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Invited Review

Lesion and regeneration in the medial cerebral cortex of lizards

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Contents

Summary. The cerebral cortex of Squamate reptiles (lizards and snakes) may be regarded as an archicortex or «reptilian hippocampus». In lizards, one cortical area, the medial cortex, may be considered as a true «fascia dentata» on grounds of its anatomy, connectivity and cyto- chemo-architectonics of its main zinc-rich axonal projection. Moreover, its late ontogenesis and postnatal development support this view. In normal conditions, it shows delayed postnatal neurogenesis and growth during the lizard's life span. Remnant neuroblasts in the medial cortical ependyma of adult lizards seasonally proliferate. The late-produced immature neurocytes migrate to the medial cortex cell layer where they differentiate and give off zinc-containing axons directed to the rest of cortical areas. This results in a continuous growth of the medial cortex and its zinc-rich axonal projection.

Perhaps the most important characteristic of the lizard medial cortex is that it can regenerate after having been almost completely destroyed. Recent experiments in our laboratory have shown that chemical lesion of its neurons (up to 95%) results in a cascade of events; first, those related with massive neuronal death and axonal-dendritic retraction and, secondly, those related with a triggered neuroblast proliferation and subsequent neo-histogenesis, and the regeneration of an almost new medial cortex that shows itself undistinguishable from a normal undamaged one.

This is the only report to our knowledge that an amniote central nervous centre may regenerate by new neuron production and neo-histogenesis. Perhaps the medial cortex of lizards may be used as a model for neuronal regeneration and/or transplant experiments in mammals or even in primates.

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The cerebral cortex of reptiles

The cerebral cortex of reptiles is formed by three main areas, the medial (MC), the dorsal (DC) and the lateral (LC) cortices. Moreover, in Squamata reptiles (lizards and snakes) there is a fourth cortical area, the dorsomedial cortex (DMC) first described as «zona piramidal curvilinea» by P. Ramón y Cajal (Ramón y Cajal, 1917). In all these areas most neuronal somata are grouped forming a principal cell layer sandwiched by two almost cell-free inner and outer plexiform layers (Fig. 1a) where afferent connections terminate in a highly laminated fashion (Martínez-Guijarro et al., 1990).

Despite this apparently simple histological pattern resembling that of the mammalian hippocampus, the cerebral cortex of reptiles has a complex neuronal population as seen by Golgi impregnations (Fig. 1b) (Martínez-Guijarro et al., 1990) which is similar to that of the hippocampal system (Lorente de No, 1934; Ramón y Cajal, 1911). Projection neurons displaying spiny bipyramidal and «double bouquet» bitufted dendritic tree patterns predominate in the cell layers. Non-spiny or sparsely spinous interneurons with diverse morphology are mainly distributed in the plexiform layers. Interneurons display GABA-immunoreactivity (Schwerdtfeger and López García, 1986), and in addition, different calcium-binding proteins (Fig. 1c) (Martínez-Guijarro et al., 1991b; Martínez-Guijarro and Freund, 1992) or a variety of neuropeptide immunoreactivities (Pérez-Clausell, 1987; Davila et al.,

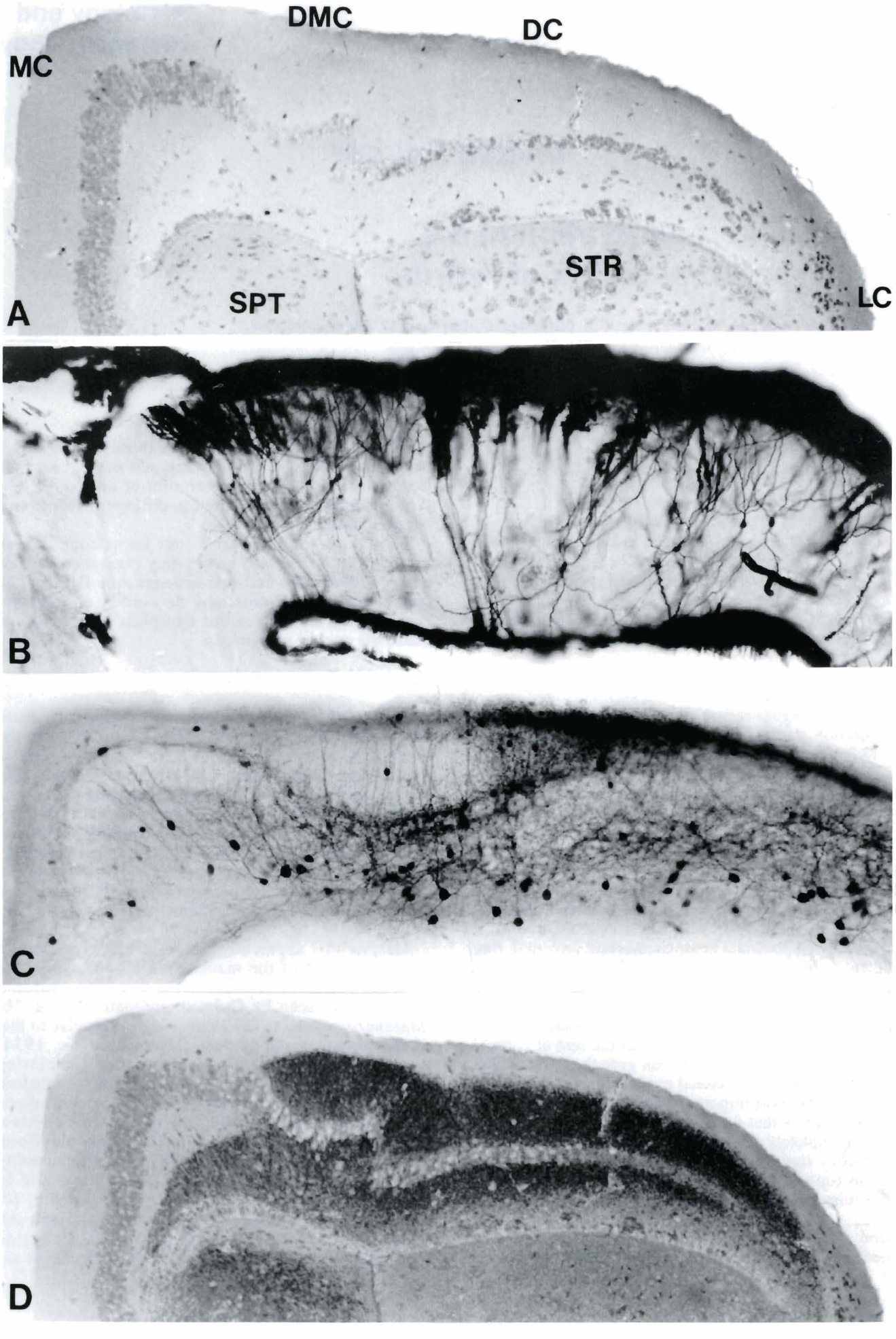
DMC
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Fig. 1. Aspect of the lizard cerebral cortex as seen in transverse sections at commissural level. **A.** Nissl staining. **B.** Golgi impregnation. **C.** Immunostained with an antibody against Parvalbumin. **D.** Zinc staining using the neo-Timm method. (DC - dorsal cortex; DMC - dorsomedial cortex; LC - lateral cortex; MC - medial cortex; SPT - septum; STR - striatum). x 100 all

1988, 199).

The intra- and extracortical connectivity of the reptilian cortex (Lohman and Van Woerden-Verkley, 1976, 1978: Bruce and Butler, 1984; Olucha et al., 1988) closely resembles that of the mammalian hippocampus (Lubbers and Frotscher, 1987; Swanson et al., 1987). The lizard medial cortex has been homologized to the mammalian fascia dentata (Molowny and López-García, 1978; López-García et al., 1983a,b, 1988c; López-García and Martínez-Guijarro, 1988). The lizard dorsomedial cortex may represent the CA3 hippocampal area (Martínez-Guijarro et al., 1984). The dorsal cortex, which is further subdivided into three subareas (Molowny et al., 1972) may represent the rest of mammalian hippocampal areas (i.e., CA2 and CA1 regions). On the other hand, the lateral cortex of lizards may be homologous to the mammalian olfactory cortex (Hoogland and Vermeulen-Van der Zee, 1988).

Cytoarchitecture of the lizard medial cortex

The lizard medial cortex or «lizard fascia dentata» occupies an interhemispheric-caudal position in the lizard telencephalon. Its cytoarchitectonic pattern is conspicuous, with a well-developed cell layer in which neuronal somata are densely packed.

The medial cortex receives axonal projections from the rest of the cortical areas and emits a recurrent axonal projection directed to the dorsal and dorsomedial cortices as well as to the dorsal septum. This axonal projection is intensely Timm-positive (Fig. 1d) due to the zinc accumulated within synaptic vesicles of its presynaptic boutons (Fig. 2). (López-García et al., 1983b; Martínez-Guijarro et al., 1987; López-García and Martínez-Guijarro, 1988). This zinc-positive axonal projection terminates in dendritic spines of bipyramidal neurons (Martínez-Guijarro et al., 1984) of the dorsomedial and the dorsal cortices.

Concurrence of zinc staining and glutamate-immunoreactivity (Fig. 2) (Martínez-Guijarro et al., 1991a) in lizard zinc-positive boutons and those of the hippocampal mossy fibres of mammals (Ottersen and Storm-Mathisen, 1985; Cotman et al., 1987; Liu et al., 1989) adds further support to the presumed homology of both systems; i.e., the lizard zinc-positive bouton system and the hippocampal mossy fibre system of mammals (Molowny and López-García, 1978; López-García et al., 1983b; Martínez-Guijarro et al., 1984).

The lizard medial cortex shows laminar organization (Fig. 3) with the following elements: a) the outer glial-limiting membrane; b) the outer plexiform layer (molecular layer); c) the principal cell layer (granular layer); d) the inner plexiform layer; e) the deep Timm-reactive layer (hilus); f) the myelinated fibre layer (alveus); and g) the ependymal layer.

Glial-limiting membrane

The glial-limiting membrane is the borderline of cortical parenchyma; it is formed by the endfoots of ependymoglial cells whose somata are located in the ependymal layer.

The outer plexiform layer

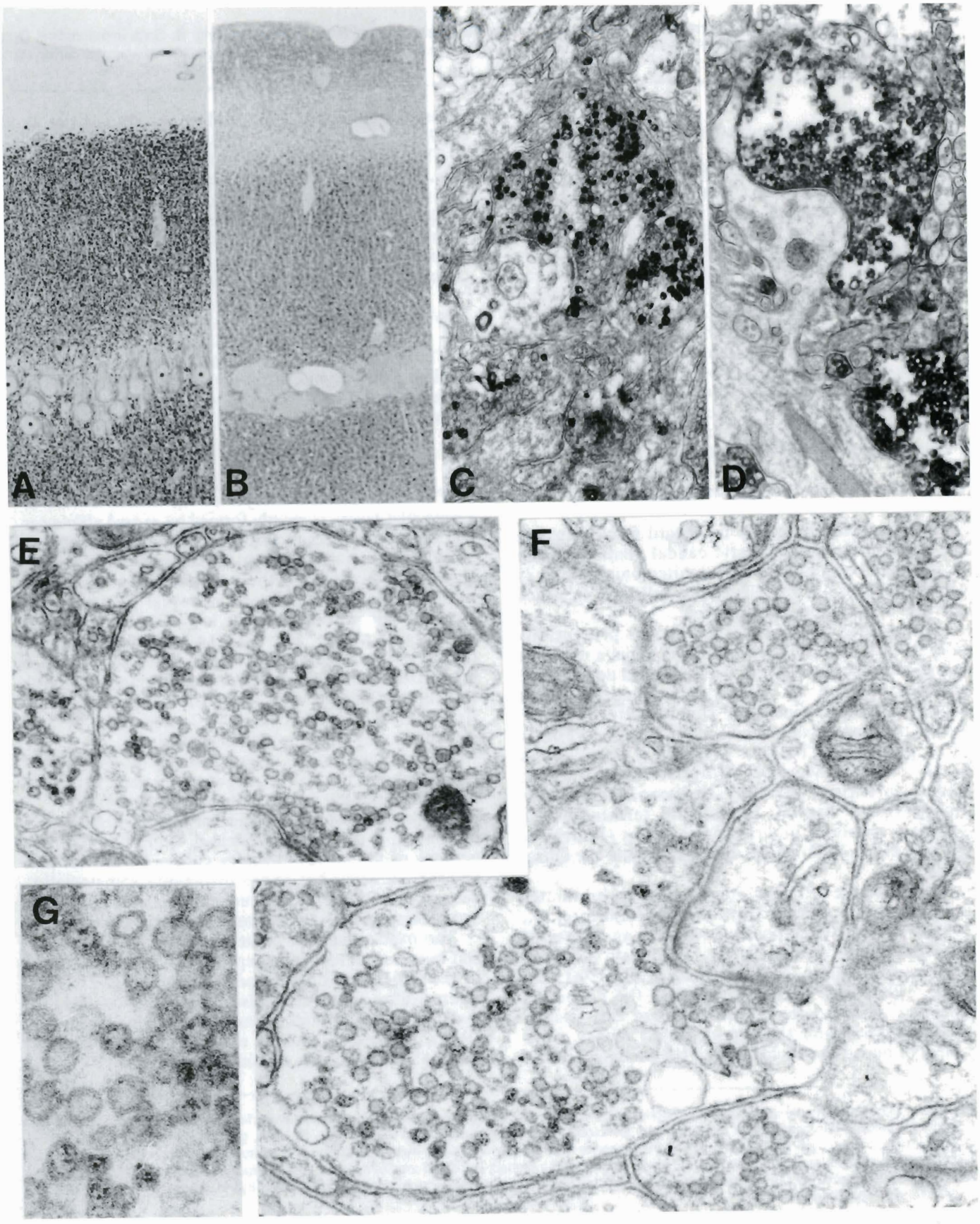
The outer plexiform layer of the medial cortex or «molecular layer» is mainly formed by axonal afferences and apical dendrites of principal neurons. Moreover, a scarce population of dispersed neurons exists in this layer. Classified by their dendritic and axonal morphologies, at least seven neuronal types may be clearly distinguished (Luis de la Iglesia et al., 1992), most of them are GABA immunoreactive (Schwerdtfeger and López-García, 1986) and/or either enkephalin-endorphin or parvalbumin immunoreactive (Martínez-Guijarro et al., 1991b). Their axons have irregular trajectories in this plexiform layer and sometimes they enter the granular cell layer.

Axonal inputs to the outer plexiform or «molecular» layer appear highly segregated in three distinct sublayers: external, intermediate and juxtagranular.

The *external sublayer*, adjacent to the glial-limiting membrane, is the projection field of axons originated in neurons of the lateral olfactory cortex. Moreover, this external sublayer has axons displaying somatostatin, neuropeptide Y, serotonin, enkephalin-endorphin-like and cholecystokinin (CCK) immunoreactivities (Fig. 3).

The *middle sublayer* is the projection field of axons and boutons coming from bipyramidal neurons located in the ipsilateral dorsal cortex (subregio D2). This intermediate sublayer is also the projection field of thalamic axons (Hoogland and Vermeulen-Vanderzee, 1991). A dense plexus of axons displaying intense Achase activity may be seen in this sublamina.

Fig. 2. The zinc-rich glutamate-immunoreactive axonal boutons emitted by the medial cortex. **A.** Neo-Timm staining for zinc; outer plexiform layer of the dorsomedial cortex. Note heavy silver staining of the juxtasomatic zones of the plexiform layer. x 600. **B.** Idem, glutamate immunostaining. x 600. **C.** Zinc-positive bouton; neo-Timm staining. Note the presence of silver granules inside synaptic vesicles of a bouton synapsing on a dendritic spine. x 40,000. **D.** Glutamate-immunoreactive bouton. Idem, electrondense product is located around synaptic vesicle profiles. x 30,000. **E, F, and G.** Zinc-positive boutons labelled by the sulphide-osmium procedure. Electrondense zinc-osmium deposits are located inside synaptic vesicles. x 40,000, x 80,000 and x 150,000 respectively.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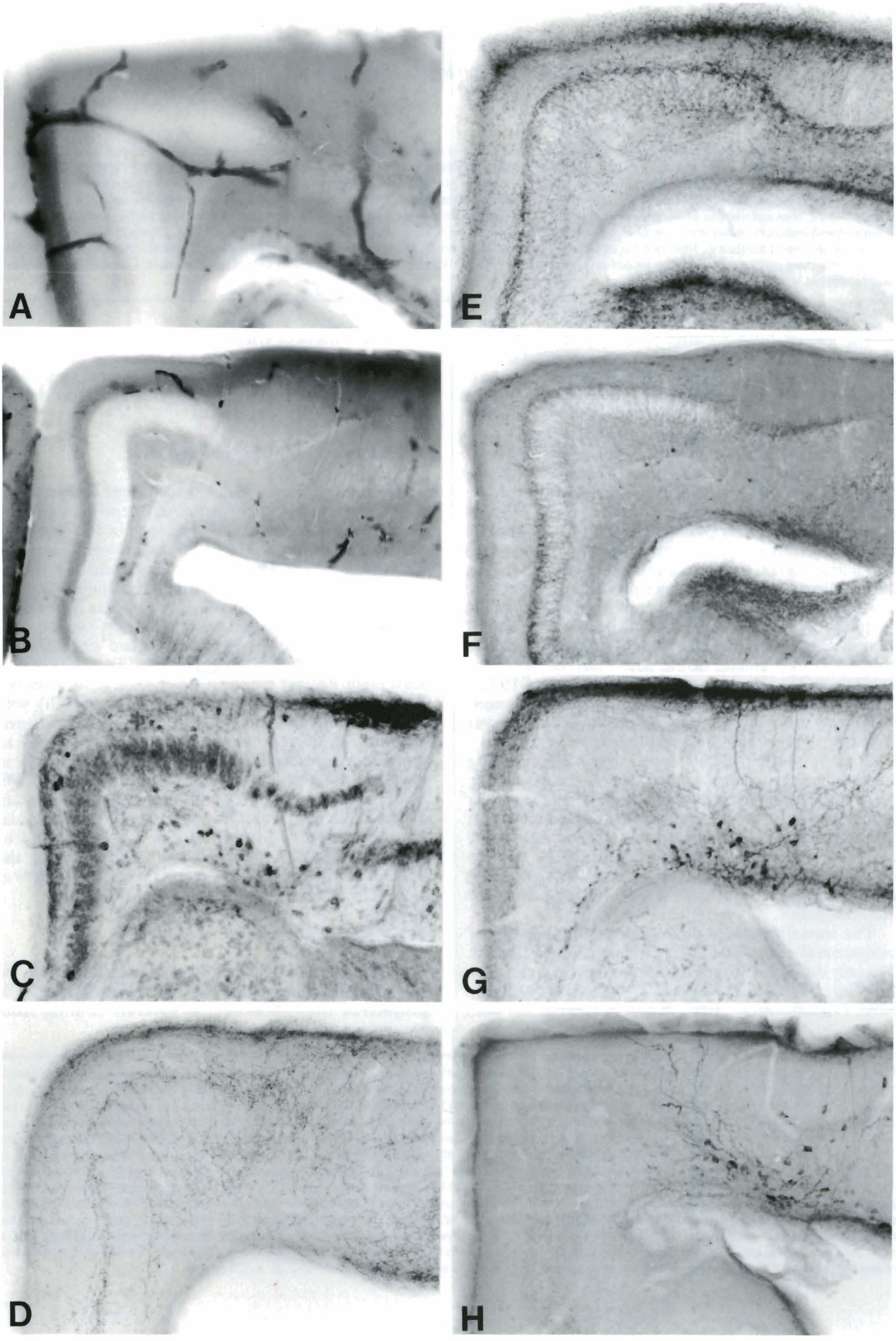
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Fig. 3. Lamination of the lizard medial cortex; distribution of intrinsic and extrinsic axonal incomings. Transverse sections at commissural level. **A.** Anterograde labelling after HRP injection in the lateral cortex. Note labelling in the subsurface lamina of the outer plexiform layer. **B.** Anterograde labelling after HRP injection in the dorsomedial cortex. Note the labelling of juxtasomatic lamina. **C.** Acetylcholinesterase staining. Note the labelling of the intermediate lamina in the outer plexiform layer. **D.** Serotonin immunostaining. Note denser plexuses of immunoreactive axons in juxtasomatic sides. **E.** Idem, cholecystokinine immunostaining. A thin sublamina close to the external side of the granular cell layer is specially developed. **F.** Idem, enkephaline-ß-endorphine-like immunostaining. Note the presence of immunoreactive somata in the inner and outer plexiform layers (arrows). **G.** Neuropeptide Y immunostaining. Note that the external sublayer is populated by an immunoreactive axonal plexus. Immunoreactive somata are located mainly in the inner plexiform layer of the medial and dorsomedial cortices. **H.** Idem, somatostatin immunostaining. The external axonal plexus appears weakly labelled. x 90 all approx.

The *juxtagranular sublayer* is the projection field of axonal collaterals of bipyramidal neurons located in both the ipsi and contralateral dorsomedial cortices. Moreover, the juxtasomatic side of this sublayer is also occupied by axons which display enkephalin-endorphin-like, serotonin and CCK immunoreactivities (Fig. 3).

Similarities of these three sublaminae with those of the mammalian fascia dentata are evident. In lizards, the lateral cortex (olfactory, paleocortex) send axons to the medial cortex (archicortex) until reaching the external sublayer of the outer plexiform layer of the medial cortex. Neurons of the medially-adjacent cortical area (the dorsal cortex, subregio D2) send axons that end in the middle sublayer of this outer plexiform layer. These two axonal projecting systems are comparable to the perforant system of mammals. In mammals, layer II neurons of the lateral entorhinal cortex give rise to perforant axons ending in the outer third of the fascia dentata molecular layer (Hjorth-Simonsen, 1972). Neurons in the medial entorhinal cortex send axons ending in the middle third of the molecular layer (Nafstad, 1967; Hjorth-Simonsen and Jeune, 1972).

This is better understood when considering embryonic development of the cerebral cortex. During early development, the mammalian cerebral cortex experiences folds which partially juxtapose archicortex and paleocortex, but they remain separated by a pial-glial borderline. The glial membrane is broken afterwards by axons coming from the entorhinal (paleo) cortex to the fascia dentata (archi) giving rise to the perforant path.

The inner/juxtaproximal sublayers are fields of commissural projections both in mammals and lizards. The only difference is that commissural projections issue from hilar neurons in mammals (Deller and Leranth, 1990; Frotscher et al., 1991) but from bipyramidal ones in lizards (Martínez-Guijarro and López-García, 1992).

It is noteworthy that in organotypic cultures of mammalian hippocampus, pyramidal neurons may send axons to the inner molecular layer of the fascia dentata (Frotscher and Ghwiler, 1988).

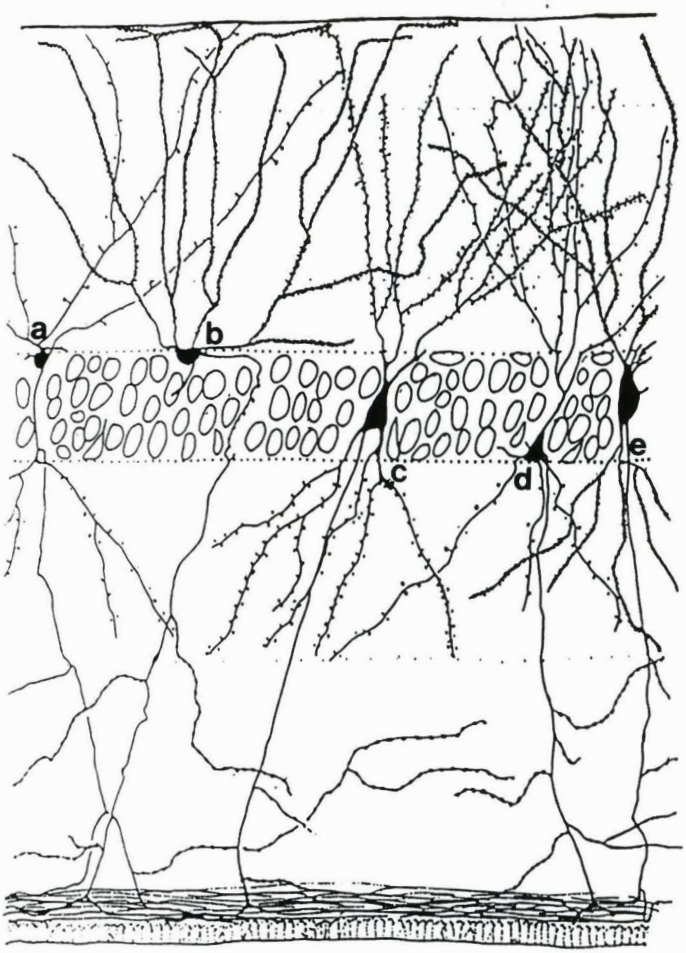
The principal cell layer or granular layer

The principal or «granular» cell layer is formed by the neuronal somata of principal neurons «granule neurons» that appear densely packed. In this layer, the number of substrata formed by somata rows increases throughout the life time of animals. Granular cell somata frequently form arrays or columns in which two sizes are clearly seen: large and small. Large somata have nuclei with dispersed chromatin. On the contrary, small somata have nuclei with clump condensed chromatine. Nevertheless, Golgi impregnations (Fig. 4) reveal at least eight distinct neuronal types. The most abundant are bitufted neurons (large, medium and small) with dendritic morphology comparable to those of the fascia dentata granule neurons of the mammalian hippocampus, especially in primates and lagomorpha in that they have basal dendrites (Buhl and Dann, 1990; Seress and Frotscher, 1990). Other types of neurons are called triangular, cup, fan, polygonal and pyramidal shaped. Some of them display GABA and/or parvalbumin immuno-reactivities specially along the borderlines of the granular stratum (Martínez-Guijarro et al., 1991b; Martínez-Guijarro and Freund, 1992).

The inner plexiform layer

Basal dendrites of principal granule neurons form the inner plexiform layer that is Timm-negative. This is the projection field of axonal collaterals coming from

Fig. 4. Drawings of the main neuronal types of the medial cortex. **A.** Principal neurons of the granular layer. Observe variability in some size (small, medium, large) and shape (a- round-granular; b- flattened; c- quadrangular; d- pyramidal; e- fusiform), as well as in dendritic tree patterns (coup-shaped in a; monopolar in b; bitufted in c, d, and e; fan-shaped, etc) and dendritic spine density (heavily spinous b, e; spinous c, d and sparsely spinous a). The closely-packed somata in the granular layer emit axons traversing the inner plexiform layer until they bifurcate in the alveus layer. When the axons reach the zinc-positive areas they give off beaded collaterals with thick boutons. **B.** Interneurons: dendritic trees. Most interneurons have their somata placed in the plexiform layers; their dendrites are smooth or studded with scarce spines of varicose appendages, except those of long-spined polymorphic neuron (m) (f- multipolar-star and «sarmentous» neurons; g- «couchant» neuron; h- «deep star» neuron; i- vertical fusiform neuron; j- horizontal fusiform neuron; k- pyriform and pyramidal-like neurons; l- large multipolar neuron; m- long-spined polymorphic neuron; n- vertical periventricular neuron; o- juxtaependymary horizontal neuron). **C.** Interneurons: axonal arborizations. Some axonal arborizations distribute in specific sublayers; i.e., those of long-spined polymorphic neurons (m) in the external sublayer of the outer plexiform; those of star-sarmentous neurons (f) in the middle sublayer; those of deep-star (h) neurons in the juxtagranular sublayer; those of pyramidal-like, big multipolar and polymorphic neurons (g, l, k, m) at both sides of the cell layer; and those of large multipolar (l) neurons in the inner plexiform layer. Observe that the deep Timm-reactive layer is free of any collateral axon from interneurons. D. Scheme summarizing inputs to every sublamina. All drawings x 500 approx.



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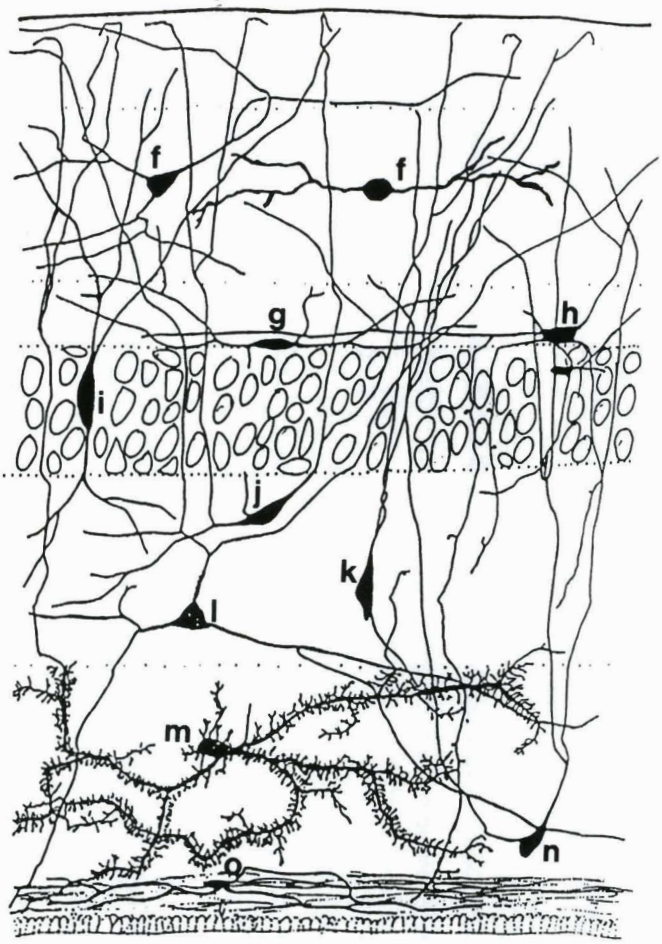

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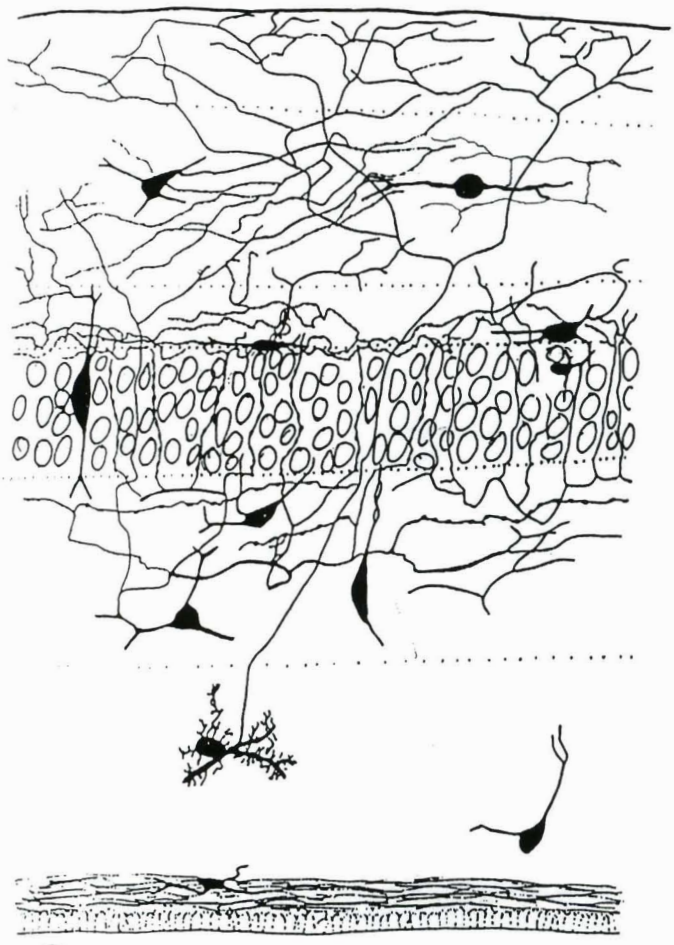

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LATERAL CORTEX
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DORSAL CORTEX
THALAMUS
Achase +

DORSOMEDIAL CORTEX
CCK, ßEND, 5OHT

DORSOMEDIAL CORTEX

ZINC+ PROJECTION

COMMISSURAL &
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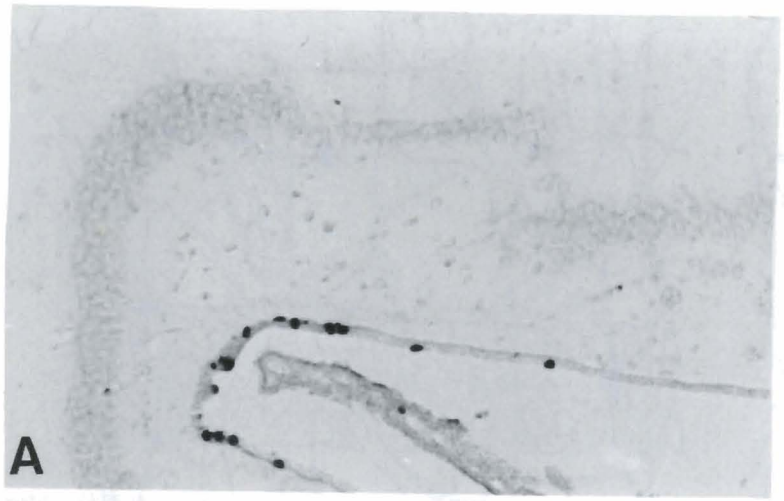
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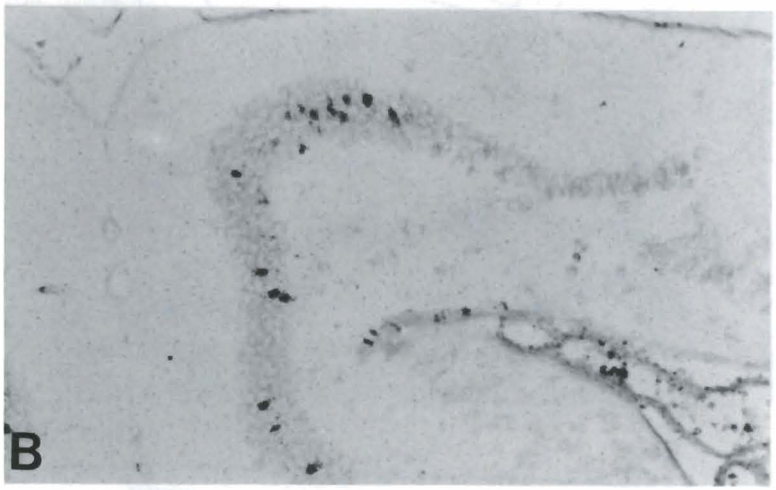
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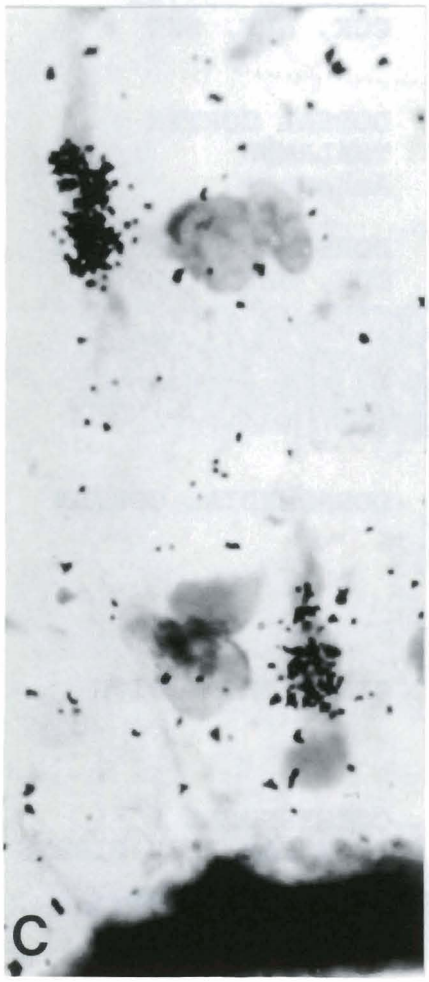
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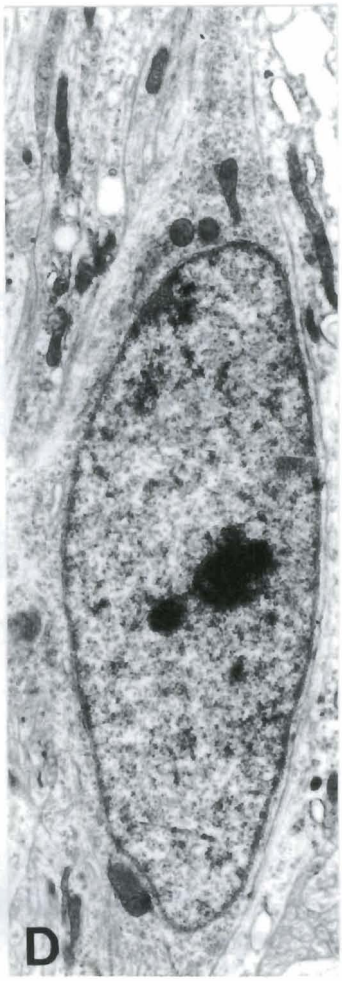
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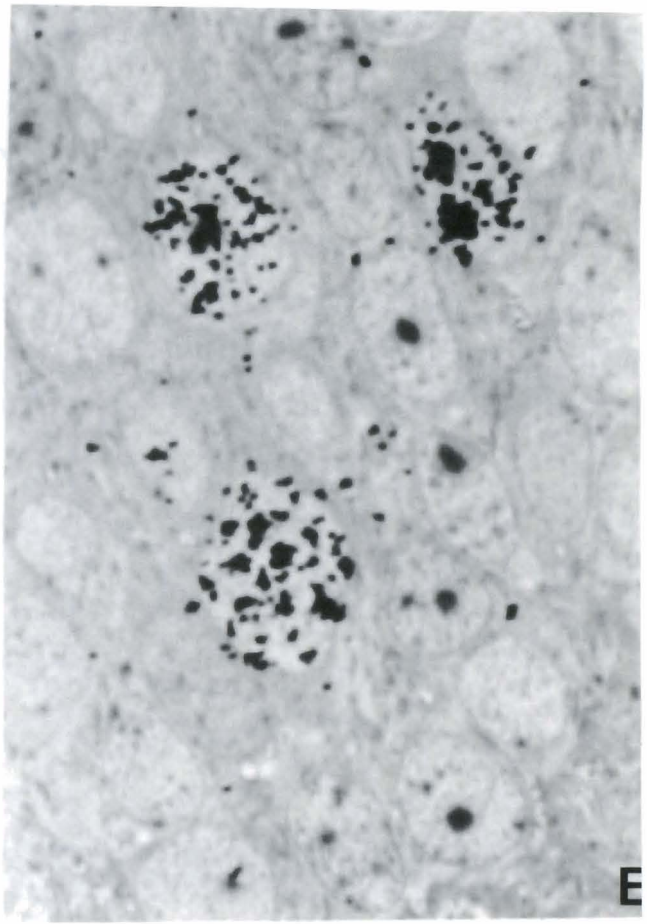
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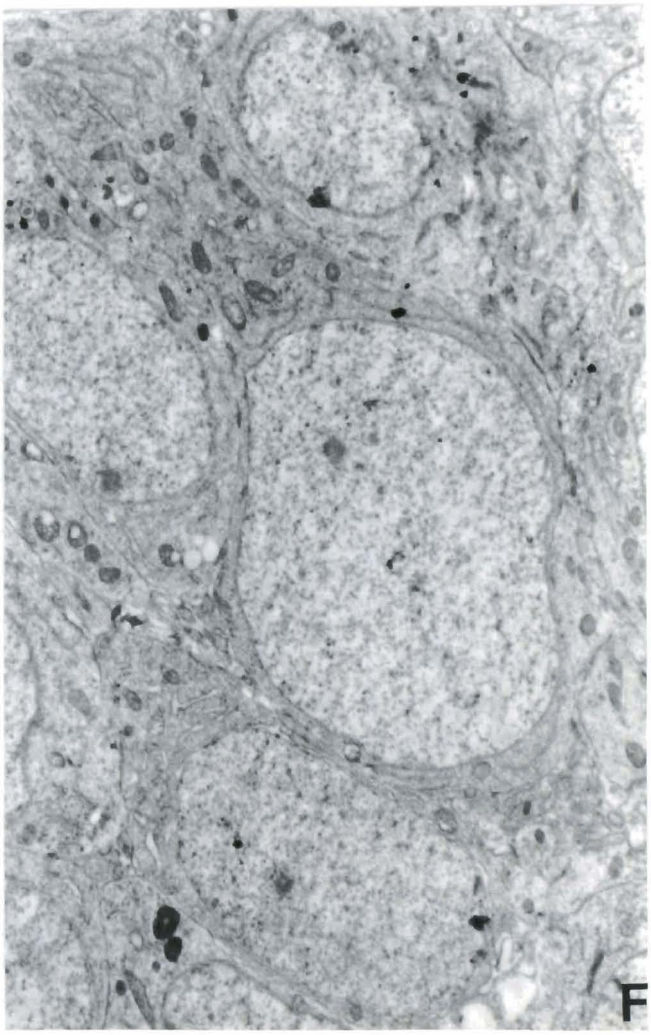
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Fig. 5. Postnatal neurogenesis shown by tritiated thymidine autoradiography. **A.** Autoradiogram of a transversal section; one day of survival after a tritiated thymidine pulse. Note that labelled cells are exclusively located in the ependyma. x 80. **B.** Idem, twenty-eight days of survival. Observe that the majority of labelled cells are located in the cell layer, but that a few of them still remain in the ependyma. x 80. **C.** Aspect of the inner plexiform layer; seven days of survival. Note the presence of heavily-labelled vertical fusiform somata belonging to migratory neurons. x 1,600. **C.** Aspect of the inner plexiform layer; seven days of survival. Note the presence of heavily-labelled vertical fusiform somata belonging to migratory neurons. x 1,600. **D.** Electron microscopic aspect of a migratory neuron. x 8,500. **E.** Aspect of the cell layer in a semithin section; one month of survival. Observe the presence of labelled nuclei from three granule neurons. x 2,600. **F.** Electron microscopic aspect of the labelled soma shown in E (middle of the picture). Observe that chromatin has disperse appearance, as in the rest of granule cell nuclei. x 6,000

bipyramidal neurons located in the ipsi- and the contralateral dorsomedial cortex.

Very rarely somata may be found in this layer; when found they are migratory immature neurons (García-Verdugo et al., 1986) or ectopic granule neurons that have not completed their migration to the principal granular layer. The latter case may be seen in caudal levels; they are frequently arranged in vertical rows which resemble a convoy. Moreover, scarce intrinsic interneurons of this sublayer are horizontal, fusiform and pyramid-like non-spinous neurons (Fig. 4) that resemble basket neurons of the fascia dentata (Seress and Pokorny, 1981; Ribak and Seress, 1983).

The deep Timm-reactive layer

The subjacent deep lamina is apparent because of its Timm-reactivity and neuropil structure, where zinc-containing boutons synapse on a scarce neuronal population (López-García et al., 1988a; Martínez-Guijarro et al., 1987). The neurons with somata located in this layer display highly heterogeneous morphology, as seen in Golgi impregnations (Fig. 4) (Berbel et al., 1987). Up to seven neuronal types may be distinguished: monopolar; horizontal bipolar; vertical bipolar and pyriform; multipolar; polymorphic and pyramidal-shaped neurons. Most of them are GABA-immunoreactive (Schwerdtfeger and López-García, 1986) and display a variety of neuropeptide immunoreactivities including somatostatin and neuropeptide Y (Pérez-Clausell, 1987; Davila et al., 1988, 1991). Based on its cyto-architechtonics, neuronal population, and Timm reactivity, this layer may be compared to the hippocampal hilus (López-García et al., 1988a).

Apart from the proper cell somata of this zone there are transient migratory immature somata that appear adhering to vertical glial shafts (García-Verdugo et al., 1986); they may appear at any level from the ependyma to the granular principal cell layer. Sometimes, vertical rows of ectopic granule neurons may be seen in the inner plexiform layer; they are specially abundant in caudal levels of the medial cortex.

The myelinated fibre layer or «alveus»

The innermost lamina contains abundant horizontal myelinated axons that connect left and right hemispheres with the septum, dorsolateral anterior thalamic nucleus, hypothalamus and raphe nuclei. It is comparable to the alveus layer of the mammalian hippocampus.

The ependymal layer

Subjacent to this lamina is the ependymal layer lining the lateral ventricles. Ependymocytes extend long fuzzy vertical cytoplasmatic shafts that reach the brain surface. They exhibit GFAP immunoreactivity; their tips form the outer glial membrane, and their fuzzy expansions become the glial envelope around all blood vessels and capillaries (García-Verdugo et al., 1981).

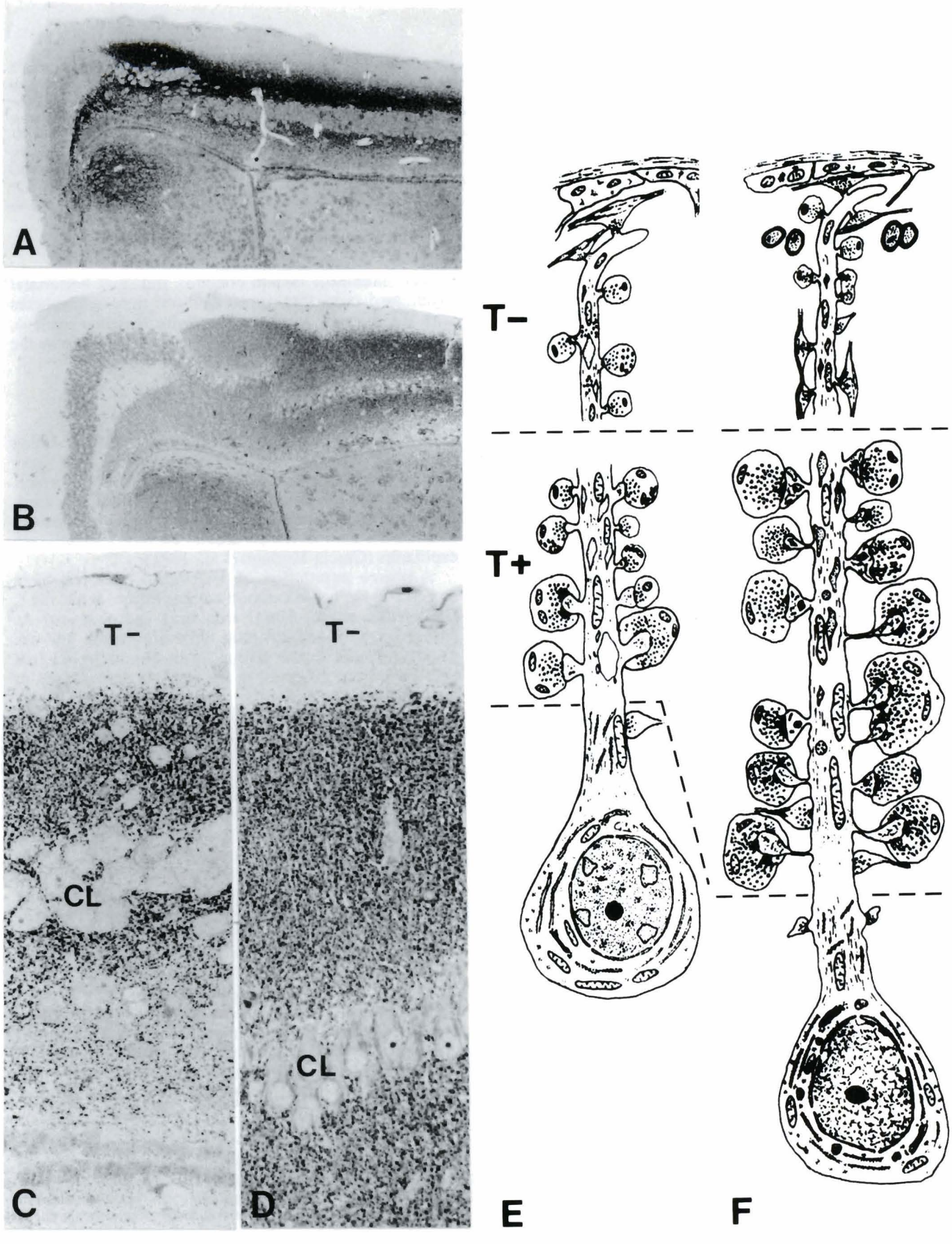
A scarce population of microglial cells has been detected in the lizard cerebral cortex (Berbel et al., 1981; Castellano et al., 1991) that may increase during pathology. Oligodendroglial cells are scarce and are usually located in the alveus layer. No astroglial free cells have been observed in the nervous parenchyma of the lizard cortex.

Postnatal neurogenesis in the medial cortex of lizards

In lower vertebrates (fish and amphibians), postnatal neurogenesis occurs in the retino-tectal system (Straznicky and Gaze, 1971, 1972; Johns and Easter, 1977; Stevenson and Yoon, 1980; Johns, 1982; Raymond and Easter, 1983) and in the inner ear (Corwin, 1981, 1985; Corwin and Warchol, 1991) during the total life span of individuals. Postnatal neurogenesis has also been detected in the spinal cord of adult fish as well as in amphibians during metamorphic stages (Richter and Kranz, 1977, 1981; Leonard et al., 1978; Birse et al., 1980; Anderson and Waxman, 1985).

In reptiles, ^{3}H-thymidine autoradiography has demonstrated postnatal neurogenesis in several brain structures; i.e., olfactory bulbs, striatal nucleus sphericus and dorsoventricular ridge, cerebral cortex and cerebellum of perinatal, young and adult lizards (López-

Fig. 6. Postnatal development of zinc-positive fields. **A.** Timm-stained semithin transversal section from a postnatal lizard. x 80. **B.** Idem, from an adult lizard. x 80. **C.** Aspect of the dorsomedial cortex; postnatal specimen. x 900. **D.** Idem, aspect of the outer plexiform layer; adult specimen. Note that the outer Timm-negative zone (T-) has similar thicknesses in the postnatal as in the adult, whereas the Timm-positive sublayer appears more developed in the adult. x 900. **E and F.** Schematic drawings of idealized apical dendrites in a postnatal (E) and and an adult (F) as seen in the electron microscope.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García et al., 1988b, c, 1990a; Pérez-Sánchez et al., 1989; García-Verdugo et al., 1989).

In birds, extensive postnatal neurogenesis occurs in the sexually dimorphic forebrain nuclei of song birds (Nottebohm, 1980; Alvarez-Buylla, 1990). These nuclei are part of a pathway that has been implicated in the production of the species-typical song. Their growth during periods of active singing is hormonally mediated and may provide increased space for storage of the species-typical song, which is different every year (Nottebohm, 1980; Goldman and Nottebohm, 1983; Burd and Nottebohm, 1985; Paton et al., 1985; Alvarez-Buylla et al., 1992).

In the mammalian brain, postnatal neurogenesis was first demonstrated with radioactive-labelled DNA precursors and autoradiographic techniques in three brain structures of perinatal laboratory animals; the hippocampal fascia dentata (Altman, 1963; Altman and Das, 1965; Angevine, 1965); the olfactory bulbs (Hinds, 1968a, b; Asztely et al., 1991); and the cerebellar cortex (Altman, 1972a,b; Rakic, 1971). Subsequently, postnatal neurogenesis was reported in the hypothalamus (Seress, 1985), spinal ganglia (Devor and Govrin-Lippman, 1985) and cerebral cortex (Kaplan and Hinds, 1977). Postnatal neurogenesis is a normal event during olfactory mucosa regeneration in mammals of all ages (Graziadei and Monti-Graziadei, 1978). In all these brain centres postnatal neurogenesis occurs during short periods after birth. Moreover, it has been recently reported that reactive mitosis of neuronal elements occurs after experimental injury of the cerebral cortex (Huang and Lim, 1990).

In lizards (Fig. 5), postnatal neurogenesis in the medial cortex has been demonstrated in perinatal, young and adult specimens, and it seems to persist throught the entire life span (López-García et al., 1988b,c). Despite the fact that postnatal neurogenetic activity is subjected to seasonal variations; it results in quadrupling the number of neurons in the medial cortex throughout the animal's life (López-García et al., 1984). Under normal circumstances, the life span of the common lizard *Podarcis hispanica* is about five years (Castanet, 1985; Caetano et al., 1986).

In rodents, the fascia dentata, acquiring up to 85% of its neurons during the first three postnatal weeks (Bayer, 1980), has extensive postnatal neurogenesis; afterwards, small numbers of neurons are still recruited during the juvenile stages (Bayer et al., 1982; Kaplan and Bell, 1983). In primates, the fascia dentata has an even more delayed period of postnatal neurogenesis that lasts throughout the first three postnatal months (Eckenhoff and Rakic, 1988; Rakic and Nowakowski, 1981).

In the lizard medial cortex the subjacent ependymary sulcus remains as a reminiscent germinative centre where neurogenesis occurs (López-García et al., 1988b, 1990c). The recently generated neurons are guided by radial ependymocytic glia to their final destination in the granular layer of the lizard medial cortex (García-Verdugo et al., 1986). This is a guiding mechanism similar to that seen in rats (Rickmann et al., 1987) and monkeys (Eckenhoff and Rakic, 1984) during hippocampal fascia dentata histogenesis. The lack of any inside-outside positional gradient of late-recruited neurons in the granular layers of both centres further supports the idea of a common origin of the mechanisms regulating the neuro-histogenesis in these cortical regions. Nevertheless, the persistence of a well-developed vertical radial glia network in the medial cortex of adult lizards (Fig. 7c) has no similar counterpart in the adult mammalian fascia dentata.

In both lizard medial cortex and mammalian fascia dentata late-generated neurons differentiate and successfully complete axonal growth to their specific targets. In juvenile rats, postnatally-generated neurons in the fascia dentata extend axons that reach the zinc-positive stratum lucidum of the CA3 hippocampal region (Stanfield and Trice, 1988). In adult lizards, the postnatally-generated neurons differentiate ultrastructurally and send their axons to the target areas; i.e., the zinc-positive zones of the cortex (López-García et al., 1990a), contributing to the dramatic postnatal growth of them.

Postnatal growth of the zinc-positive projection

In lizards, the volume of the cortical zinc-positive zones, which are the targets for the medial cortex axonal projection, undergo a dramatic increase with the age of lizards (Fig. 6) (Rodríguez-Serna, 1987; Pérez-Cañellas, 1989). Quantiative stereological studies have demonstrated that this volumetric increase is accompanied by a significant increase in the number of zinc-positive synapses in those areas (Rodríguez-Serna, 1987). Considering that zinc-positive boutons synapse mainly on the dendritic spines of bipyramidal neurons (Martínez-Guijarro et al., 1984), it follows that the number of dendritic spines in the dorsal and dorsomedial cortices must be expected to increase with age.

In fact, growth in lizards results in dramatic growth of the dendritic trees of cortical spiny bipyramidal neurons (Figs, 6, 7a) (Martínez-Guijarro, 1985) but does not significantly influence the number of them (López-García et al., 1984). In other words, while the number of neurons giving rise to zinc-reactive axons quadruples with age, the number of receptive neurons does not increase significantly; a similar phenomenon occurs in some sensory systems of lower vertebrates; namely, the increase in the number of inner hair cells is not paralleled by spiral ganglion neuron increase (Corwin, 1985).

The dendritic growth of bipyramidal neurons, which seems to occur in the soma-proximal dendritic segments (Martínez-Guijarro, 1985), may account for new synaptic sites. In the hippocampal formation juxtasomatic dendritic segments of pyramidal neurons form the so-called «stratum lucidum», which is the projection site of zinc-reactive mossy fibres (Lorente de No, 1934; Blackstad and Kjaerheim, 1961). In lizards,

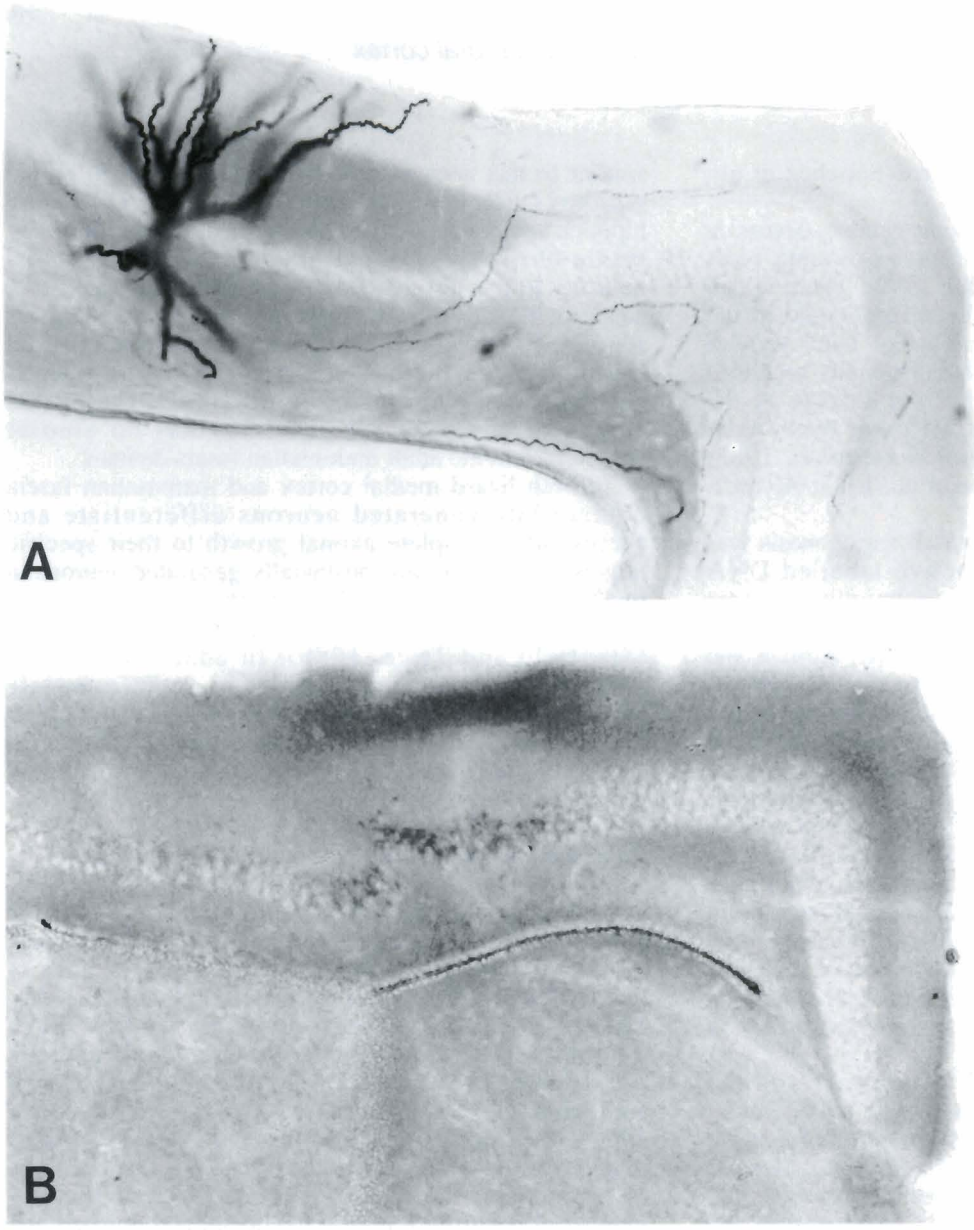


Fig. 7. A. Intracellularly-HRP injected bipyramidal neuron of the dorsomedial cortex. Observe that juxtasomatic dendritic segments are in the zinc-rich field, whereas distal ones are in a zinc-negative zone. x 100. **B.** Aspect of the lizard cortex after immunostaining with an NGF antibody. Note conspicuous juxtasomatic immunostaining in the dorsomedial cortex as well as in the distal zinc-negative outer plexiform layer. x 100. **C.** Idem, immunostaining with an antibody against glial fibrillar acid protein. Observe the dense radial network of ependymocyte processes that delineate cortical subfields. There are no immunostained glial somata in the cortical parenchyma. x 100

this very juxtasomatic dendrite zone of the dorsomedial cortex bipyramidal neurons («lizard stratum lucidum») displays nerve growth factor immunoreactivity (Fig. 7b) that could act as an attraction for incoming axons.

Although there is presently no direct evidence on the guiding mechanism/s of newly generated axons, they may take advantage of preexisting axons that frequently appear fasciculated when observed in the electron microscope (García-Verdugo et al., 1986). The presence of filopodial profiles in immature axons randomly distributed by the wide zinc-reactive zones of the plexiform layers supports this possibility. Finally, the incoming axons may contact novel dendritic spines generated in the juxtaproximal dendritic segment of bipyramidal neurons, a zone that display NGF immunoreactivity.

Our work hypothesis

The biological meaning of the continuous growth of the medial cortex in lizards is an enigma. It is commonly accepted that the regenerative capacity of different nervous centres depends on whether they have finished their neurogenetical schedules.

Fish show postnatal neurogenesis in their spinal cord and they can regenerate their spinal cord after its transection. This regenerative phenomenon is age-dependent (Berstein, 1964). In larval frogs (amphibians), spinal cord transection leads to regenerative events that restore initial motor control but spinal cord transection in adult frogs causes final impairment (Holder and Clarke, 1988). In chick embryos spinal cord transection prior to E15 results in no discernible motor impairment in the hatched animals, but after E15 spinal cord transection results in abnormal or no motor activity (Shimizu et al., 1990).

In lizards, according to this hypothesis, the delayed postnatal neurogenesis of the cortex must confer upon it an extraordinary regenerative capacity. In fact, it has been reported that lizards can regenerate their cerebral cortex after its partial surgical ablation (Minelli et al., 1977).

Specific neurotoxic lesion of the lizard medial cortex

In a search for specific substances which would injure the lizard medial cortex without severely affecting the rest of cortical areas and brain centres, we found the antimetabolite 3-acetylpyridine (AP) (Font et al., 1991; López-García et al., 1990b). Up to 90-95% of neurons of the medial cortex were destroyed after intraperitoneal injections of 3-acetylpyridine leaving the rest of brain structures slightly affected. The nucleus sphericus of the archistriatum also displayed lesioned cells located in its mural stratum but never surpassing 30% of them. Moreover, very few scattered pyknotic cells could be seen in the inner plexiform layer of the dorsal cortex and in certain neostriatal areas.

Immediately after lesions, the lizards showed behavioural disorders including lack of prey catch fitness, intense exploratory movements, and maze test impairments; these symptoms are interpreted as severe impairment of their spatial memory. In mammals, hippocampus and fascia dentata are concerned with spatial memory (Roullet and Lassalle, 1990). Again, these neurobehavioural observations support analogy of both nervous centres. Nevertheless, the most dramatic phenomena observed was that 3AP-lesioned lizards progressively recovered their initial behavioural capacities (Font et al., 1989).

The histological examination of 3AP-treated lizards at different times of survival (Fig. 8) demonstrated that medial cortex destruction was followed by a neo-histogenetic regenerative process. Thus, we must deduce that the ependymal sulcus is induced to produce thousands of new neurons that migrate to the granular layer, differentiate, emit axons, etc., repeating a new histogenetical process that finishes by rebuilding a new medial cortex.

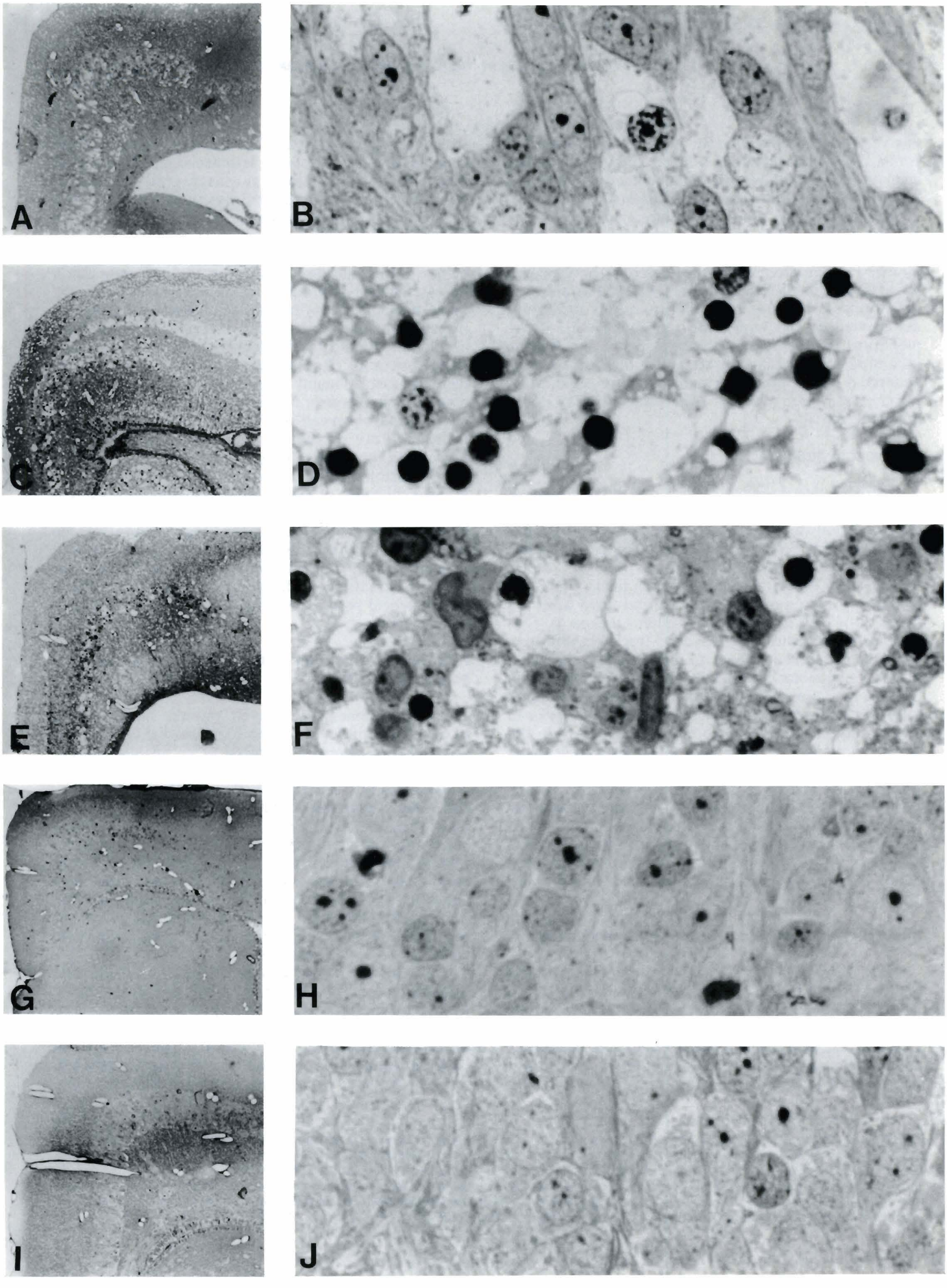
Histological changes in the medial cortex after 3-acetylpyridine lesion

Both the principal cell layer (granular layer) as well as the plexiform layers of the lizard medial cortex were directly and severely affected after intraperitoneal injection of 3-acetylpyridine.

As soon as twelve hours after 3AP injection (Fig. 8a,b) many neuronal somata in the medial cortex cell layer showed initial lesion symptoms; i.e., chromatin clumping and acute cytoplasmic swelling that even affected the perinuclear cisternae (Fig. 9c). Medial cortex plexiform layers displayed a spongy appearance caused by swelling of dendrites and some axonal profiles. Nevertheless, Timm-positive areas, which are the projection fields of medial cortex axonal projection, displayed normal appearance.

One day after 3AP injection (Figs. 8c,d, 9a,b), the granular layer of the medial cortex was crowded by highly abundant somata with pyknotic nuclei and disorganized cytoplasm. This is unequivocal symptoms of necrosis. Moreover, some neuronal somata displayed variable lesioning symptoms; i.e., chromatin clumping, but not dramatic cytoplasmic swelling. Medial cortex

Fig. 8. The medial cortex in 3-acetylpyridine-lesioned lizards. **A.** tolouidine blue-stained transversal semithin section showing the medial cortex twelve hours after 3AP injection. Observe moderate swelling in the medial cortex plexiform layers as well as in the granular cell layer. **B.** Idem; aspect of the cell layer. Several granule cell somata appear swollen. **C.** One day after 3AP injection. **D.** Idem;aspect of the cell layer. Most nuclei are pyknotic. **E.** Eight days after 3AP injection. **F.** Idem; aspect of the cell layer. Observe that immature fusiform nuclei have reached the cell layer. **G.** One month after 3AP injection. **H.** Idem; aspect of the cell layer. Although pyknotic nuclei are still present, most cell somata appear normal. **I.** Two months after 3AP injection. **J.** Idem; aspect of the cell layer displaying normal histological pattern. x 80 for A, C, E, G and I. x 1,500 for B, D, F. H and J.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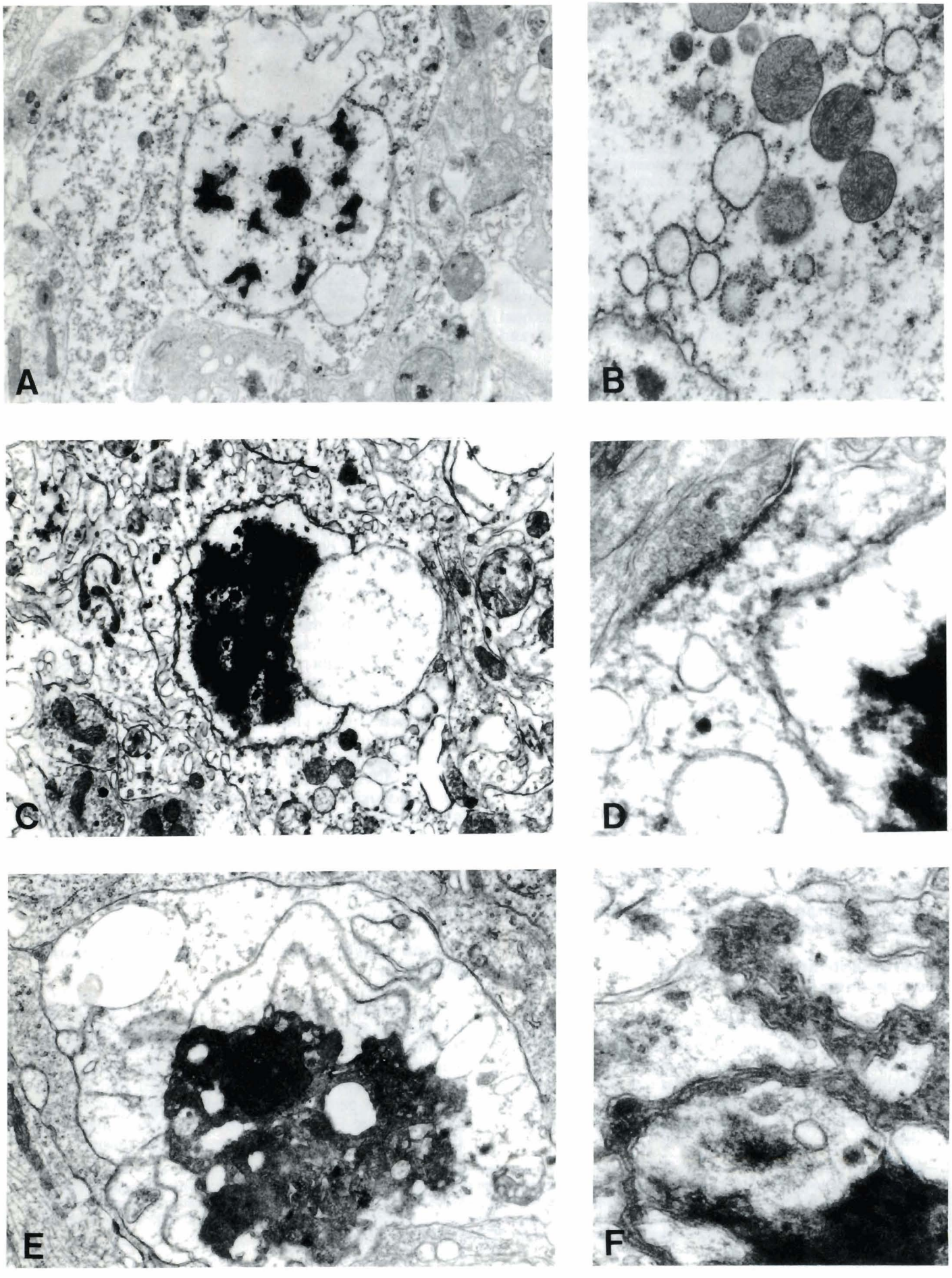
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Fig. 9. Lesioned neurons of the medial cortex cell layer. **A.** Slightly lesioned granule neuronal soma one day after 3AP injection. Chromatin clumping and swelling of perinuclear cisternae are dramatic. Cytoplasm appears disorganized. x 12,000. **B.** Idem; aspect of cytoplasmic organelles. Granular endoplasmic reticulum appears vacuolated; mitochondria appear contracted. x 23,000. **C.** Idem, ten days after 3AP lesion. Chromatin forms a unique clump and the perinuclear cisterna is still evident but swollen in a pole. x 17,000. **D.** Idem, axo-somatic synaptic contacts are still visible on some lesioned somata. x 45,000. **E.** Idem, a month after 3AP lesion. Both pyknotic nucleus and disorganized cytoplasm appear engulfed by ependymocytic processes. x 19,000. **F.** Aspect of ependymocytic processes engulfing fragmented debris x 48,000

plexiform layers appeared completely distorted. Scattered neuronal somata in the plexiform layers displayed a normal healthy appearance, apparently unaffected by the neurotoxin. The medial cortex axonal projection areas evidenced initial symptoms of axonal degeneration; i.e., moderate swelling of axonal and dendritic profiles.

From days two to eight after 3AP injection, the medial cortex cell appeared severely distorted; e.g., many pyknotic nuclei, residual debris and scarce normal somata populated the medial cortex granular layer. Swelling in the plexiform layers of the medial cortex became less prominent. In these zones, ependymocytic processes removed cellular debris by phagocytosis (Fig. 9e, f). At this time, the medial cortex projection areas displayed the most extensive lesion symptoms.

Approximately on day eight after 3AP injection (Fig. 8e,f) many recruited immature neuronal somata appeared in the medial cortex cell layer. They displayed fusiform or polarized cytoplasm and fine grain, with disperse chromatin inside their nuclei. This is the same morphology of migratory immature neurons that could be frequently seen in the inner plexiform layer of these animals. This phenomenon continued until approximately one month later.

From days 8 to 30 after 3AP injection (Fig. 8g, h) the rate of pyknotic nuclei in the medial cortex cell layer progressively decreased as the number of newly-immature plus normal somata increased.

Finally, two months after 3AP injection both the granular and the plexiform layers of the medial cortex displayed a histological pattern that was undistinguishable from that of non-injected animals (Fig. 8i, j). The axonal projection areas of the medial cortex also appeared normal and displayed Timm-reactivity, even in specimens in which the dorsomedial cortex was secondarily affected and not regenerated.

Neuroblast-induced proliferation after 3-acetylpyridine lesion

Pulses of tritiated thymidine (3HT) (López-García et al., 1988b, 1990a,c) and 5-bromodeoxiuridine (BrDU) (Soriano et al., 1991), both being DNA markers of cellular proliferation (posteriorly identified by autoradiography or immunocytochemistry), confirmed that the ependymal sulcus was activated after 3AP lesion.

Both 3HT or 5BrDU pulses in 3AP-injected animals resulted in labelled cells located mainly in the ependyma or the inner plexiform layer of the medial cortex (Fig. 10). Labelled cells in the inner plexiform layer displayed fusiform morphologies and were easily identified as migratory neurons. Due to short survival period (two to five days) after DNA marker pulses, only few labelled cells could be recruited in the medial cortex cell layer. Here, marked cells sometimes displayed less intense labelling (i.e., less silver grain density or DAB immunostaining).

Counts revealed that 3AP-injected animals had more labelled cells than controls. Large numerical differences were detected between animals with different survival times after 3AP injection. Until two days after 3AP injection, numbers of labelled cells per hemisphere were in the range of control animals, nevertheless notorious interindividual differences were detected in this initial response period.

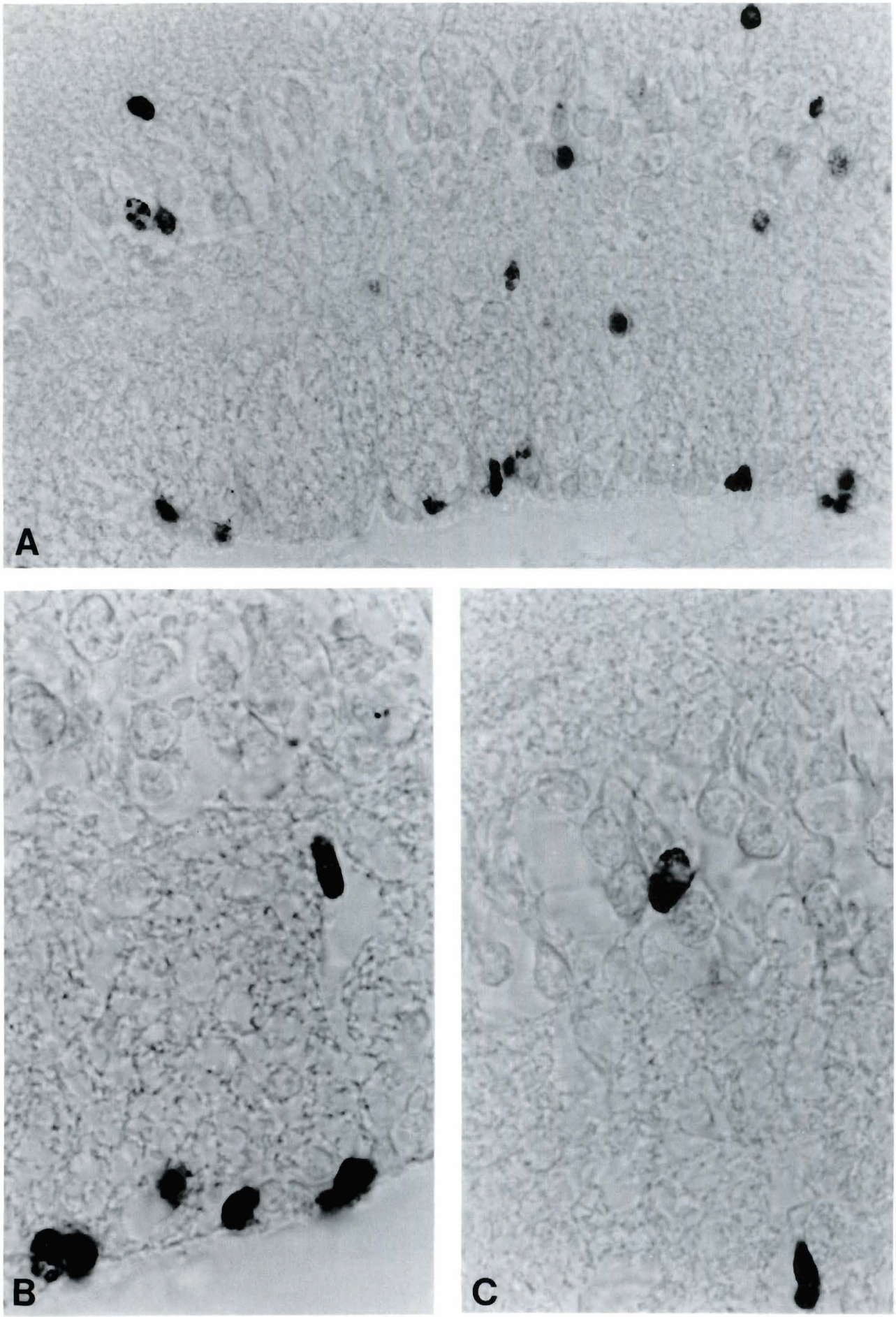
A second period of maximal proliferative activity was detected from the third day after 3AP injection until 5-7; in this period numbers of labelled cells per hemisphere were ten fold higher than in controls. A third period of stabilized proliferation activity continued until a month later; in this period the number of labelled cells per hemisphere was roughly similar to controls.

Most, if not all, 3HT- or 5BrDU-labelled cells in the cortex of lesioned lizards were neurons in an immature state of differentiation. Their preferential distribution in the ependyma and their characteristic fusiform migratory morphology when located in the inner plexiform layer of the medial cortex are indirect evidence. The electron microscopic examination of autoradiographically-labelled cells revealed unequivocal immature neuronal morphology (Font et al., 1991). In any case, it is possible that some labelled cells are glial.

Lesion regeneration of zinc-containing axonal projection

As expected, 3AP neurotoxin also lesioned the zinc-containing axons and boutons of granule cells, thus leading to a subsequent partial bleaching of Timm staining in the lizard cortex. In the electron microscope, the zinc-rich lesioned boutons showed two distinct degeneration patterns. Some boutons appeared swollen and disrupted (Fig. 11a); others appeared contracted (Fig. 11b) with dense and disorganized cytoplasm.

Fig. 10. Reactive neurogenesis in the medial cortex after 3AP lesion. **A.** Immunocytochemical staining with 5BrDU antibody; 5BrDU pulse on a lizard 4 days after 3AP lesion; five days of survival after 5BrDU pulse. Observe the presence of clusters of labelled nuclei in the ependyma as well as scattered labelled nuclei in the inner plexiform layer and in the cell layer. Some nuclei placed in the cell layer display weak labelling. x 1,500. **B.** Idem, aspect of labelled nuclei in the ependyma and in the inner plexiform layer. x 1,500. **C.** Idem, aspect of the cell layer. x 1,500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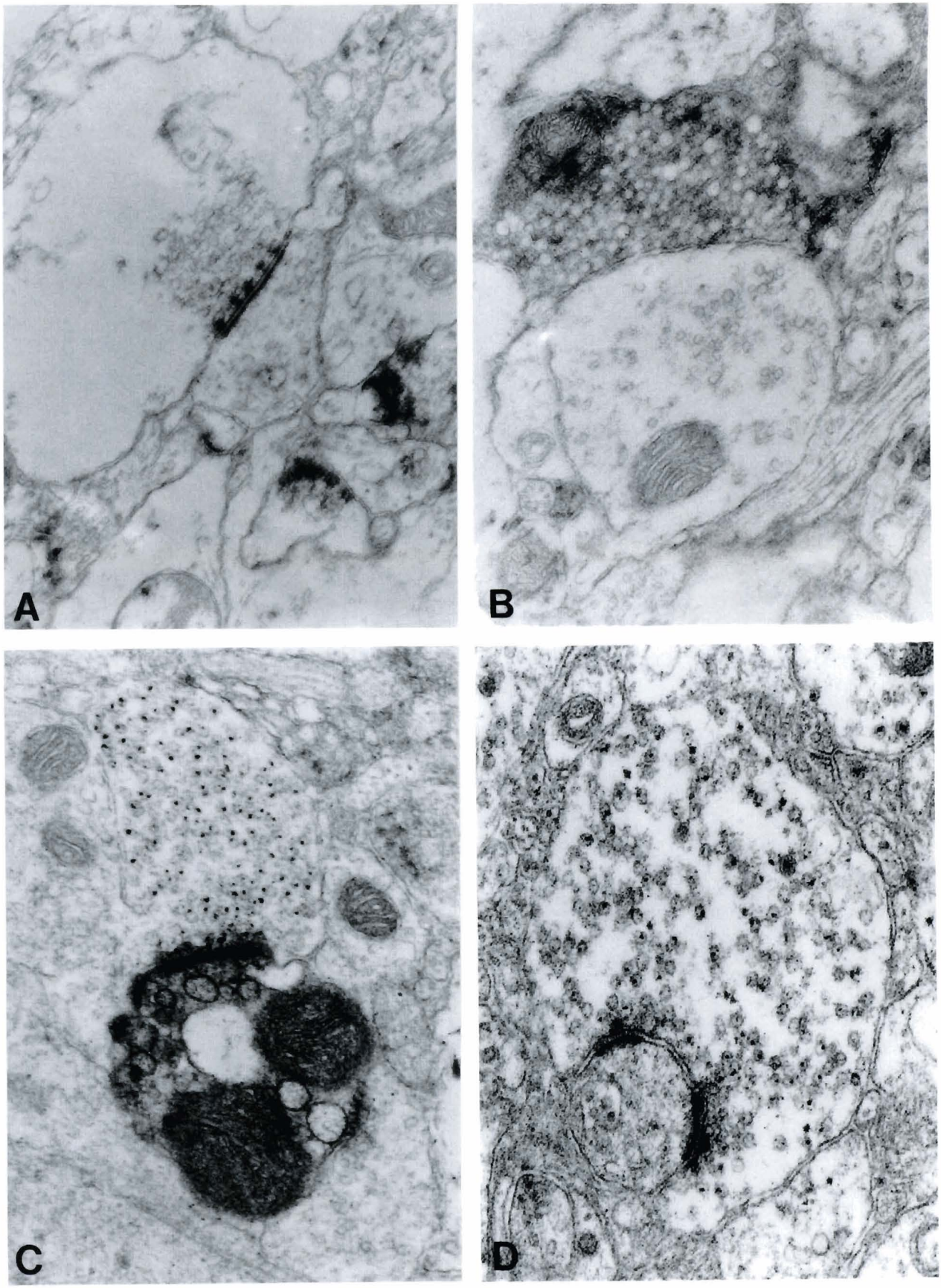
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Fig. 11. Zinc-positive boutons in 3-acetylpyridine-lesioned lizards. **A.** Boutons in the outer plexiform layer of the dorsomedial cortex one day after 3AP injection. Observe the pronounced swelling of the presynaptic bouton x 32,000. **B.** Idem, observe the contracted matrix of a bouton and the initial swelling of the adjacent one. x 32,000. **C.** Undamaged zinc-positive boutons in the outer plexiform layer of the dorsomedial cortex ten days after 3AP injection. Observe a zinc-positive bouton synapsing on a lesioned postsynaptic structure x 32,000. **D.** Idem, the zinc-positive bouton synapses on an apparently normal dendritic spine. x 32,000

Resorption of the lesioned boutons was observed to be carried out by phagocytic activity of ependymal processes and by microglia-macrophage-like cells that appeared very late. The cleaning process appears incomplete even after two months after lesion.

Simultaneous to the glial cleaning activity new axons reached the dorsomedial and dorsal cortices reestablishing a new zinc-containing projection by intensive neosynaptogenetic activity (Molowny et al., 1992). The arrival of new incoming zinc-containing boutons was detected in the Timm-reacting areas by day 10-15 after 3AP injection (Fig. 11d). Sometimes, they were identified as closely-grouped axons and boutons displaying immature characteristics. Although two months later the neuropil ultrastructure of zinc projection fields appeared undistinguishable from non-lesioned lizards, quantitative data indicated that reactive neo-synaptogenesis must have continued for six months after 3AP injection without reaching the synaptic density levels of non-lesioned lizards (Molowny et al., 1992).

Concluding considerations

In summary, the histological examination of brains after 3-acetylpyridine lesion reveals that the germinal ependymary sulcus is activated very soon after lesion of the medial cortex cell layer. The newly-generated neurons migrate to the almost totally destroyed cell layer where they mature into a new cell layer and give rise to a new zinc-containing axonal projection. Simultaneous with this reactive neo-histogenetic process there is a demolition of the residual network formed by the dying neurons. These two processes, demolition and neohistogenesis, overlap in time.

Perhaps the most exciting aspect in the regeneration process is that it takes place in adult aged specimens. In the telencephalon of old lizards there are residual ependymal areas with high cubic pseudostratified morphology; every ependymal area displaying such morphology is called «sulcus» (Kirsche, 1967; Tineo et al., 1987). Although the residual «sulcus» lining the medial cortex of adult-aged lizards is almost atrophic, as deduced by their tritiated thymidine capture rate (López-Garcia et al., 1988c, 1990b,c), tritiated thymidine autoradiography shows that it still has a few stem cells that can be activated to produce thousands of new neurons that regenerate a new medial cortex (López-García, 1992).

Repetitive treatments with 3-acetylpyridine result in several lesion-regeneration cycles with the subsequent once, twice and successive activations of this sulcus (unpublished data). It appears to have unlimited proliferation capacity. This dramatic prolific neurogenetic potential of the ependyma was unexpected, and it raises crucial questions about the mechanisms regulating its activity: what is/are the trigger/s for reactive neurogenesis? What is/are the signals to stop it? What is the role of the glial network during degenerative-regenerative processes?

Interesting questions arise about how the reorganization of neural connectivity during regeneration of the medial cortex takes place. Do the rest of cortical areas projecting to the medial cortex outer plexiform layer re-arrange in the same laminar pattern? Do the medial cortex granule neurons really re-constitute the precendent circuitry?

In any case, the initial hypothesis that delayed postnatal neurogenesis of the medial cortex of lizards may be conducive to dramatic regenerative events has been proven. With the restrictions due to the phylogenetical distances, regenerative phenomena in the lizard medial cortex may give some optimism to hypothetical regenerative abilities in the fascia dentata of the mammalian brain.

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References

Altman J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat. Rec. 145, 573-592.

Altman J. (1972a). Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular. J. Comp. Neurol. 145, 353-398.

Altman J. (1972b). Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and the molecular layer. J. Comp. Neurol. 145, 399-464.

Altman J. and Das G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol. 124, 319-335.

Alvarez-Buylla A. (1990). Mechanism of neurogenesis in adult avian brain. Experientia 46, 948-955.

Alvarez-Buylla A., Ling C.-Y. and Nottebohm F. (1992). High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries. J. Neurobiol. 23, 396-406.

Anderson M.J. and Waxman S.G. (1985). Neurogenesis in tissue cultures of adult teleost spinal cord. Dev. Brain Res. 20, 203-212.

Angevine J.B. (§965). Time of neuron origin in the hippocampal region, an autoradiographic study in the mouse. Exp. Neurol. 2, 1-70.

Asztely F., Hanse E., Wigström H. and Gustafsson B. (1991). Synaptic potentiation in the hippocampal CA1 region induced by application of N-methyl-D-aspartate. Brain Res. 558, 153-156.

Bayer S.A. (1980). Development of the hippocampal region in the rat. I.

Neurogenesis examined with ^{3}H-thymidine autoradiography. J. Comp. Neurol. 190, 87-114.

Bayer S.A., Yackel J.W. and Puri P.S. (1982). Neurons in the dentate gyrus granular layer substantially increase during juvenile and adult life. Science 216, 890-892.

Berbel P.J., López-García C., García-Verdugo J.M., Regidor J. and Marín-Girón F. (1981). Microglia in the cerebral cortex of Lacerta. A combined Golgi-electron microscopic study. Morf. Norm. Patol. 5, 261-270.

Berbel P.J., Martínez-Guijarro F.J. and López-García C. (1987). Intrinsic organization of the medial cerebral cortex of the lizard *Lacerta pityusensis*. A Golgi study. J. Morphol. 194, 275-286.

Berstein J.J. (1964). Relation of spinal cord regeneration to age in adult goldfish. Exp. Neurol. 9, 161-174.

Birse S.C., Leonard R.B. and Coggeshall R.E. (1980). Neuronal increase in various areas of the nervous system of the guppy, Lebistes. J. Comp. Neurol. 194, 291-301.

Blackstad T.W. and Kjaerheim A. (1961). Special axon-dendritic synapses in the hippocampal cortex: electron and light microscopic studies on the layer of mossy fibers. J. Comp. Neurol. 117, 133-146.

Bruce L.L. and Butler A.B. (1984). Telencephalic connections in lizards. I. Projections to cortical areas. J. Comp. Neurol. 229, 585-604.

Buhl E.H. and Dann J.F. (1990). Basal dendrites are a regular feature of hippocampal granule cells in flying fox hippocampus. Neurosci. Lett. 116, 263-228.

Burd D.B. and Nottebohm F. (1985). Ultrastructural characterization of synaptic terminals formed on newly generated neurons in a song control nucleus of the adult canary forebrain. J. Comp. Neurol. 240, 143-152.

Caetano M.H., Castanet J. and Crespo E.G. (1986). Estimation a l'aide de la squelettchronologie de l'age de *Podarcis hispanica*, (Steindnacher, 1870), Sauria, Lacertidae, provenant d'una population portugaise. Rev. Suisse Zool. 93, 117-127.

Castanet J. (1985). La squelettochronologie chez les reptiles. I. Resultats experimentaux sur la signification des marques de croissance squelettiques chez les Lezards et les Tortues. Ann. Sci. Natl. Zool. Paris. 7, 23.

Castellano B., González B., Dalmau I. and Vela J.M. (1991). Identification and distribution of microglial cells in the cerebral cortex of the lizard: A histochemical study. J. Comp. Neurol. 311, 434-444.

Corwin J.T. (1981). Postembryonic production and aging of inner hair cells in sharks. J. Comp. Neurol. 201, 541-553.

Corwin J.T. (1985). Perpetual production of hair cells and maturational changes in the hair cell ultrastructure accompany postembryonic growth in an amphibian ear. Proc. Natl. Acad. Sci. USA 82, 3911-3915.

Corwin J.T. and Warchol M.E. (1991). Auditory hair cells: structure, function, development and regeneration. Annu. Rev. Neurosci. 14, 301-333.

Cotman C.W., Monaghan D.T., Ottersen O.P. and Storm-Mathisen J. (1987). Anatomical organization of excitatory amino acid receptors and their pathways. TINS 7, 273-280.

Davila J.C., Guirado S. and De la Calle A. (1988). Immunocytochemical localization of somatostatin in the cerebral cortex of lizards. Brain Res. 447, 52-59.

Davila J.C., De la Calle A., Gutiérrez A., Megias M., Andreu M.J. and Guirado S. (1991). Distribution of neuropeptide Y (NPY) in the cerebral cortex of the lizards *Psammodromus algirus* and *Podarcis hispanica*: Co-localization of NPY, somatostatin, and GABA. J. Comp. Neurol. 308, 397-408.

Deller T. and Leranth C. (1990). Synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. J. Comp. Neurol. 300, 433-447.

Devor M. and Govrin-Lippmann R. (1985). Neurogenesis in adult rat dorsal ganglia. Neurosci. Lett. 61, 189-194.

Eckenhoff M.F. and Rakic P. (1984). Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural and immunocytochemical analysis in the developing rhesus monkey. J. Comp. Neurol. 223, 1-21.

Eckenhoff M.F. and Rakic P. (1988). Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. J. Neurosci. 8, 2729-2747.

Font E., García Verdugo J.M., Martínez-Guijarro F., Alcántara S. and López-García C. (1989). Neurobehavioural effects of 3-acetylpiridine in the lizard *Podarcis hispanica*. Eur. J. Neurosci. 2S, 148.

Font E., García-Verdugo J.M., Alcántara S. and López-García C. (1991). Neuron regeneration reverses 3-acetylpyridine-induced cell loss in the cerebral cortex of adult lizards. Brain Res. 551, 230-235.

Frotscher M. and Gghwiler B.H. (1988). Synaptic organization of intracellularly stained CA3 pyramidal neurons in slice cultures of rat hippocampus. Neuroscience 24, 541-551.

Frotscher M., Seress L., Schwerdtfeger W.K. and Buhl E. (1991). The mossy cells of the fascia dentata: a comparative study of their fine structure and synaptic connections in rodents and primates. J. Comp. Neurol. 312, 145-163.

García-Verdugo J.M., Berbel P. and López-García C. (1981). A Golgi and electron microscopic study of the ependymal cells of the cerebral cortex of the lizard *Lacerta galloti*. Trab. Inst. Cajal Inv. Biol. 72, 269-278.

García-Verdugo J.M., Farina I., Molowny A. and López-García C. (1986). Ultrastructure of putative migrating cells in the cerebral cortex of *Lacerta galloti*. J. Morphol. 189, 189-198.

García-Verdugo J.M., Llahi S., Ferrer I. and López-García C. (1989). Postnatal neurogenesis in the olfactory bulbs of a lizard. A tritiated thymidine autoradiographic study. Neurosci. Lett. 98, 247-252.

Goldman S.A. and Nottebohm F. (1983). Neuronal production, migration and differentiation in a vocal control nucleus of the adult female canary brain. Proc. Natl. Acad. Sci. USA 80, 2390-2394.

Graziadei P.P.C. and Monti-Graziadei G.A. (1978). The olfactory system: a model for the study of neurogenesis and axon regeneration in mammals. In: Neuronal Plasticity. Cotman C.W. (ed). Raven Press. New York. pp 131-153.

Hinds J.W. (1968a). Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia. J. Comp. Neurol. 134, 305-322.

Hinds J.W. (1968b). Autoradiographic study of histogenesis in the mouse olfactory bulb. II. Cell proliferation and migration. J. Comp. Neurol. 134, 305-322.

Hjorth-Simonsen A. and Jeune B. (1972). Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. J. Comp. Neurol. 144, 215-232.

Hjorth-Simonsen A. (1972). Projection on lateral part of the entorhinal area to the hippocampus and fascia dentata. J. Comp. Neurol. 146, 219-232.

Holder N. and Clarke J.D.W. (1988). Is there a correlation between continuous neurogenesis and directed axon regeneration in the vertebrate nervous system? TINS 11, 94-99.

Hoogland P.V. and Vermeulen-Van der Zee E. (1988). Intrinsic and

extrinsic connections of the cerebral cortex of lizards. In: The forebrain of reptiles. Current concepts of structure and function. Schwerdtfeger W.K. and Smeets W.J.A. (eds). Karger. Basel. pp 20-29.

Hoogland P.V. and Vermeulen-Vanderzee E. (1991). The dentate area in the lizard *Gekko gecko* is divided in a rostral thalamic and a caudal olfactory part. Eur. J. Neurosci. 4S , 21.

Huang L. and Lim R. (1990). Identification of injury-induced mitotic cells in adult rat cerebral cortex by neuron-specific markers. Dev. Brain Res. 51, 123-127.

Johns P.R. (1982). Formation of photoreceptors in larval and adult goldfish. J. Neurosci. 2, 178-198.

Johns P.R. and Easter S.S. (1977). Growth of the adult goldfish eye. II. increase in retinal cell number. J. Comp. Neurol. 176, 331-342.

Kaplan M.S. and Hinds J.W. (1977). Neurogenesis in the adult rat, electron microscopic analysis of light radiographs. Science 197, 1092-1094.

Kaplan M.S. and Bell D.H. (1983). Neuronal proliferation in the 9-month-old rodent radioautographic study of granule cells in the hippocampus. Exp. Brain Res. 52, 1-5.

Kirsche W. (1967). Über postembryonale Matrix-Zonen im Gehirn verschiedener. Z. Mikrosk-Anat. Forsch. 76, 313-406.

Leonard R.B., Coggeshall R.E. and Willis W.D. (1978). A documentation of an age related increase in neuronal and axonal numbers in the stingray. J. Comp. Neurol. 179, 13-22.

Liu C.Y., Grandes P., Matute C., Cuenod M. and Streit P. (1989). Glutamate-like immunoreactivity revealed in rat olfactory bulb, hippocampus and cerebellum by monoclonal antibody and sensitive staining method. Histochemistry 90, 427-445.

Lohman A.H.M. and Van Woerden-Verkley I. (1976). Further studies on the cortical connection of the Tegu lizard. Brain. Res. 103, 9-28.

Lohman A.H.M. and Van Woerden-Verkley I. (1978). Ascending connections to the forebrain in the tegu lizard. J. Comp. Neurol. 182, 555-594.

López-García C., Molowny A. and Pérez-Clausell J. (1983a). Volumetric and densitometric study in the cerebral cortex and septum of a lizard (*Lacerta galloti)* using the Timm method. Neurosci. Lett. 40, 13-18.

López-García C., Soriano E., Molowny A., García-Verdugo J.M., Berbel P.J. and Regidor J. (1983b). The Timm-positive system of axonic terminals of the cerebral cortex of *Lacerta*. In: Ramón y Cajal's contributions to the neurosciences. Grisolia S., Guerri C., Samson F., Norton S. and Reinoso-Suarez F. (eds). Elsevier Sci. Pub. Amsterdam. pp 137-148.

López-García C., Tineo P. and Del Corral J. (1984). Increase of the neuron number in some cerebral cortical areas of a lizard, *Podarcis hispanica* (Steind, 1870) during postnatal periods of life. J. Hinforsch. 25, 255-259.

López-García C., Martínez-Guijarro F.J., Berbel P.J. and García-Verdugo J.M. (1988a). Long-spined polymorphic neurons of the medial cortex of lizards. A Golgi, Timm and electron-microscopic study. J. Comp. Neurol. 272, 409-423.

López-García C., Molowny A., García-Verdugo J.M. and Ferrer I. (1988b). Delayed postnatal neurogenesis in the cerebral cortex of lizards. Dev. Brain Res. 43, 167-174.

López-García C., Molowny A., Rodríguez-Serna R., García-Verdugo J.M. and Martínez-Guijarro F.J. (1988c). Postnatal development of neurons in the telencephalic cortex of lizards. In: The forebrain of reptiles: Current concepts of structure and function. Schwerdtfeger W.K. and Smeets W. (eds). Karger. Basel. pp 122-130.

López-García C. and Martínez-Guijarro F.J. (1988). Neurons in the medial cortex give rise to Timm-positive boutons in the cerebral cortex of lizards. Brain Res. 463, 205-217.

López-García C., Molowny A., García-Verdugo J.M., Martínez-Guijarro F.J. and Bernabeu A. (1990a). Late generated neurons in the medial cortex of adult lizards send axons that reach the Timm-reactive zones. Dev. Brain. Res. 57, 249-254.

López-García C., Molowny A., García-Verdugo J.M., Martínez-Guijarro F.J., Font E. and Alcántara S. (1990b). Neurogenesis postnatal tardia y regeneración en la corteza cerebral de la lagartija comun. Biol. Desenvolup. 8, 183-192.

López-García C., Molowny A., García-Verdugo J.M., Pérez-Sánchez F. and Martínez-Guijarro F.J. (1990c). Postnatal neurogenesis in the brain of the lizard, *Podarcis hispanica*. In: The forebrain in non-mammals. New aspects of structure and development. Schwerdtfeger W.K. and Gemroth P. (eds). Springer-Verlag. Berlin. pp 103-117.

López-García C. (1992). Postnatal neurogenesis and regeneration in the lizard cerebral cortex. Restor. Neurol. (in press).

Lorente de No R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the Ammonic System. J. Psychol. Neurol. 46, 113-177.

Lubbers K. and Frotscher M. (1987). Fine structure and synaptic connections of identified neurons in the rat fascia dentata. Anat. Embryol. 177, 1-14.

Luis de la Iglesia J.A., Martínez-Guijarro F.J. and López-García C. (1992). The neurons of the molecular layer of the lizard fascia dentata. Golgi and immunocytochemical study (in preparation).

Martínez-Guijarro F.J., Berbel P.J., Molowny A. and López-García C. (1984). Apical dendritic spines and axonic terminals in the bipyramidal neurons of the dorsomedial cortex of lizards (Lacerta). Anat. Embryol. 170, 321-326.

Martínez-Guijarro F.J. (1985). Organización de las áreas dorsomedial y dorsal de la corteza cerebral de *Podarcis hispanica* (Steindachner, 1870), Doctoral Thesis. University of Valencia.

Martínez-Guijarro F.J., Molowny A. and López-García C. (1987). Timm staining intensity is correlated with densities of Timm-positive presynaptic structures in the cerebral cortex of lizards. Histochemistry 86, 315-319.

Martínez-Guijarro F.J., Desfilis E. and López-García C. (1990). Organization of the dorsomedial cortex in the lizard *Podarcis hispanica*. In: The forebrain in non-mammals. New aspects of structure and development. Schwerdtfeger W.K. and Germroth P. (eds). Springer-Verlag. Berlin. pp 77-92.

Martínez-Guijarro F.J., Soriano E., Del Rio J.A. and López-García C. (1991a). Zinc-positive boutons in the cerebral cortex of lizards show glutamate immunoreactivity. J. Neurocytol. 20, 834-843.

Martínez-Guijarro F.J., Soriano E., Del Rio J.A. and López-García C. (1991b). Parvalbumin-immunoreactive neurons in the cerebral cortex of the lizard *Podarcis hispanica*. Brain Res. 547, 339-343.

Martínez-Guijarro F.J. and López-García C. (1992). Ultrastructure of the commissural boutons in the hippocampal cortex of the lizard *Podarcis hispanica*. (in preparation).

Martínez-Guijarro F.J. and Freund T.F. (1992). Distribution of gabaergic interneurons immunoreactive for calretinin, calbidin D28K and parvalbumin in the cerebral cortex of the lizard *Podarcis hispanica*. J. Comp. Neurol. (in press).

Minelli G., Del Grande P. and Mambelli M.C. (1977). Preliminary study

of the regenerative processes of the dorsal cortex of the telencephalon of *Lacerta viridis*. Z. Mikrosk-Anat. Forsch. 91, 241-246.

Molowny A. and López-García C. (1978). Estudio citoarquitectónico de la corteza cerebral de reptiles. II. Localización histoquímica de metales pesados y definición de subregiones Timm-positivas en la corteza de *Lacerta*, *Chalcides*, *Tarentola* y *Malpolon*. Trab. Inst. Cajal Inv. Biol. 70, 55-74.

Molowny A., López-García C. and Marín Girón F. (1972). Estudio citoarquitectónico de la corteza cerebral de reptiles. I.Areas y nucleos corticales en *Lacerta galloti*, Chalcides sp. y Tarentola mauritanica. Trab. Inst. Cajal. Inv. Biol. 24, 125-158.

Molowny A., Danscher G. and López-García C. (1992). Restore of Zinc containing boutons in lizard cerebral cortex after 3-acetylpiridine lesion of the medial cortex. (In preparation).

Nafstad P.H.J. (1967). An electron microscope study on the termination of the perforant path fibers in the hippocampus and the fascia dentata. Z. Zellforsch. 76, 532-542.

Nottebohm F. (1980). Testosterone trigers growth of brain vocal control nuclei in adult female canaries. Brain Res. 189, 429-436.

Olucha F., Martínez-García F., Poch L., Schwerdtfeger W.K. and López-García C. (1988). Projections from the medial cortex in the brain of lizards: correlation of anterograde and retrograde transport of horseradish peroxidase with Timm staining. J. Comp. Neurol. 276, 469-480.

Ottersen O.P. and Storm-Mathisen J. (1985). Different neuronal localization of aspartate-like and glutamate-like immunoreactivities in the hippocampus of rat, guinea pig and Senegalese baboon (*Papio Papio*) with a note on the distribution of GABA. Neuroscience 16, 589-606.

Patón J.A., O'Loughlin B.E. and Nottebohm F. (1985). Cells born in the adult canary forebrain are local interneurons. J. Neurosci. 5, 3088-3093.

Pérez-Cañellas M. (1989). Desarrollo postnatal tardio del sistema Timm-positivo septocortical de *Chalcides sexlineatus*. Minor Thesis, University of Valencia.

Pérez-Clausell J. (1987). Estudi histoquimic del cervell de la sargantana. Doctoral Thesis. University of Valencia.

Pérez-Sánchez F., Molowny A., García-Verdugo J.M. and López-García C. (1989). Postnatal neurogenesis in the nucleus sphericus of the lizard *Podarcis hispanica*. Neurosci. Lett. 106, 71-75.

Rakic P. (1971). Neuron-glia relationship during granule cell migration in developing cerebellar cortex: A Golgi and electron microscopic study in the Macacus rhesus. J. Comp. Neurol. 141, 283-312.

Rakic P. and Nowakowski R.S. (1981). The time of origin of neurons in the hippocampal region of the rhesus monkey. J. Comp. Neurol. 196, 99-128.

Ramón y Cajal P. (1917). Nuevo estudio del encefalo de los reptiles. Trab. Lab. Inv. Biol. Univ. Madrid 15, 83-99.

Ramón y Cajal S. (1911). Histologie du Systeme Nerveux de l'homme et des vertebres, A. Maloine. Paris.

Raymond P.A. and Easter S.S. (1983). Postembryonic growth of the optic tectum in goldfish. I. Location of germinal cells and numbers of neurons produced. J. Neurosci. 3, 1077-1091.

Riback C.E. and Seress L. (1983). Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. J. Neurocytol. 12, 577-597.

Richter W. and Krantz D. (1977). Uber die Bedeutung der Zellproliferation fur die Hirnregeneration bei niederen Vertebraten. Autoradiographische Untersuchungen. Verch. Anat. Ges. 71, 439-445.

Richter W. and Kranz D. (1981). Autoradiographische Untersuchungen der postnatalen Proliferantionsaktivitat der Matrixzonen des Gehirns der Forelle (*Salmo irideus*). Z. Mikrosk. Anat. Forsch. 95, 491-520.

Rickman M., Amaral D.G. and Cowan W.M. (1987). Organization of radial glia cells during the development of the rat dentate gyrus. J. Comp. Neurol. 264, 449-479.

Rodríguez-Serna R. (1987). Desarrollo postnatal del sistema Timm-positivo del telencefalo de *Podarcis hispanica*, (Steindachner, 1870). Minor Thesis. University of Valencia

Roullet P. and Lassalle J.M. (1990). Genetic variation, hippocampal mossy fibres distribution, novelty reactions and spatial representation in mice. Behav. Brain Res. 41, 61-70.

Schwerdtfeger W.K. and López-García C. (1986). GABA-ergic neurons in the cerebral cortex of the brain of a lizard (*Podarcis hispanica*). Neurosci. Lett. 68, 117-121.

Seress L. (1985). Postnatal neurogenesis in the rat hypothalamus. Dev. Brain Res. 22, 156-160.

Seress L. and Frotscher M. (1990). Morphological variability is a characteristic feature of granule cells in the primate fascia dentata: a combined Golgi/electron microscope study. J. Comp. Neurol. 293, 253.

Seress L. and Pokorny J. (1981). Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. J. Anat. 133, 181-195.

Shimizu I., Oppenheim R.W., O'Brien M. and Shneiderman A. (1990). Anatomical and functional recovery following spinal cord transection in the chick embryo. J. Neurobiol. 21, 918-937.

Soriano E., Del Río J.A., Martínez-Guijarro F.J. and López-García C. (1991). Immunocytochemical detection of 5'-bromodeoxyuridine in fluoro-gold-labeled neurons: A simple technique to combine retrograde axonal tracing and neurogenetic characterization of neurons. J. Histochem. Cytochem. 39, 1565-1570.

Stanfield B.B. and Trice J.E. (1988). Evidence that granule cells generated in the dentate gyrus of adult rats extended axonal projections. Exp. Brain Res. 72, 399-406.

Stevenson J. and Yoon M.G. (1980). Kinetics of cell proliferation in the halved tectum of adult goldfish. Brain Res. 184, 11-22.

Straznicky K. and Gaze R.M. (1971). The growth of the retina in *Xenopous laevis*, an autoradiographic study. J. Embryol. Exp. Morphol. 26, 67-79.

Straznicky K. and Gaze R.M. (1972). Development of the optic tectum in *Xenopus laevis*, an autoradiographic study. J. Embryol. Exp. Morphol. 28, 87-115.

Swanson L.W., Kohler C. and Bjorklund A. (1987). The limbic region. I. The septohippocampal system. In: Handbook of chemical neuroanatomy, vol 5, Integrated systems of the CNS, part I. Bjorklund A., Hokfelt T. and Swanson L.W. (eds). Elsevier. Amsterdam. pp125-277.

Tineo P., Planelles M.D. and Del Corral J. (1987). Modifications in cortical ependyma of the lizard *Podarcis hispanica* during postnatal development. J. Hirnforsch. 28, 485-489.