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Ultrastructure of Putative Migrating Cells in the Cerebral Cortex of *Lacerta galloti*

J.M. GARCIA-VERDUGO, I. FARINAS, A. MOLOWNY, AND C. LOPEZ-GARCIA
Citologia e Histologia, Facultad de Ciencias, Universidad Autonoma de Barcelona (J.M.G.-V., I.F.) and Catedra de Citologia e Histologia, Facultad de Ciencias Biologicas, Universidad de Valencia (A.M., C.L.-G.), Spain

ABSTRACT Cells considered to be migratory in the cerebral cortex of adult lizards are ultrastructurally of two types. Nuclei in the first type have highly dispersed chromatin, creating a spongy appearance, whereas in the second type the chromatin is irregularly clumped. Both types of cells are closely associated with processes of radial ependymal glia cells, which perhaps orient their migratory pathways. Cells with spongy chromatin show an increase in cytoplasmic organelles and progressive chromatin condensation as they travel from the ependymal layer to the granular layer. Possibly these cells account for the neuronal increase that takes place in the granular layer during postnatal life. Cells with chromatin clumps are very scarce; ultrastructurally they resemble immature reptilian astroglia cells.

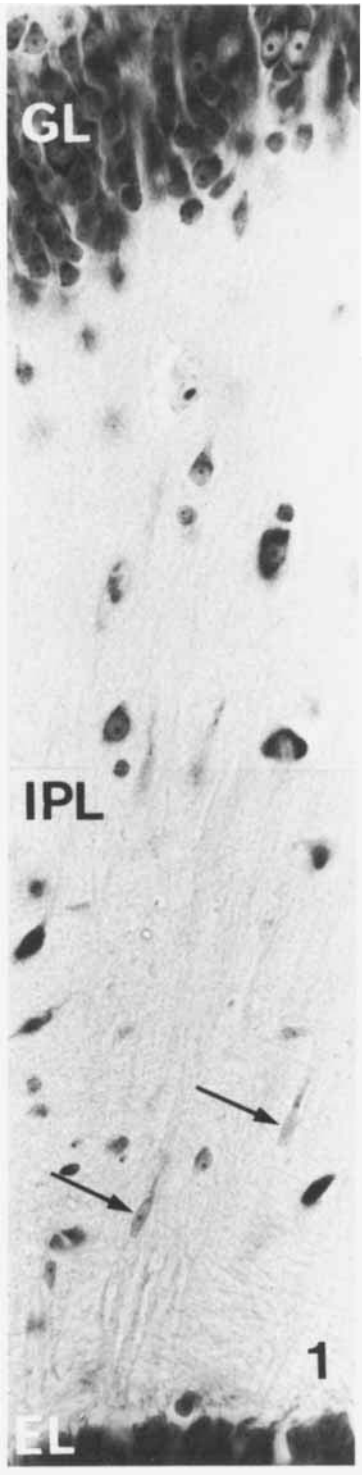
The cerebral cortex of squamate reptiles is composed of four cortical regions: medial cortex, dorsomedial cortex, dorsal cortex, and lateral cortex. Following topographical criteria, the medial cortex has been subdivided into three portions: ventral portion (M1-1), medial portion (M1-2), and dorsal portion (M1-3) (Platel, '69; Platel and Bauchot, '70; Martin-Trujillo, '76), each one presenting, from the glial surface to the ependymal layer, three basic strata: the outer plexiform layer (OPL), the granular layer (GL), and the inner plexiform layer (IPL). In *Lacerta galloti*, the M1-3 portion of the inner plexiform layer is divided from the granular to the ependymal layers into three layers: dendritic layer, profound layer, and alveus layer (Garcia-Verdugo et al., '84).

In *Lacerta*, the ependymal zone facing the dorsal portion (M1-3) of the medial cortex has been described as a proliferative center and called the *dorsal germinative zone* (Kirsche, '74; Hetzel, '77). It presents a pseudostratified aspect and has some ultrastructural characteristics different from those of other cerebral cortex ependymal zones (Garcia-Verdugo et al., '81).

Since neurons are generated only in proliferative areas, they usually have to undergo a migration. Neuronal migration is a well-established process, and, when neuronal increase takes place during development, migrating cells have been described in several areas of the mammalian brain, such as olfactory bulbs (Hinds, '68), cortex (Rakic, '71a), and cerebellum (Rakic, '71b). In the monkey cerebral cortex, migrating cells appear tightly associated with glial extensions, which would serve them as a guide on their way through the neuropil (Rakic, '71a).

Recent studies have shown a neuronal increase in some areas of the medial cortex of the lizard *Podarcis hispanica* (formerly *Lacerta hispanica*) from neonatal to adult stages (Lopez-Garcia et al., '84); thus, the possibility of a migratory process of cells coming from the ependymal germinative zone to the granular strata can be hypothesized in this species. Cells with an immature aspect directed toward the granular layer have already been described in the M1-3 portion of the inner plexiform layer of *Lacerta galloti* (Garcia-Verdugo et al., '84), a species in which an increase in the number of cortical neuronal cells after the postnatal period of life is also expected (Felip, '76; Martin-Trujillo, '76).

The aims of this work are to characterize the fine structure of these immature putative migrating cells found in the lizard cerebral cortex, especially in M1-3 levels during postnatal life, and to describe their relationship with the surrounding neuropil.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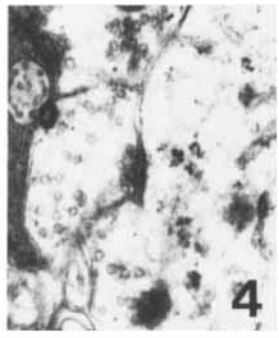
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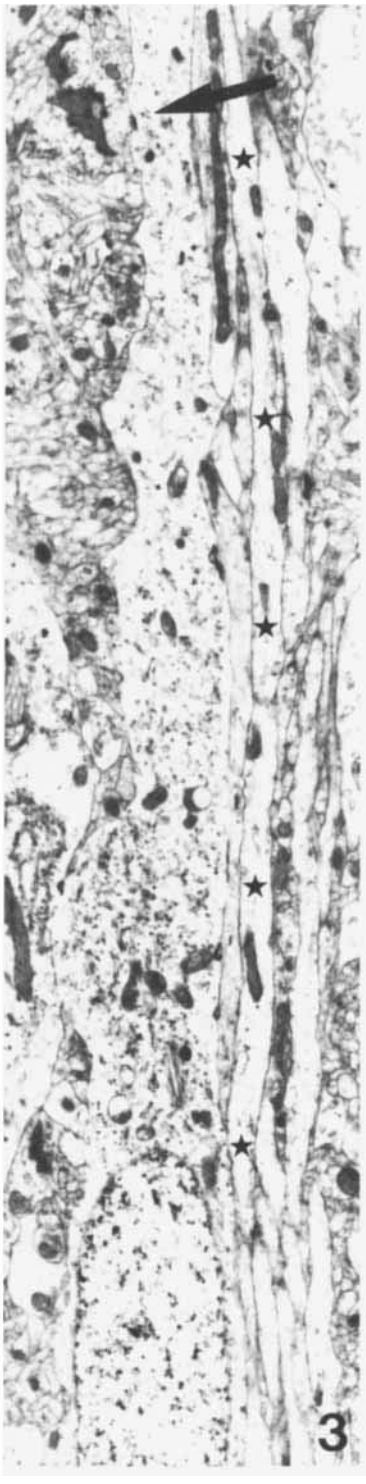
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MATERIALS AND METHODS

For this work, 20 adult specimens of *Lacerta galloti* (head–cloaca size ranging from 8 to 12 cm) from the Canary Islands were used. The animals were sacrificed immediately after being captured in their natural habitat. After being anesthetized with ether, the lizards were perfused transcardially with 4% glutaraldehyde and 3% paraformaldehyde in 5% phosphate buffer (0.13 M, pH 7.3). The brains were removed and kept in the same fixative solution for 4 hours. Fixed brains were then cut into 0.2-mm-thick transverse slices, which were postfixed with 2% OsO_4 in the above-mentioned buffer, dehydrated with acetone, stained in block with 2% uranyl acetate, and embedded in Araldite.

For topographical controls, thick sections (1 μm) were cut with an LKB-III ultratome and stained with 0.5% toluidine blue in 0.5% borax. After trimming each block to include only the M1-3 portion to detect cells interpreted as "migratory," ultrathin sections were obtained, stained with lead citrate, and examined with a Hitachi-12A electron microscope.

Up to 36 different "migratory" cells were studied at the electron microscopic (EM) level. Four "migratory" cells with spongy chromatin located near the ependyma and another four of the same type located near the granular stratum were selected for comparison of their chromatin particle size; a straight line traversing the nucleus in each micrograph was drawn, and the size of the segments superposed on chromatin fragments was measured.

In addition, some brains were processed by the Golgi-Colonnier procedure (Colonnier '64). Fixation was carried out by perfusion with a fixative of 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). After Golgi impregnation, brains were encapsulated in paraffin, sectioned into 200-μm-thick transverse sections, and mounted in Araldite between two coverslips.

For light microscope study, five brains were embedded in paraffin, sectioned at 10 μm, and Nissl stained with cresyl violet or toluidine blue.

RESULTS

Electron microscopic examination of the medial cortex inner plexiform layer of adult *Lacerta galloti* lizards revealed the existence of some elongated immature cells, identified on the basis of chromatin aspect, small nucleolus, polyribosomes, few cytoplasmic organules, and so forth. These were oriented perpendicularly to the ventricular surface and frequently arranged along bundles formed by radial glia and unmyelinated axons. Moreover, few well-differentiated neuronal somata, oligodendroglia, and microglia cells were detected.

These elongated immature cells can be seen in Nissl-stained sections (Fig. 1). Cell counts in series of sections revealed no more than 1,000 immature, apparently migratory cells per hemisphere of adult specimens; they were especially abundant at caudal levels where the medial cortex shows its greatest thickness (600 μm) and where the inner plexiform layer measures 400 μm.

Using conventional electron microscopy, we distinguished two types of these elongated immature cells based on their different nuclear appearances in the same preparations: type A, elongated immature cells with finely dispersed chromatin (95%); and type B, elongated immature cells with large chromatin clumps (5%). Besides this difference in chromatin dispersion, type A cells differ from type B cells in their cytoplasmic appearance and organelle population.

In portion M1-3, elongated immature cells were seen at different levels of depth in the inner plexiform layer. They were oriented perpendicularly to the dorsal germinative zone, and most of their outline contacted unmyelinated axons and ependymoglial extensions that were also arranged perpendicularly to the ependymal surface. In this M1-3 inner plexiform layer the glial network had a distinctive appearance in Golgi impregnations, namely, some segments of ependymoglial extensions did not show the char-

Fig. 1. Transverse paraffin section (10 μm thick) of the cerebral cortex of *Lacerta galloti* showing some migrating cells (arrows) in the inner plexiform layer (IPL). GL, granular layer; EL, ependymal layer. ×400.

Fig. 2. Golgi-impregnated ependymocytic shaft in the inner plexiform layer of the M1-3 portion of the medial cortex. The upper segment appears smooth, although it has not reached the granular layer. ×1,230.

Fig. 3. Electron micrograph of type A cell (putative migrating neuron) in the inner plexiform layer close to a vertical bundle of interlaced unmyelinated axons (stars). The arrow shows a synaptic contact on its surface. ×8,200.

Fig. 4. Detail of the synaptic contact in Figure 3. ×25,000.

Fig. 5. Type A cell (putative migrating neuron) in the inner plexiform layer close to the alveus layer, showing dispersed chromatin, scarce cytoplasmic organelles, and a centriole in an apical position (arrow). Some ependymoglial extensions with electron-dense corpuscles (arrowheads) can also be seen. ×10,500.

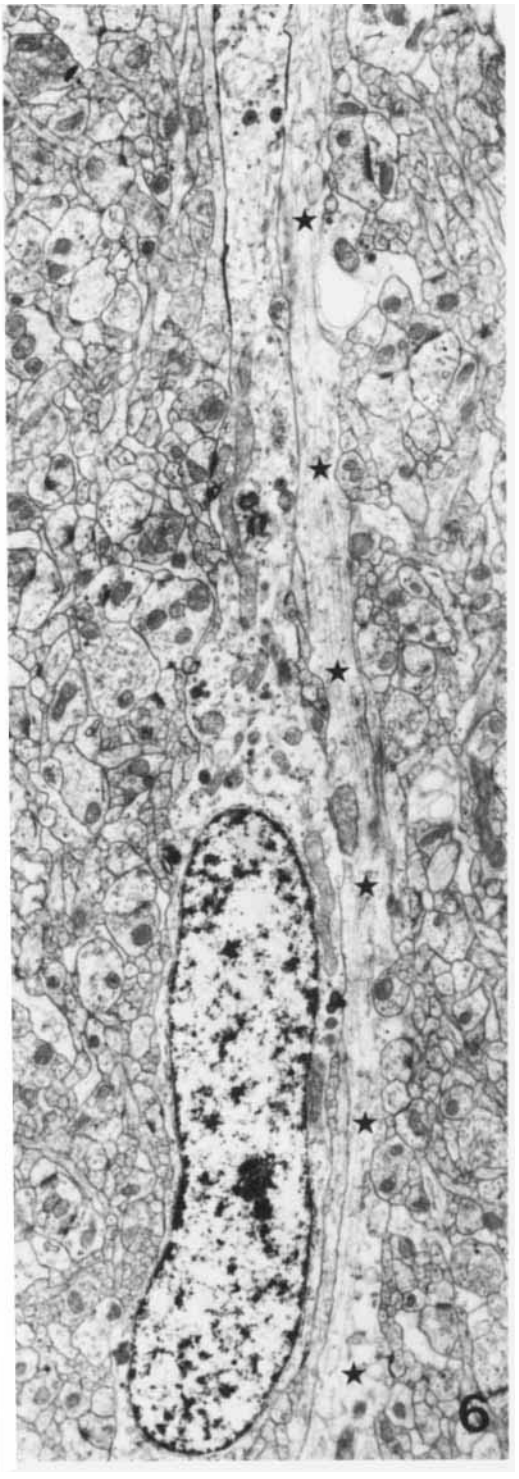

Fig. 6. Type B cell in the inner plexiform layer, closely attached to a vertical ependymocytic extension (radial glia) (stars). Chromatin is clumped, and cytoplasmic organelles, including elongated mitochondria, are more abundant than in type A cells. ×8,200.

acteristic varicosity of such cell processes but instead a rather smooth appearance (Fig. 2). When viewed by electron microscopy, ependymoglial extensions presented a slightly irregular outline with some very short lateral prolongations. Their fine structure revealed some microtubules, microfilaments, small cisternae of smooth endoplasmic reticulum, and vacuoles. The presence of characteristic clear vacuoles with an electron-dense corpuscle may also be noted. Unmyelinated axon bundles were closely associated with these elongated immature cells (Fig. 3).

Elongated, type A immature cells with spongy chromatin (Fig. 5) were always located in the dendritic and the profound layers of the medial and dorsomedial cortices, especially at caudal levels, whereas type B cells with chromatin clumps (Fig. 6) were widely distributed throughout all the cortical areas, both in the inner and outer plexiform layers. No intermediate forms between cell types A and B were observed in the electron microscope.

Type A cells (with spongy chromatin) measured from 3 to 4 μm in diameter at the nuclear level. The nucleus was elongated and had a central nucleolus. The chromatin was spongy and homogeneously distributed throughout the nucleus. The cytoplasm was clear and preferentially occupied the apical part of the cell. Small associations of polyribosomes (four to 10 ribosomes each) were observed throughout the cytoplasm. Mitochondria varied from spherical to very elongated shapes. Some dictyosomes were located in the apical cytoplasmic portion, very close to the nucleus. The number of saccules per dictyosome varied from four to seven and were arranged in the direction of the apical cytoplasmic sprout. A population of vesicles was frequently observed, mostly composed of coated vesicles that surrounded the saccules or contacted them. Vacuoles, distributed throughout the cytoplasm, and cisternae of smooth endoplasmic reticulum were numerous. Some dense bodies, lipofuscin, microtubules and microfilaments, and, frequently, one or two centrioles far from the nucleus could also be detected. Synaptic contacts on some migrating cells were observed; these synaptic contacts were asymmetric, and the presynaptic components contained spherical vesicles 45 nm in diameter (Fig. 4).

Type B cells (with large chromatin clumps) measured from 2 to 3 μm in diameter at the nuclear level; they also appeared to be associated with ependymal processes and unmyelinated axons. Their nucleus, located at the basal end of the soma, was elongated and sometimes presented some invaginations. Chromatin formed clumps that were both distributed throughout the nucleus and adherent to the inner side of the nuclear membrane, forming a dense chromatin envelope complex 100–110 nm thick. A centric nucleolus was also observed. The cytoplasm was electron lucent, and most organelles were located in the apical part of the soma. There were polyribosomes, numerous mitochondria, dictyosomes near the nucleus, vacuoles, cisternae of smooth endoplasmic reticulum, some cisternae of rough endoplasmic reticulum, numerous dense bodies, and, occasionally, a pair of centrioles. Synaptic contacts on these cells have never been detected. Cells resembling these immature cells with large chromatin clumps, except for the elongated shape, were observed in the outer and inner plexiform layers of all the cortical areas either as free cells or associated, as satellites, with neurons around blood vessels or just under the superficial limiting glial membrane. No kind of morphological gradient in distribution of chromatin or abundance of organelles could be observed among type B cells wherever they were located, i.e., either in inner or outer plexiform layers.

Type A cells (spongy chromatin) displayed subtle morphological differences depending on their location with respect to the ependymal layer and the granular stratum. In the alveus layer, close to the ependymal cells (Fig. 7), type A cells had a larger cell body than elsewhere, and their nuclei were subspherical and irregular in shape with abundant dispersed chromatin (50 $\pm$ 9 mean particle profile size). Near the granular stratum (Fig. 8) the nucleus was located in the basal pole of the cell soma; the chromatin showed small chromatin clumps (110 $\pm$ 30 mean particle profile size), suggesting a stage of growth. The surface to volume ratio of chromatin in sections of nuclei decreased from 60–70% when they were located near the ependyma to 40–50% when located near the granular stratum. This disparity in size of chromatin particles is correlated with the nuclear volume increase of 24–35% that occurs during cell migration. Frequent nuclear membrane invaginations were seen in somata located near the granular stratum. Concurrent with this nuclear development along the path of cell migration there was an

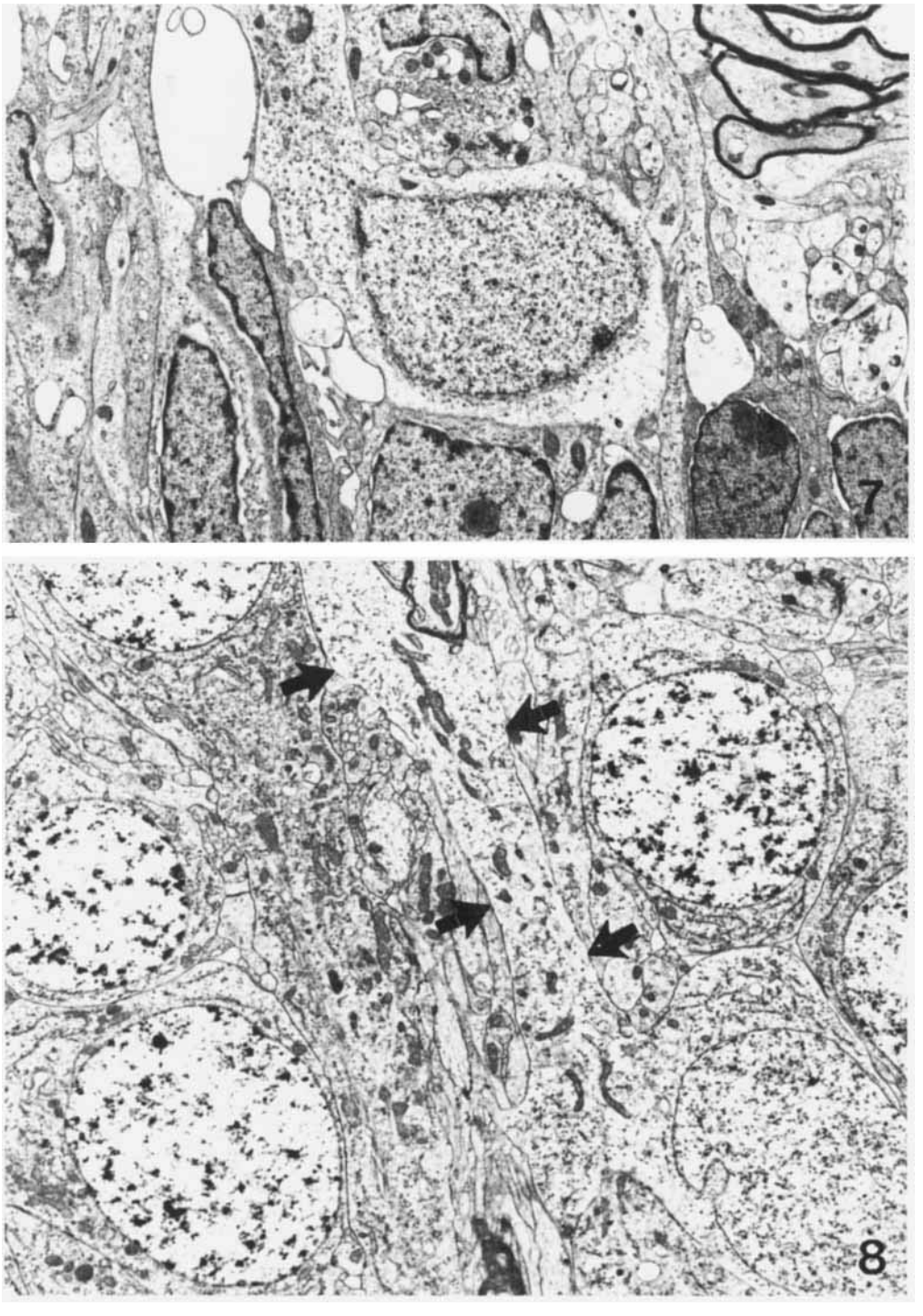

Fig. 7. Type A cell (center of micrograph) located at the level of the dorsal germinative zone of the ependymal layer (along bottom of micrograph). ×8,300.

Fig. 8. Type A cell (chromatin dispersed) located in the granular layer of the medial cortex. A long apical extension (arrows) can be seen protruding between granular neuronal somata (chromatin clumped). ×5,700.

unmistakable increase in granular endoplasmic reticulum (ER) membranes. Near the ependyma there were only one or two profiles of the ER per soma profile, whereas near the granular stratum there were six to 11 per soma profile. The number of Golgi dictyosomes also increased from one dictyosome per soma profile near the ependyma to two or three dictyosomes per soma profile near the granular stratum.

In type A cells located near the granular stratum, a thick apical cytoplasmic prolongation protruded from the apical pole of the soma and extended outward into the granular stratum; sometimes short, thick branches ramify from this apical cytoplasmic extension.

DISCUSSION

In spite of the numerous previously published Golgi studies on the subject of the adult lizard cortex, elongated immature cells have never before been identified by light microscopy, possibly because of their low number in the reptilian adult cortex or because of inferiority in preparative methods used. However, we have clearly observed such cells in paraffin or semithin resin sections.

By electron microscopy, we have identified elongated immature cells, designated *type A* cells, which have spongy chromatin and show immature cytoplasmic characteristics. They differ from all the neuronal somata described for *Lacerta galloti* medial cortex (Garcia-Verdugo et al., '84). Type A cells are small and have few cytoplasmic organelles. The presence of centrioles indicates the growth potential of these cells, since centrioles are involved in microtubule synthesis (Gonatas and Robbins, '65; Tennyson, '65). In general, the ultrastructure of type A cells resembles that of young migrating cells described in the monkey during the development of the neocortex (Rakic, '72, '78), cerebellar cortex (Rakic, '71b), and the last stages of prenatal hippocampal development (Nowakowski and Rakic, '79). These studies on the monkey have also described the association of migrating cells to glial extensions and bundles of unmyelinated axons.

In lizards, ependymoglial prolongations are responsible for almost the entire glial network, the covering of blood vessels, and the formation of the superficial limiting glial membrane (Garcia-Verdugo et al., '81). Recently, close associations between migrating cells and radial glia have been described in several embryonic stages of the reptilian cerebral cortex during development (Goffinet, '83). Since glial extensions act as guides, as demonstrated in monkeys by Rakic ('72, '78, '81), it seems even more reasonable that elongated immature cells should migrate along preformed paths in adult lizards, whose cerebral cortex is already organized. In relation to this supposition it is interesting to note that the ependymoglial extensions in M1-3 posterior levels have rather smooth profiles, which might facilitate the directed movement of the elongated immature (putative migrating) cells during their migration.

Since some of these type A elongated immature cells reach the base of the granular layer of the medial cortex, they could be responsible, after completion of their presumably migratory pathway, for the great numerical increase that this area experiences during postnatal life (Lopez-Garcia et al., '84). An "outside–in" sequence of cellular increase similar to that in the fascia dentata of mouse, rat, and monkey (Angevine, '65; Schlessinger et al., '75; Stanfield and Cowan, '79) could be assumed for this area. In effect, the medial cortex of *Lacerta galloti* seems to be homologous to the mammalian fascia dentata (Garcia-Verdugo et al., '84). An "outside–in" sequence for the cerebral cortex histogenesis of some reptiles has also been suggested (Goffinet, '83). Tritiated thymidine autoradiographic studies in a related species of lizard (*Podarcis hispanica*) have shown that in animals with short survival time after dosage almost all labeled cells were located in the ependyma. Longer survival time (8 to 15 days) resulted in labeling of many elongated and vertical cells of the inner plexiform layer of the medial cortex. After survival times of longer than 1 month, most of the labeled cells were located in the granular stratum of the medial cortex (Lopez-Garcia et al., manuscript in preparation).

The so-called dorsal germinative zone has been described as a proliferative area in *Lacerta agilis* (Kirsche, '74). It seems to be homologous to the subventricular zone of dogs (Blakemore and Jolly, '72) and rats (Blakemore, '69). In the *Lacerta galloti* ependymal layer, this zone presents a pseudostratified cellular packing of ependymal cells with a dense matrix (Garcia-Verdugo et al., '81). Furthermore, ectopic ependymocytes and immature glial cells have been found close to the ependymal layer (Garcia-Verdugo, '80). Elongated immature cells with spongy chromatin can be detected in this zone. In con-

trast, the ependymal cells have clumps of chromatin sticking to the nuclear membrane, a dilated perinuclear cisterna, and some characteristic vacuoles with small electron-dense corpuscles that serve for identification of ependymal cells (Garcia-Verdugo et al., '81).

The progressive increase in nuclear density and number of organelles of elongated immature cells with spongy chromatin as they travel from the ependymal layer to the granular may be considered the result of a progressive maturation, similar to that during monkey hippocampal development (Nowakowski and Rakic, '79).

It is difficult to interpret the presence of synaptic contacts on some of these elongated immature cells. Some synapses on migrating cells that have not reached their final position have been previously described in the monkey hippocampus (Nowakowski and Rakic, '79). In our material, though, elongated immature cells that receive synaptic contacts could also have finished their migration at that point of the inner plexiform layer despite maintaining an immature aspect, and they would then be responsible for the numerical increase of thc scattered neuronal population already described for this layer in *Lacerta* (Lopez-Garcia et al., '84).

Several facts, such as fine structure and association to radial glia, liken type A migrating cells with spongy chromatin to other migrating cells described in the cerebral areas of other animals. Recently described neuronal increases in the medial and dorsomedial cortices of a lizard (Lopez-Garcia et al., '84) could be explained by the arrival of these cells from the ependymal layer. Furthermore, the continuous postnatal growth of the Timm-positive system of synapses of the cerebral cortex of lizards, whose axons come from the medial cortex (Molowny and Lopez-Garcia, '78; Molowny, '80; Lopez-Garcia et al., '83), can be explained in part by the arrival of these cells at the granular stratum of the medial cortex and the expected growth of their new axons.

Elongated cells with large chromatin clumps, designated *type B* cells, show ultrastructural characteristics similar to those of cells described previously as immature astroglial cells in this species (Garcia-Verdugo, '80). Thus, these cells could be responsible for the progressive increase that the initially scarce glial population undergoes during postnatal life.

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