, whereas in migrating cells and in the neurons of the cortical plate the centrosome is found at the level of the main dendrite – usually on the external side of the cell. This suggests that a translocation of the centrosome occurs concomitantly to cell migration. This could be a general feature of cortical histogenesis, for it has also been described in mice (Shoukimas and Hinds, '78).

In both species studied, synapses appear first and are the most abundant in the marginal zone. A large majority of them are asymmetrical. Early synaptogenesis, which has been studied in the rat (König et al., '75) and in mice (Derer et al., '77; Goffinet, '80), thus follow a comparable course in the reptilian and mammalian cortex.

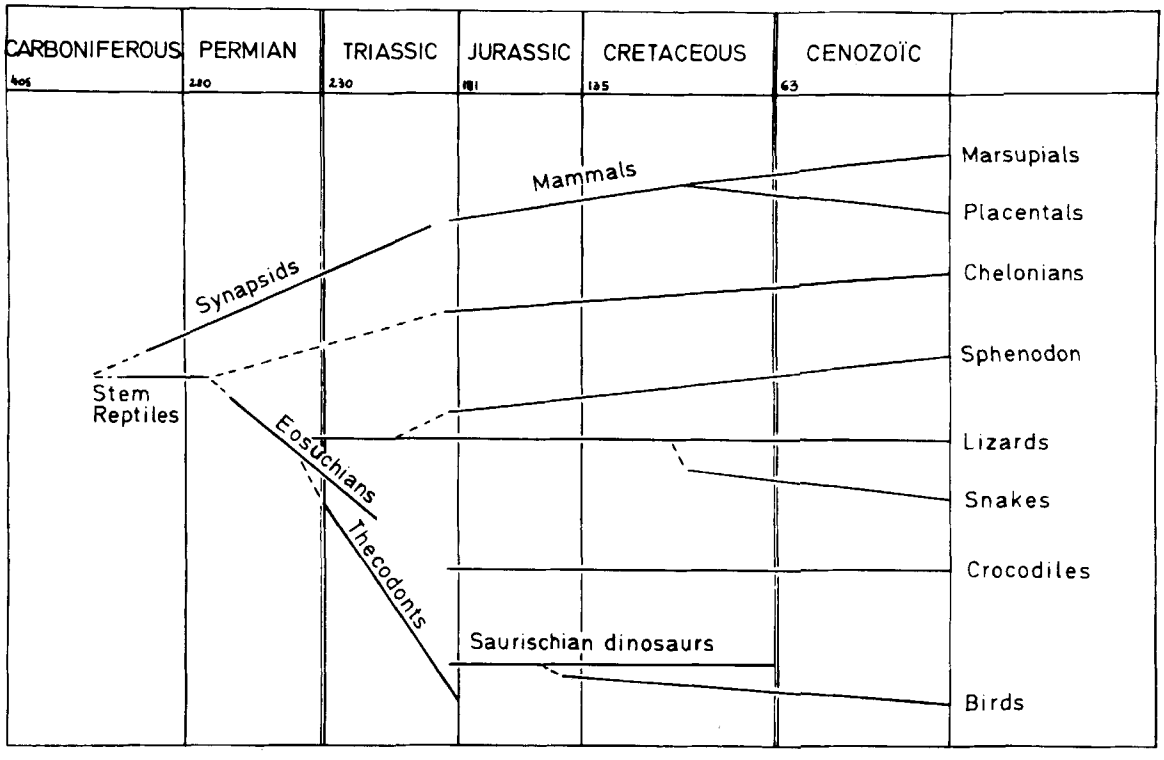
In *Emys* and *Lacerta* a very immature cortical plate is observed in the telencephalon in the absence of any synaptic profile, whereas in specimens slightly more advanced in development, synapses and oblique axons appear in the MZ. The appearance of synapses thus closely follows the onset of differentiation of the cortical plate. This demonstrates that, in these species at least, the formation of connections, as judged by the presence of synapses, is not a condition necessary to the early differentiation of the cortical plate.

Developmental differences in the cortical plate between *Emys* and *Lacerta*

The second part of the present discussion is focused on the cytological differences that we have observed between *Emys* and *Lacerta* – particularly the density and radial orientation of neurons in the cortical plate.

The developmental literature cited seems to have overlooked the differences in the architectonic and cytological organization of the cortical plate among reptilian groups. Differences are mentioned in the neuroanatomical review of Goldby and Gamble ('57) and are quite apparent when the histology of the cerebral cortex in turtles (Ebner and Colonnier, '78) is compared with the structure of the cortex in squamates (Northcutt, '78; Ulinski, '79; Lohman and Van Woerden-Verkley, '78). The well-defined cytoarchitectonics in the lizard cortex is stressed by Minelli ('66) and Wouterlood et al. ('81). According to Goldby and Gamble ('57), variations in cortical cytoarchitectonics are common, of minor character, and do not seem to be related in any systematic way to taxonomic position. Our observations suggest that the differences are more significant than previously reported. Possibly their importance has been minimized because they are more obvious during embryogenesis and tend to become less striking as development proceeds. This interspecies variation in the development of the cortical plate raises several questions concerning (1) possible underlying biological mechanisms and (2) their evolutionary significance.

Biological mechanisms involved in the histogenesis of the cortical plate. The role of afferent connections in the early development of the cortical plate in reptiles is not crucial, for in both species studied the cortical plate appears before the first synapses can be seen in the MZ. In



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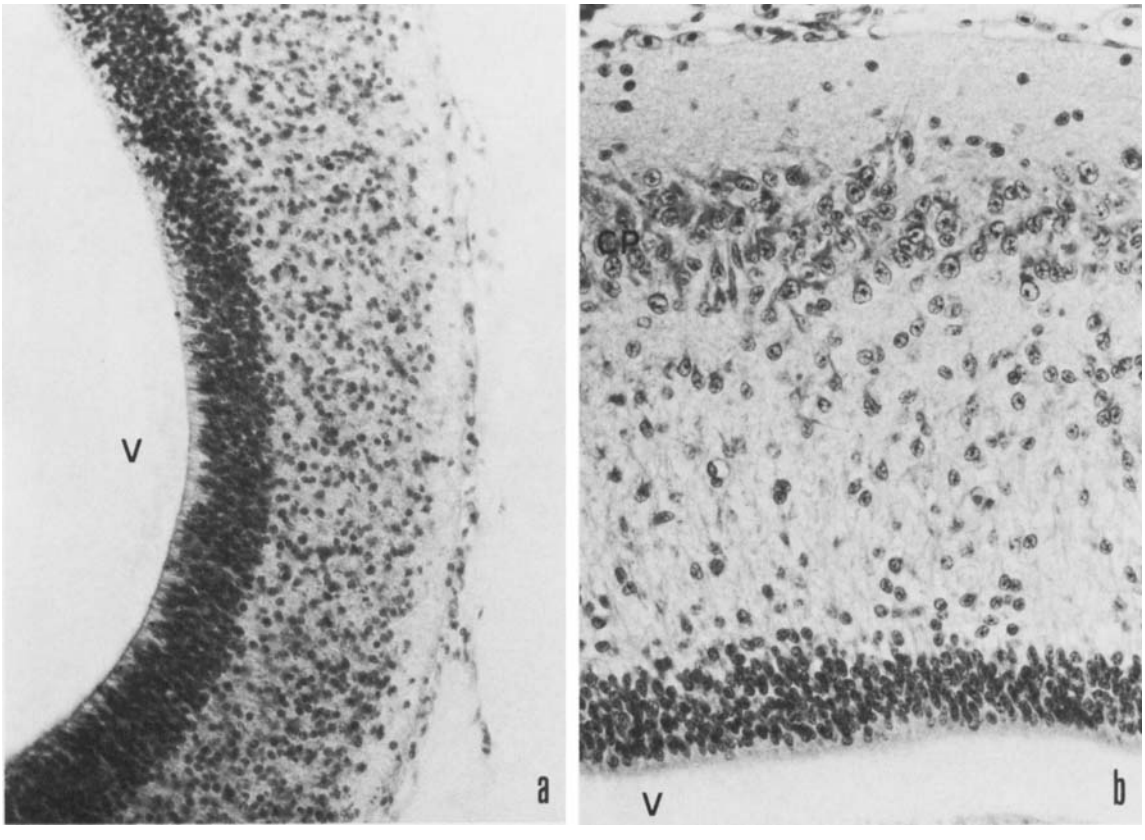

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Fig. 7. Schematic, "minimal consensus" cladogram of reptilian filiations, including the ancestry of mammals and birds. Geological time from the beginning of each era is noted in Myrs.

Fig. 8. Light photomicrographs of the cerebral cortex in embryos from *Crocodilus niloticus*. a. At stage 1 (40–50 mm), the telencephalon is composed of a prominent ventricular zone near the ventricle (V) and a poorly defined cortical plate. Hematoxylin-eosin, × 170. b. At stage 2 (80–90 mm), the cortical plate (CP) is well defined but the radial organization of neurons is rudimentary. Hematoxylin-eosin, × 260.

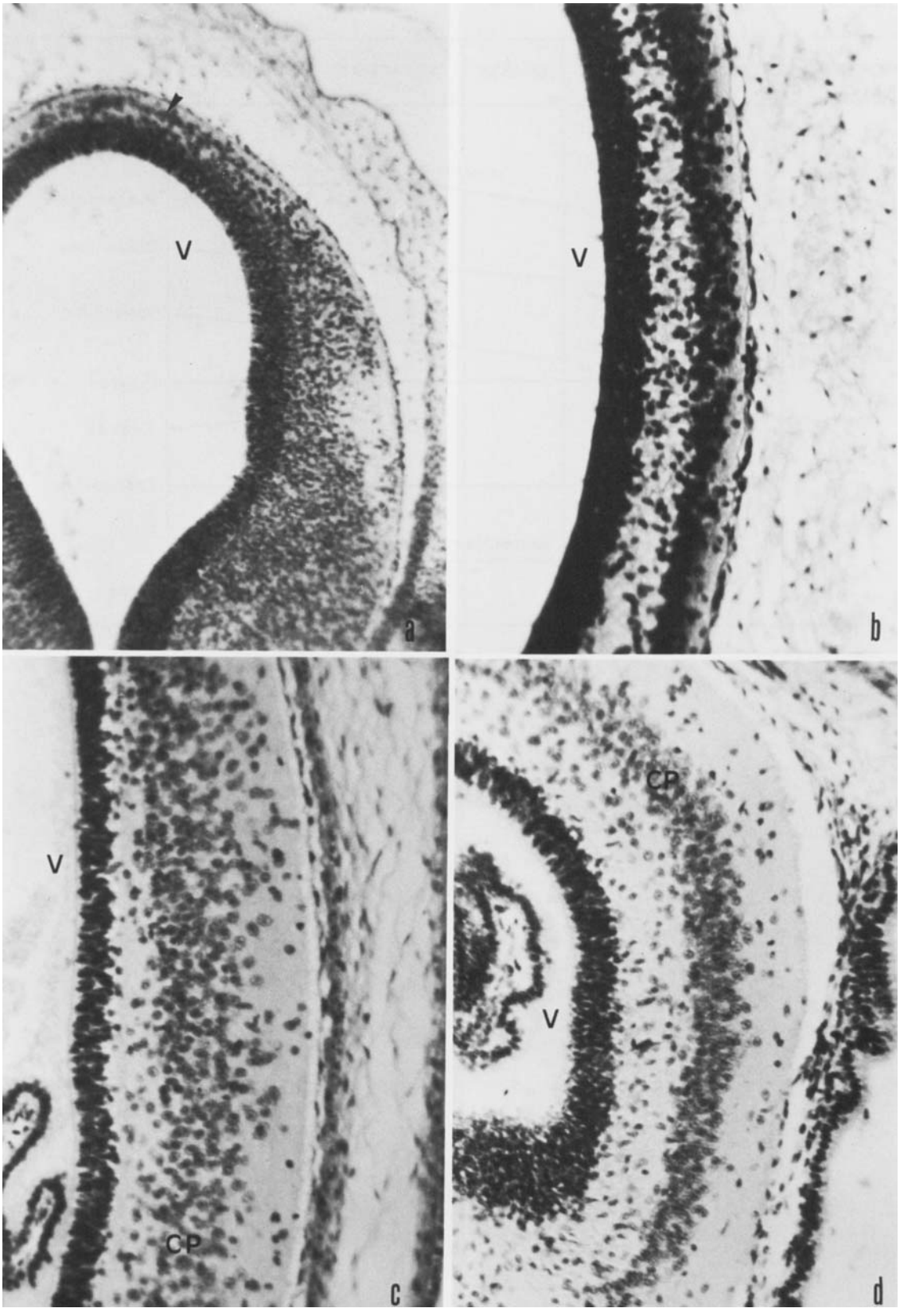

Fig. 9. The development of the cerebral cortex in *Sphenodon punctatus*. a. Photomicrograph of the telencephalic vesicle with a very immature cortical plate (arrowhead; stage P-Q of Dendy). × 120. b. Photomicrograph of the cortex at developmental stage 1 (stage Q of Dendy): The cortical plate is relatively well defined, especially at the level of the future medial cortex. × 150. c. Photomicrograph of the dorsal pallium at stage 2 (stage R of Dendy): The cortical plate (CP) is better defined than in *Emys* but less than in *Lacerta* (compare with Fig. 3a,c). × 150. d. Photomicrograph of the hippocampal cortex at stage 2: The cortical plate is sharply defined, like in *Lacerta*, and in continuity with the dorsal cortical plate; the superpositions of de Lange are not present (compare with Fig. 3d). × 150. Photographed from King's College collection, courtesy of Prof. C.B. Cox.

addition, the sequence of synaptogenesis is identical in *Emys* and *Lacerta*, irrespective of the obvious differences in the development of the cortical plate. In mammals, it is not possible to separate the events of synaptogenesis from the appearance of the cortical plate, for they are concurrent (Derer et al., '77). Our observations do not exclude a nonsynaptic role of fiber systems in the histogenesis of the cortical plate. For example, monoaminergic fibers have been demonstrated in the rat cortex, before the condensation of the cortical plate (Schlumpf et al., '77).

The differences in the organization of the cortical plate between *Emys* and *Lacerta* are associated with subtle variations in the cytology of the radial glial fibers. In *Emys*, these follow a less strictly radial, more undulating course, and branch at more variable and usually deeper levels than in *Lacerta*. These morphological features might reflect differentiation along the axis of the radial guides responsible for differences in the disposition of postmigratory cells within the cortical plate. In addition to providing the ideal substrate for neuronal migration, radial fibers could be important in the determination of cytoarchitectonic patterns. This is in agreement with the hypothesis proposed by Rakic ('80)—that glial fibers play a role in the areal differentiation of the central nervous system—and would generalize this theory to include reptilian species.

In mice, it has been shown that the radial organization of embryonic cortical neurons is related and possibly a necessary condition to the establishment of a correct inside-out gradient of histogenesis within the cortical plate (Caviness and Rakic, '78; Goffinet, '79). If this is true, one might expect to observe different gradients of histogenesis in *Emys* (presumably outside-in) and *Lacerta* (inside-out). Our observations suggest that the maturation within the cortex of *Emys* is more advanced at inner than outer cortical level, indicating a possible outside-in gradient. No differences in maturation have been observed in the lacertilian cortex. Autoradiographic dating of cortical neurons should provide an answer to this question.

Evolutionary considerations. We have attempted to correlate the observed developmental differences in the cortical plate in *Emys* and *Lacerta* with the lineage of reptiles and their relation to mammalian ancestors. Many points in the paleontology of reptilian evolution remain unknown and cannot be deduced from the study of living species. Biochemical studies (mainly protein sequences) are of relatively little help as they lead to cladograms which show substantial divergence from paleontological data and are often very different from each other (compare, for example, Perutz et al., '81, and Maeda and Fitch, '81). For these reasons only widely accepted facts will be considered as a basis for discussion, and we will refer to the "minimal consensus" cladogram shown in Figure 7 (C.B. Cox, personal communication). A common reptilian ancestor (probably during the Pennsylvanian) gave rise to several independent lines, four of which led to living reptiles, mammals, and birds. A first branch, the synapsids, separated early and gave rise to mammals; a second branch led to chelonians, via a poorly understood lineage; a third branch led to Rynchocephalia (of which *Sphenodon punctatus* is the only living representative) and to lizards and their ophidian derivatives; the fourth branch gave rise to the crocodilians (via thecodonts), and to birds (via saurischian dinosaurs). The third and fourth branches form the diapsid group, the ancestors of which correspond to eosuchians; relationships between diapsids are still incompletely known.

The present results, together with observations on members of other reptilian groups, suggest that the radial organization of the embryonic cortical plate is not an all or nothing phenomenon and that, among reptiles, *Emys* and *Lacerta* represent two extremes of this cytologic feature. In *Crocodilus*, the neurons in the cortical plate are not well packed (as in *Lacerta*) but the cortical plate is still better organized than in turtles (Fig. 8). In *Sphenodon*, the architectonics of the cortical plate is elaborate at the level of the hippocampus but the dorsal pallium appears poorly organized (Fig. 9). In snake embryos (not shown) the cytoarchitectonics in the various sectors of the cortical plate appears more highly organized than in turtles but less than in lizards.

The radial orientation of developing cortical neurons is thus expressed differently in the various reptilian radiations and has been acquired independently, gradually, and to variable extents, after phyletic divergence. It is thus a case of homoplasy, probably due to evolutionary convergence, for it "involves the independent evolution of similar characters in organisms possessing distant common ancestry" (Northcutt, '81). As in other cases of homoplasy, it reveals that "similar solutions to biological problems have occurred independently" (Northcutt, '81) and probably correspond to efficient solutions. Among reptiles, lizards could be considered the most successful in the evolutionary sense since they have the widest geographical distribution, the largest number of species, and occupy the greatest diversity of environment (Carroll, '77).

In conclusion, the radial organization of cortical neurons, so characteristic of mammalian development, is not a general property of every cerebral cortex but rather is the product of an evolutionary process. As such, it must have its own independent genetic basis. In addition, the present work shows that the evolution of the nervous system and of its components occurs by changing the geometry of neuronal distribution within the various brain structures as well as by an increase in cell number and differentiation.

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