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Neurogenesis and Neuronal Regeneration in the Adult Reptilian Brain

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Adult neurogenesis · Neuronal regeneration · Stem cell · Radial glia · Brain · Telencephalon · Reptile · Lizard · Turtle

Abstract
Evidence accumulated over the last few decades demonstrates that all reptiles examined thus far continue to add neurons at a high rate and in many regions of the adult brain. This so-called adult neurogenesis has been described in the olfactory bulbs, rostral forebrain, all cortical areas, anterior dorsal ventricular ridge, septum, striatum, nucleus sphericus, and cerebellum. The rate of neuronal production varies greatly among these brain areas. Moreover, striking differences in the rate and distribution of adult neurogenesis have been noted among species. In addition to producing new neurons in the adult brain, lizards, and possibly other reptiles as well, are capable of regenerating large portions of their telencephalon damaged as a result of experimentally-induced injuries, thus exhibiting an enormous potential for neuronal regeneration. Adult neurogenesis and neuronal regeneration take advantage of the same mechanisms that are present during embryonic neurogenesis. New neurons are born in the ependyma lining the ventricles and migrate radially through the brain parenchyma along processes of radial glial cells. Several lines of evidence suggest that radial glial cells also act as stem cells for adult neurogenesis. Once they reach their final destination, the young neurons extend axons that reach appropriate target areas. Tangential migration of neurons alongside the ventricular ependyma has also been reported. Most of these tangentially migrating neurons seem to be destined for the olfactory bulbs and are, thus, part of a system similar to the mammalian rostral migratory stream. The proliferation and recruitment of new neurons appear to result in continuous growth of most areas showing adult neurogenesis. The functional consequences of this continuous generation and integration of new neurons into existing circuits is largely conjectural, but involvement of these phenomena in learning and memory is one likely possibility.

Introduction

Reports of abundant adult neurogenesis in representatives of all major vertebrate taxa have forced a revision of the long-standing dogma that new neurons are not added to the adult brain [Alvarez-Buylla and Lois, 1995; Gross, 2000; Alvarez-Buylla et al., 2001]. Although neurogenesis in adult mammals appears to be limited to a few areas in the brain [Gould et al., 1999a; Peretto et al., 1999; Hastings et al., 2001], numerous studies have documented

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widespread occurrence of this phenomenon in the brain of non-mammals. Reptiles, in particular, offer some of the best examples of adult neurogenesis in vertebrates. Every day thousands of new cells are added to the adult reptilian telencephalon, the majority of which differentiate into neurons and are recruited into pre-existing neural circuits. In addition, reptiles exhibit an enormous potential to replace damaged neurons by generating new ones after injuries. In particular, regeneration in the cerebral cortex of some lizards stands out as one of the best examples of structural plasticity in vertebrates studied thus far [Font et al., 1991, 1997].

In this paper, we will review the literature on adult neurogenesis and neuronal regeneration in the brains of reptiles. It is important, however, to clarify at the outset that the available evidence is based on research conducted on just a handful of species and that many features of reptilian neurogenesis and regeneration are still largely unexplored.

The monophyletic group that encompasses all living reptilian taxa also includes birds, which are the sister group of crocodiles. However, as commonly used, the term 'reptiles' refers to a paraphyletic assemblage consisting of approximately 7,200 species of turtles, crocodiles, lizards, snakes, and tuatara. Despite their highly corroborated position among the reptiles, birds are usually not considered reptiles. In this review, we will follow the traditional usage and examine adult neurogenesis and regeneration in diapsid reptiles and turtles, but not in birds. However, where appropriate we will draw comparisons with results obtained with birds and mammals in an attempt to find commonalities and differences among all three groups of amniotic vertebrates.

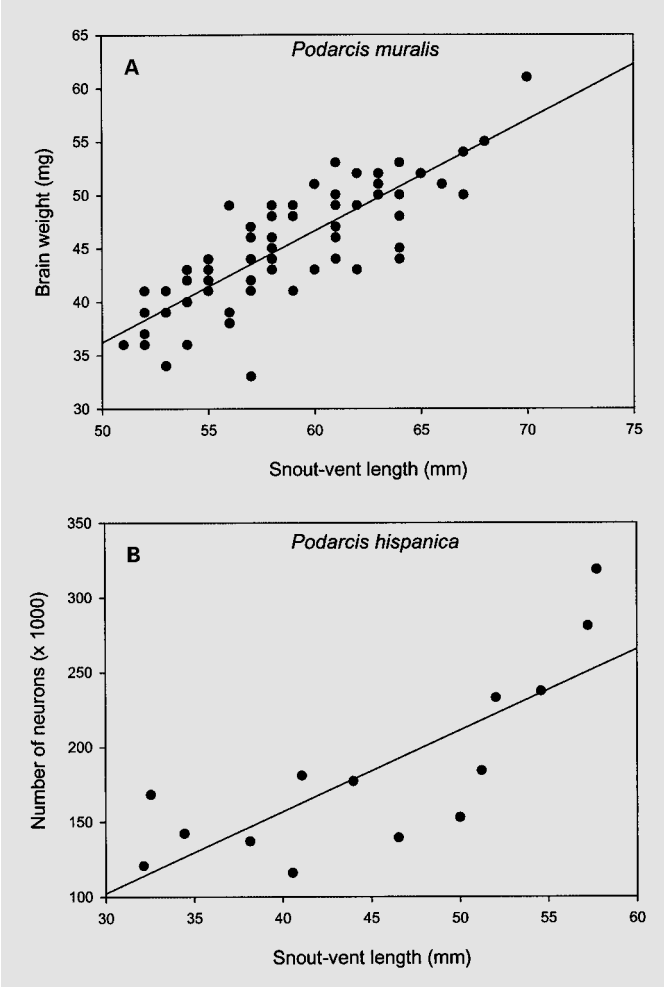

Fig. 1. Brain growth in lacertid lizards. A Regression plot of brain weight versus snout-vent length in *Podarcis muralis*; data from Platel [1974]. B Number of neurons in the medial cerebral cortex plotted against snout-vent length in *P. hispanica*; data from López-García et al. [1984]. Both data sets include male and female lizards.

Research on Adult Neurogenesis in Reptiles

During the 1950s and 1960s, occasional reports raised the possibility that neurogenesis may continue in the brain of adult reptiles. Källen [1951], Fleischhauer [1957], Kirsche [1967], and Schulz [1969] claimed that specialized 'matrix areas' containing undifferentiated cells are present in the ependymal lining of the ventricular walls in adult specimens of several reptile species. These matrix areas, usually located in or near sulci of the ventricular system, were assumed to be remnants of the embryonic germinative tissue that retained their proliferative potential into adulthood. In agreement with these observations, mitotic figures were described in the sulcal ependyma of adults of two lacertid lizards, *Lacerta agilis* [Schulz, 1969] and *Podarcis* (formerly *Lacerta*) *sicula* [Hetzel, 1974]. However, in these studies it was unclear whether proliferation in the ependyma led to the production of any surviving neurons or glial cells. Two lines of evidence provided support for the suggestion that reptiles may show adult neurogenesis. Firstly, morphometric studies revealed persistent growth of the reptilian brain well past pre- and perinatal stages of development [Platel, 1974]. Secondly, cell counts demonstrated that, at least in the cerebral cortex of the lizard *Podarcis hispanica*, the age-related size increase is the result of continuous addition of newly generated neurons [López-García et al., 1984] (fig. 1). At about the same time, work on another

Table 1. Brain areas exhibiting adult neurogenesis in reptiles

Species	MOB	AOB	RF	MC	DMC	DC	LC	Sp	St	ADVR	NS	Cb	Source
Podarcis hispanica	+	+	+	+	+	+	+	+	+	+	+	+	López-García et al., 1988a, 1990b; García-Verdugo et al., 1989; Pérez-Sánchez et al., 1989; Font et al., 1997
Psammodromus algirus	+	+	+										Peñafiel et al., 1996
Gallotia galloti				+	+								García-Verdugo et al., 1986
Tarentola mauritanica	+	+	+	+	+	+	+	+	+	+	+		Pérez-Cañellas and García-Verdugo, 1992, 1996
Anolis carolinensis				+									Duffy et al., 1990
Trachemys scripta	+	+	+	+	+	+	+	+	+	+	n.a.	+	Pérez-Cañellas and García-Verdugo, 1992; Pérez-Cañellas et al., 1997

Brain areas in which proliferation and/or recruitment of new neurons have been reported are indicated with a '+' sign. The evidence for adult neurogenesis in the cerebral cortex of the lizard *G. galloti* is based on observation of cells presumed to be radially migrating neuroblasts. In every other case, the evidence for neurogenesis was obtained using proliferation markers. Blanks represent areas for which data are not available. Our own unpublished data have been used to supplement published reports of adult neurogenesis in *P. hispanica*, *T. mauritanica*, and *T. scripta*. Turtles do not have a nucleus sphericus.

ADVR, anterior dorsal ventricular ridge; AOB, accessory olfactory bulb; Cb, cerebellum; DC, dorsal cortex; DMC, dorsomedial cortex; LC, lateral cortex; MC, medial cortex; MOB, main olfactory bulb; NS, nucleus sphericus; RF, rostral forebrain; Sp, septum; St, striatum.

lacertid, *Gallotia* (formerly *Lacerta*) *galloti*, revealed the presence of immature cells in the inner plexiform layer of the medial and dorsomedial cortices, in close association with processes of radial glial cells. These cells were elongated or fusiform, with their major axis oriented perpendicular to the ventricular surface. According to their ultrastructural characteristics, they were identified as immature radially migrating neuroblasts [García-Verdugo et al., 1986]. Further studies found mitoses in the sulcal ependyma of adults of the same species [Yanes-Méndez et al., 1988a].

An important step forward in the study of neurogenesis was the introduction of [^{3}H]thymidine in the late 1950s. This tritiated thymidine analogue is incorporated into the DNA of dividing cells and can be detected in their progeny by means of autoradiography. This technique was successfully applied to the study of embryonic brain development in reptiles [e.g., Schwab and Durand, 1974; Goffinet et al., 1986], but until the late 1980s it was not used to examine adult neurogenesis in representatives of this vertebrate class. In 1988, López-García and his colleagues published the first of a series of reports describing areas of adult neurogenesis in the brain of the Iberian wall lizard, *Podarcis hispanica* [López-García et al., 1988a]. Combining autoradiography and electron microscopy, they also showed that those [^{3}H]thymidine-labeled cells that are recruited into several telencephalic areas in lizards of all ages have ultrastructural characteristics of neurons, such as dendrites and synapses, but not of glial cells.

Areas Showing Adult Neurogenesis: Regional and Interspecific Variation

Systematic surveys of adult neurogenesis using modern proliferation- and cell-type-specific markers have been conducted in only two lizards and one turtle species. There are scattered reports of adult neurogenesis in other lizards, but the available data are limited to one or a few brain regions (table 1). Data are entirely lacking for the majority of lizard families, crocodiles, and snakes, although it has been hypothesized that postnatal neurogenesis may also occur in the brain of natricine snakes [Holtzman and Halpern, 1991; Holtzman, 1993].

In this section, we will review the rather limited information available on the distribution of areas showing adult neurogenesis in the brain of reptiles. Mitotically active cells in S-phase have been detected by [^{3}H]thymidine autoradiography, 5-bromo-2′-deoxyuridine (BrdU) labeling, or proliferating cell nuclear antigen (PCNA) immunohistochemistry. Confirmation of the phenotype of neurons born in the adult brain has been sought in some, but not all, cases by examining the ultrastructure of [^{3}H]thymidine-labeled cells.

Neurons born in adulthood have been found in all the major subdivisions of the lacertilian telencephalon, including the main and accessory olfactory bulbs, rostral forebrain, all four cortical areas, septum, anterior dorsal ventricular ridge, striatum, and nucleus sphericus [López-García et al., 1988a, b, 1990b; García-Verdugo et al., 1989; Pérez-Sánchez et al., 1989; Pérez-Cañellas and García-Verdugo, 1992, 1996; Font et al., 1997; E. Font, E. Desfilis, M.M. Pérez-Cañellas, J.M. García-Verdugo,

unpublished observations]. This widespread neurogenesis contradicts claims that adult neurogenesis in lizards is restricted to the medial cerebral cortex [Molowny et al., 1995; Nacher et al., 1996].

Where quantitative data are available, these brain regions display regional, as well as interspecific, differences in their neurogenic capacity. In the telencephalon of the lacertid *Podarcis hispanica* the rate of incorporation of new neurons is highest in the nucleus sphericus and lowest in the septum (fig. 2). In the geckonid *Tarentola mauritanica*, on the other hand, the majority of neurons born in adulthood are destined for the medial cerebral cortex (fig. 2). Because different telencephalic areas vary in the size of their neuronal populations, neurogenesis can be relatively intense even in areas that, in absolute terms, display a rather low rate of mitoses. In *Tarentola mauritanica*, for example, the highest labeling index (calculated as the ratio of the number of labeled to unlabeled neurons) is found in the anterior dorsal ventricular ridge, an area where relatively few neurons are produced [Pérez-Cañellas and García-Verdugo, 1996]. The reasons for these interspecific differences are not clear.

Proliferative areas in the ependyma of turtles were described half a century ago [Fleischhauer, 1957]. Recently, work with hatchling and juvenile red-eared slider turtles, *Trachemys scripta elegans*, has demonstrated the existence of neurogenesis in many areas that also display adult neurogenesis in lizards [Pérez-Cañellas et al., 1997] (table 1). However, in contrast to lizards, postnatal neurogenesis in the turtle telencephalon appears to be a residual phenomenon given the small number of neuroblasts that are recruited into telencephalic neuronal populations (fig. 2). The olfactory bulbs are exceptional in this respect, as they incorporate large numbers of new neurons, but the mechanisms responsible for olfactory bulb neurogenesis may be different from those in the rest of telencephalon (see below).

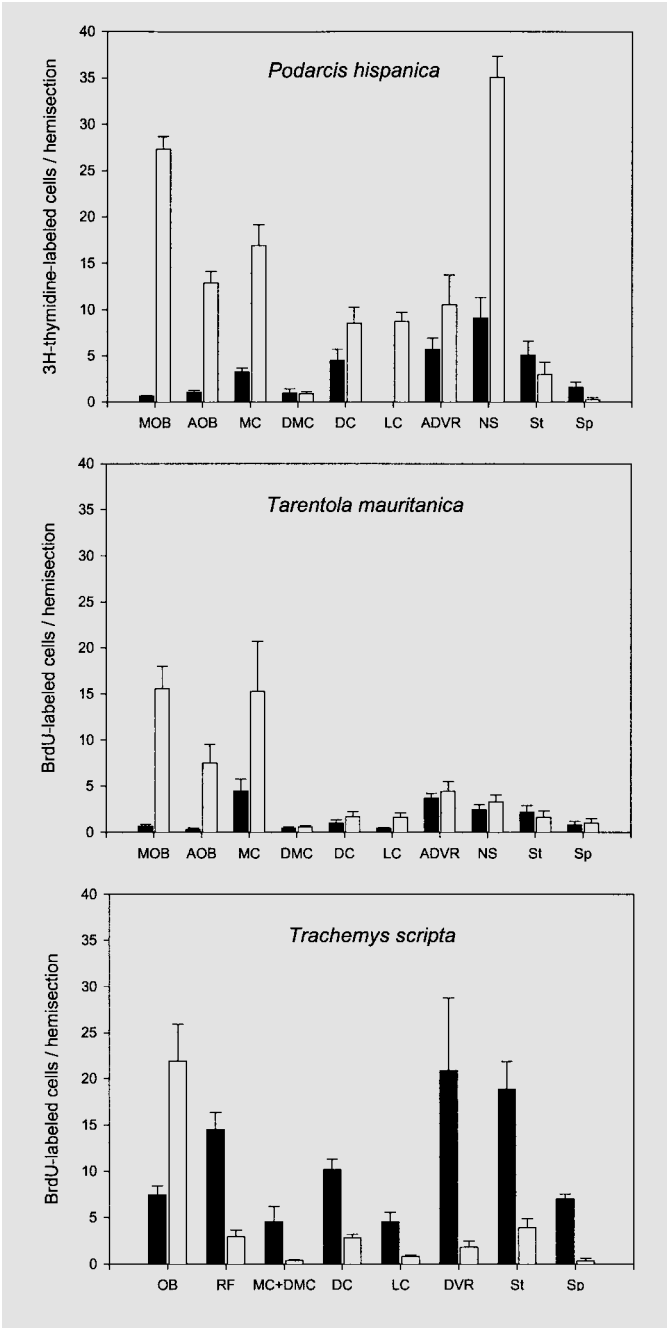

Fig. 2. Mean number of [^{3}H]thymidine or BrdU-labeled cells in the telencephalon of two lizard species (*P. hispanica* and *T. mauritanica*) and one turtle species (*T. scripta*). Black bars correspond to labeled ventricular zone (VZ) cells, whereas clear bars represent the number of labeled neuron-like cells outside the VZ (obvious endothelial and glial cells were excluded from the counts, as were putative migrating neurons). Sample sizes are four (*P. hispanica* and *T. scripta*) and eight (*T. mauritanica*) individuals. Survival times are roughly 30 days for the two lizard species and six months for the turtle. In all cases, labeled cells were counted in evenly spaced transverse paraffin hemisections covering all major subdivisions of the telencephalon. Counts of labeled cells were used to derive an average for each region of interest and are expressed as mean number of [^{3}H]thymidine or BrdU-labeled cells per hemisection. Note difference in distribution of labeled cells between the turtle and the two lizard species. Although more cells are labeled in the brain of *P. hispanica* than in the other two species, the difference could be due to the use of different proliferation markers. Standard error is shown by the vertical lines over the bars. ADVR, anterior dorsal ventricular ridge; AOB, accessory olfactory bulb; DC, dorsal cortex; DMC, dorsomedial cortex; DVR, dorsal ventricular ridge; LC, lateral cortex; MC, medial cortex; MOB, main olfactory bulb; NS, nucleus sphericus; OB, olfactory bulbs; RF, rostral forebrain; Sp, septum; St, striatum.

Outside the telencephalon, adult neurogenesis has been described only in the cerebellum of *Podarcis hispanica* [López-García et al., 1990b]. The cerebellum may be a site of adult neurogenesis in other reptiles, as we have found labeled cells in the cerebellum of *Trachemys scripta* turtles previously injected with BrdU [E. Font, E. Desfilis, M.M. Pérez-Cañellas, J.M. García-Verdugo, unpublished observations]. The BrdU-labeled cells are morphologically indistinguishable from neighboring granular cerebellar neurons, which suggests that they are neurons rather than glial cells. However, ultrastructural confirmation of the phenotype of cells born in the adult cerebellum is lacking for these two species.

Ventricular Zone Origin of Neurons Born in Adulthood

Neurogenesis in the adult reptilian brain follows a pattern similar to that described for the embryonic development of the brain [Goffinet, 1983]. New neurons are born in the ependyma lining the ventricular walls, here referred to as the ventricular zone (VZ), and migrate through the brain parenchyma to their final destination [López-García et al., 1988a, 1990a; Pérez-Cañellas and García-Verdugo, 1996] (fig. 3). The VZ ependyma shows marked regional variation and includes areas of pseudostratified epithelium with ultrastructural features that suggest proliferative capacity [Yanes-Méndez et al., 1988a, b]. In the telencephalon, these areas of specialized ependyma are located in or near sulci of the lateral ventricular system [Kirsche, 1967; Schulz, 1969; Tineo et al., 1987; Yanes-Méndez et al., 1988a]. Although four proliferative sulci have been recognized (sulcus lateralis, sulcus septomedialis, sulcus ventralis, and sulcus terminalis), only the sulcus septomedialis, which supplies neurons for the overlying medial cerebral cortex, has been studied in some detail in relation to adult neurogenesis. The sulcus septomedialis has been considered a proliferative 'hot spot', similar to the neurogenic centers responsible for adult neurogenesis in birds [Alvarez-Buylla et al., 1990a]. However, comparative evidence suggests that the role of ventricular sulci in relationship to adult neurogenesis in reptiles may have been overrated.

In lizards and turtles, shortly after injection of a proliferation marker labeled cells are found throughout the walls of the lateral ventricles, suggesting that neurons are born by division of VZ cells (fig. 4). Only rarely are labeled cells seen in the walls of the third, tectal, and fourth ventricles. The distribution of labeled cells in the VZ of the lateral ventricles is not homogeneous. In *Tarentola mauritanica*, the highest density of labeling occurs precisely in the telencephalic sulci. In contrast, there seems to be no clear correspondence between areas of proliferative VZ and ventricular sulci in *Podarcis hispanica* and *Trachemys scripta*. Moreover, the proliferative VZ supplying neurons for some telencephalic areas with intense neurogenic activity in lizards (e.g., dorsal cortex or nucleus sphericus) is located in areas of non-sulcal ependyma. Thus, the available evidence is not entirely consistent with the proposal of hot spots in reptiles. Rather, it suggests that most regions of the telencephalic VZ are neurogenic.

Recently, PCNA immunohistochemistry has been used to reveal the extent and the distribution of proliferative ependyma in the telencephalon of adult *Podarcis sicula* lizards [Margotta et al., 1999]. In keeping with the results obtained with other reptiles, PCNA-positive cells are found in all four ventricular sulci, but some are also present in areas of non-sulcal ependyma.

Development of Neurons Born in the Adult Telencephalon

The cerebral cortex of reptiles is usually divided into four major cytoarchitectonic areas: medial cortex, dorsomedial cortex, dorsal cortex, and lateral cortex. These cortical areas appear in transverse sections of the telencephalon as longitudinal strips extending from the dorsal septum medially to the ventrolateral surface of the hemispheres laterally (fig. 3). Each cortical area is a trilaminate structure, with a tightly packaged cell layer sandwiched between relatively cell-poor outer and inner plexiform layers [Ulinski, 1990]. In the lizard *Podarcis hispanica*, approximately 14% of all new neurons are generated in the medial cerebral cortex (fig. 2). Neurogenesis in the medial cortex VZ is followed by daughter cell migration into the parenchyma along the distal processes of radial glial cells, as in forebrain development [Goffinet, 1983]. One day after administration of a proliferation marker, no labeled cells are found outside the VZ. With survival times of 1–3 weeks, many fusiform cells with labeled nuclei, thought to be young migrating neurons, are found in the inner plexiform layer. These cells are found at increasing distances from the ventricular wall with increasing survival time. Labeled cells that look like mature neurons are also located, at this time, in the inner plexiform layer and in the cell layer. With still longer survival times (more than 1 month), most labeled cells are found in

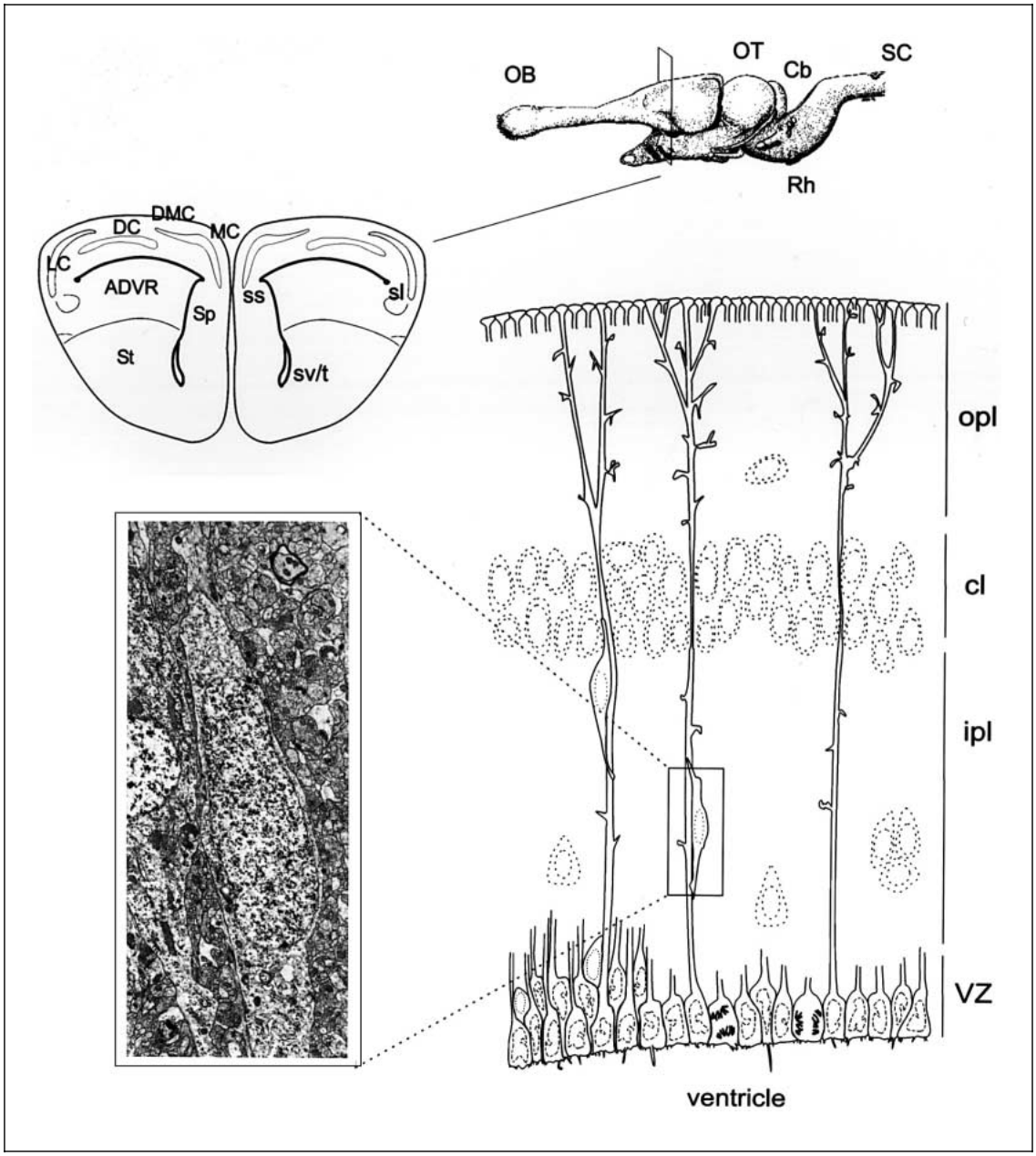

Fig. 3. Summary of the major developmental processes involved in adult neurogenesis in the reptilian telencephalon. Top: The drawing on the right shows a lateral view of the brain of a lizard indicating the position of a transverse section through the telencephalon, which is depicted in schematic form on the left. Bottom: The figure on the right side illustrates the main events that take place during adult neurogenesis in the cortex of lizards. The ependymal cells in the ventricular zone (VZ) form a monostratified or pseudostratified epithelium with apical expansions (radial glial fibers) that traverse the brain parenchyma and reach the pial surface, where they terminate as subpial endfeet. The glial fibers are covered by lamellate excrescences which decrease in density as the fibers traverse the cortical cell layers. Radial glial cells in contact with the ventricular lumen divide and give rise to immature neurons. The latter migrate in association with radial glial fibers until they reach their final destination in the inner plexiform layer (ipl), or in the cell layer (cl), where they become indistinguishable from the surrounding neurons. An electron micrograph of a migrating neuron from the cerebral cortex of *Gallotia galloti* is shown in the left inset. ADVR, anterior dorsal ventricular ridge; Cb, cerebellum; DC, dorsal cortex; DMC, dorsomedial cortex; LC, lateral cortex; MC, medial cortex; OB, olfactory bulbs; opl, outer plexiform layer; OT, optic tectum; Rh, rhombencephalon; SC, spinal cord; sl, sulcus lateralis; Sp, septum; ss, sulcus septomedialis; St, striatum; sv/t, sulcus ventralis/terminalis.

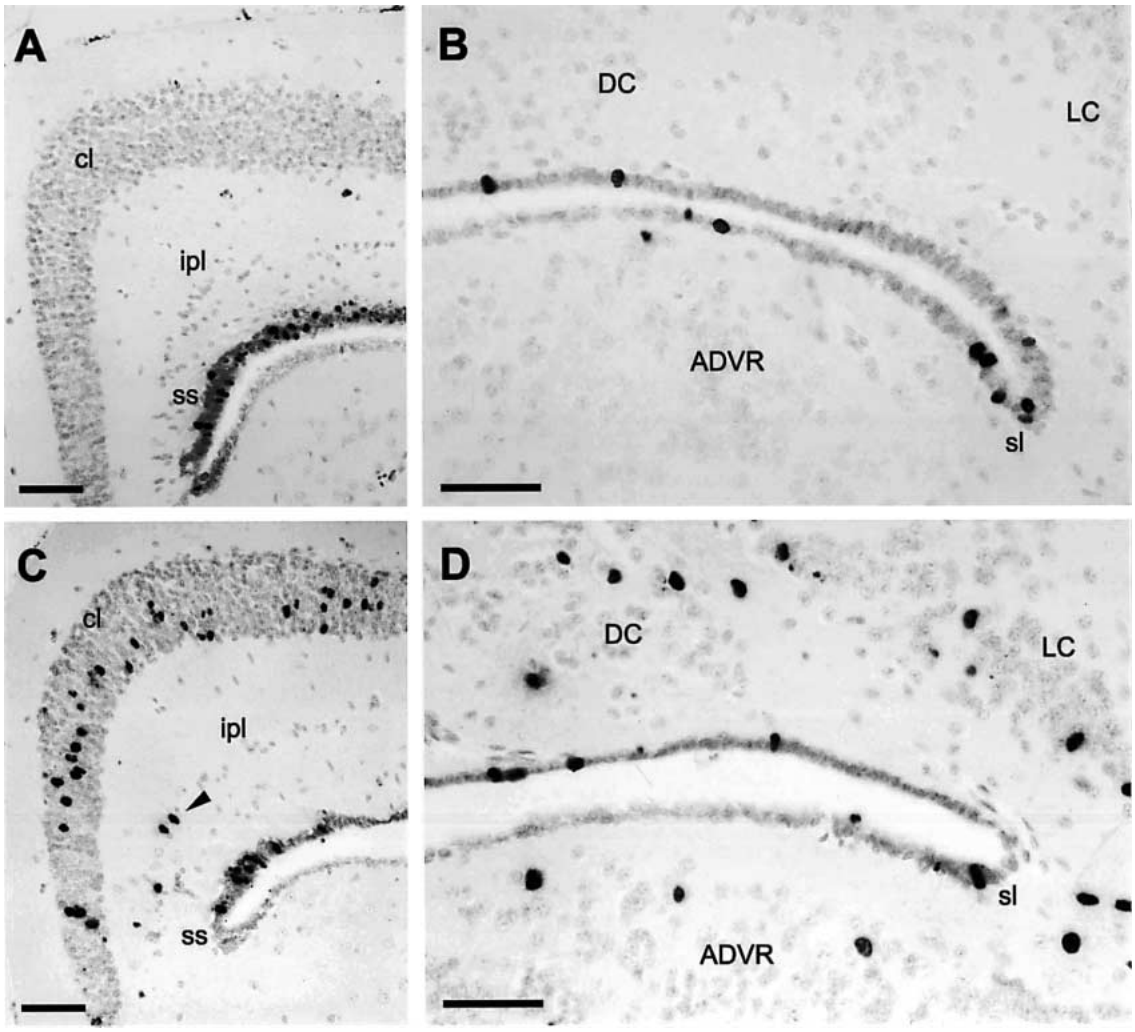

Fig. 4. Photomicrographs illustrating the distribution of BrdU-labeled cells in transverse hemisections through the telencephalon of adult lizards (*T. mauritanica*). Shortly after BrdU administration, most labeled cells are found in areas of specialized ventricular zone (VZ) ependyma located in or near sulci of the ventricular system. With longer survival times after BrdU administration, labeled cells are also found in the inner plexiform layer (ipl) and in the cell layer (cl) of most telencephalic areas. A, B Labeled VZ cells in the sulcus septomedialis, ss (A) and sulcus lateralis, sl (B) seven days after BrdU administration (note the presence of a few labeled cells in areas of non-sulcal ependyma). C, D Labeled cells in the medial cortex (C) and in other pallial areas (D) 30 days after BrdU administration. At this time, labeled cells are still present in the sulcal ependyma. Cells resembling migrating neurons are also labeled in the ipl of the medial cortex (arrowhead). ADVR, anterior dorsal ventricular ridge; DC, dorsal cortex; LC, lateral cortex. Scale bars, 50 μm.

the cell layer, where they are indistinguishable from the surrounding neurons. Based on this evidence, we infer that it takes a minimum of seven days for neurons to be born, to migrate, and to differentiate in the adult lacertilian brain [López-García et al., 1990a].

Though the preceding details were obtained from the medial cortex of *Podarcis hispanica*, the overall process resembles that observed in other telencephalic areas and in other lizard species (fig. 4). In telencephalic areas with a well-defined cell layer (e.g., cortex or nucleus sphericus), most new neurons are recruited into the main cell layer of the corresponding structures. One exception to this pattern is the dorsal cortex, where the new neurons do not reach the cell layer, but remain instead in the inner plexiform layer (fig. 4D). In fact, the inner plexiform layer may also be the final destination for at least part of the neurons born in the remaining cortical areas, as some labeled cells resembling mature neurons are found at this location even with the longest survival times. Neuronal recruitment in the septum, striatum, and anterior dorsal ventric-

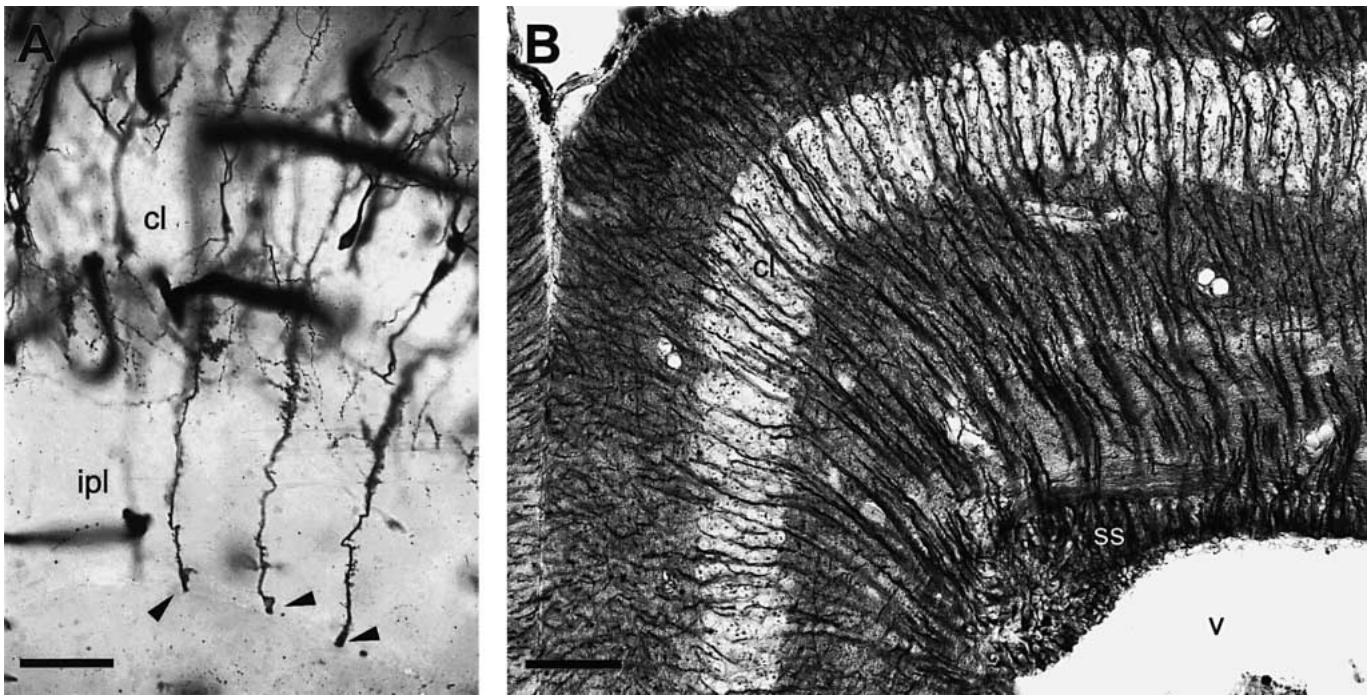

Fig. 5. Radial glial cells in the medial cortex of adult lizards. A Photomicrograph of Golgi-stained radial glial cells in the medial cortex of *Gallotia galloti*. Arrowheads point to radial cell perikarya. B Photomicrograph of transverse section through the telencephalon of *T. mauritanica* immunostained for GFAP. The medial cortex, which is a paired structure, lies on the dorsomedial surface of the telencephalon, above the lateral ventricle. Only the right side is shown here, but part of the left side can also be seen. Note radial orientation of the stained fibers in this area. cl, cell layer; ipl, inner plexiform layer; ss, sulcus septomedialis; v, ventricle. Scale bars, 50 μm.

ular ridge does not seem to be restricted to any anatomical subdivision within these structures. Interestingly though, new neurons in the anterior dorsal ventricular ridge of *Tarentola mauritanica* are added only to its dorsalmost portion, close to the overlying proliferative VZ [Pérez-Cañellas and García-Verdugo, 1996].

In most telencephalic areas, migration of new neurons away from the VZ usually follows a radial path. The cells generated in the VZ migrate within the first few days of their life towards specific target areas in close association with the processes of radial glial cells. Ependymal or radial glial cells are adult derivatives of the embryonic radial glia, the somata of which form a monostratified or pseudostratified epithelium lining the cerebral ventricles [Ulinski, 1990; Yanes et al., 1990]. Radial glia are generated before neurogenesis and guide neuronal migration in the developing brain [Rakic, 1990]. In mammals, radial glial cells disappear or become astrocytes within days to weeks after birth [Voigt, 1989; Chanas-Sacre et al., 2000], but in non-mammals they persist into adulthood as radial glia.

The morphology and distribution of radial glia in the adult reptilian brain has been investigated with traditional methods, such as Golgi staining and electron microscopy [Stensaas and Stensaas, 1968; García-Verdugo et al., 1981], as well as immunohistochemically with antibodies against glial fibrillary acidic protein (GFAP) or glutamine synthetase [Onteniente et al., 1983; Dahl et al., 1985; Kriegstein et al., 1986; Monzón-Mayor et al., 1990; Yanes et al., 1990; Kálmán et al., 1994]. Radial glia cell bodies are elongated or cuboidal and join those of neighboring ependymal cells via junctional complexes. A long distal process (radial glial fiber) emerges from the cell body and usually divides up into two or more separate branches. In the cerebral cortex, radial glial processes span the full width of the cortical parenchyma and reach the pial surface where they terminate as subpial endfeet to form a glial limiting membrane (fig. 5). Elsewhere in the telencephalon, radial processes end either subpially or on blood vessels, where they form perivascular endfeet. Radial glia provide a scaffolding of processes that guide the radial migration of new neurons. Radial glial cells may also be

multipotent progenitor cells that give rise to various cell types, including neurons (see below). Despite their critical role in relationship to adult neurogenesis and regeneration, very little is known about cell-cell interactions between radial glia and newly generated neurons in reptiles [García-Verdugo et al., 1986].

Migrating neurons undergo subtle morphological changes as they travel along the glial fibers. Near the VZ, newly formed neurons in the cerebral cortex of adult *Gallotia galloti* have an elongated nucleus 3–4 μm in diameter with a central nucleolus and spongy chromatin. As they move away from the VZ, the chromatin becomes more clumped and the nuclear volume increases by up to 35%. Concurrent with these nuclear changes, there is also an increase in granular endoplasmic reticulum membranes and Golgi dictyosomes with increasing distance from the proliferative VZ [García-Verdugo et al., 1986]. These morphological changes are indicative of neuronal maturation and suggest that migrating neurons undergo some maturational changes while in transit toward their final destination. There is no evidence that the new neurons undergo further division in the course of their migration.

Once they reach the cell layer of the medial cortex, the young neurons extend axons that reach appropriate target areas. This was shown by applying the retrograde tracer horseradish peroxidase (HRP) to the dorsal and dorsomedial cortices of lizards injected with [^{3}H]thymidine one to two months earlier [López-García et al., 1990a]. Following a short survival time after HRP application, the lizards were sacrificed and their brains processed, first for histochemical detection of HRP, and then for autoradiography. As neurons in the cell layer of the medial cortex project to the dorsal and dorsomedial cortices [Ulinski, 1990], the finding of double-labeled neurons in the medial cortex ipsilateral to the site of HRP injection provides evidence that at least some neurons born after the [^{3}H]thymidine pulse had time to migrate to the cell layer and extend an axon that reached as far as the areas injected with HRP. This suggests that cells born in the adult medial cortex become adult neurons that are incorporated into pre-existing functional circuits.

Neurogenesis in the brains of adult birds follows a course similar to that described in reptiles. However, in adult birds radial glial fibers do not span the full width of the telencephalic parenchyma so that young neurons are guided by glial fibers only during the first part of their migratory journey [Alvarez-Buylla et al., 1988; Alvarez-Buylla, 1990]. A further difference between birds and reptiles is that in the former only one third of the migrating young neurons become neurons, while the remaining two thirds fail to differentiate and degenerate [Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla, 1990]. Because no degenerating cells labeled with [^{3}H]thymidine have been found in lizards or turtles, it seems reasonable to conclude that the majority of neurons produced in the adult reptilian telencephalon complete their development and become part of existing neuronal populations.

The Reptilian Rostral Migratory Stream

In most areas undergoing adult neurogenesis, the newly produced cells migrate a short distance to their final destination which is usually located in the parenchyma facing the proliferative VZ. One striking exception to this rule is the long-distance migration of neurons destined for the olfactory bulbs. In adult rodents and primates, new neurons are constantly recruited to the olfactory bulbs from progenitor cell populations located in the rostral forebrain [Luskin, 1993; Lois and Alvarez-Buylla, 1994; Kornack and Rakic, 2001]. The postnatally generated cells originate in a discrete region of the subventricular zone lining the lateral ventricles and migrate anteriorly to form a highly restricted migratory route known as the rostral migratory stream (RMS), which runs from the rostral forebrain into the olfactory bulbs [Lois and Alvarez-Buylla, 1994; Doetsch and Alvarez-Buylla, 1996]. Unlike the radial glial-guided migration used by most migrating neurons in the adult telencephalon of reptiles and birds, cells undergoing chain migration in the mammalian RMS migrate in a neurophilic mode, with neurons moving upon one another and surrounded by tubes of astrocytes that demarcate the RMS [Lois et al., 1996]. This neurogenic migratory system is presumed to be a generic feature of all adult mammalian brains. However, its existence in other vertebrate taxa is insufficiently documented.

The main and accessory olfactory bulbs are among the most important sites for adult neurogenesis in all reptilian species examined to date (fig. 2). Work on two lizards and one turtle species indicates that, as in mammals, the new olfactory bulb neurons are not produced in situ, but instead migrate to their final destination from distant proliferative zones located in the telencephalon caudal to the olfactory bulbs. In the turtle *Trachemys scripta*, olfactory bulb neurons make up almost two thirds of all neurons born in adulthood. Yet, proliferative activity in the ependyma of the olfactory bulbs is very scant. In contrast, proliferation is very intense in the rostral forebrain, particularly in the VZ facing the anterior olfactory nucleus, but

few neurons are recruited into the surrounding parenchyma (fig. 2). This finding, together with the observation of elongated cells presumed to be tangentially migrating neuroblasts in the layer immediately subjacent to the VZ provides suggestive evidence for a migratory system similar to the mammalian RMS [Pérez-Cañellas et al., 1997]. Assuming these conclusions about olfactory bulb neurogenesis, it remains unknown whether the rostral forebrain VZ is the sole supplier of neurons destined for the olfactory bulbs in turtles.

In the lizard *Tarentola mauritanica*, shortly after BrdU administration labeled cells are very abundant in the sulcus ventralis/terminalis. Thirty days after application of BrdU there are almost no labeled cells in this sulcus, yet the surrounding striatum contains very few labeled cells. This mismatch suggests that either the newly generated neuroblasts die shortly after birth, or they migrate to distant brain areas. Because no cell death has been detected in this area, it has been suggested that at least part of the neurons born in this sulcus could undergo tangential migration to the olfactory bulbs in a manner resembling the mammalian RMS [Pérez-Cañellas and García-Verdugo, 1996]. As in turtles, proliferation, but not recruitment, is also very intense in the rostral forebrain VZ [E. Font, E. Desfilis, M.M. Pérez-Cañellas, J.M. García-Verdugo, unpublished observations], raising the possibility that both these areas contribute neurons to the olfactory bulbs of adult *Tarentola mauritanica*. Recently, proliferation in the rostral forebrain VZ has also been described in the lizard *Podarcis sicula* [Margotta et al., 1999].

Tangential migration of neuroblasts from distant proliferative zones was directly addressed in a study examining the lizard *Psammodromus algirus* [Peñafiel et al., 1996]. Lizards were injected with [^{3}H]thymidine and sacrificed after survival times of 2, 15, and 33 days. Shortly after the tracer injection, most labeled cells were located in the VZ of the rostral forebrain. With increasing survival times, labeled cells became more abundant first in the olfactory peduncle and then in the olfactory bulbs, where labeled cells were indistinguishable from adjacent neurons in the granule cell layer. Further, Nissl-stained sections of lizards with intermediate survival times revealed the presence of elongated cells, some of which were labeled with [^{3}H]thymidine, with their major axes oriented parallel to the VZ of the olfactory peduncle. These elongated cells form chains in the parenchyma immediately subjacent to the VZ and are presumed to be migrating neuroblasts *en route* to their final destination in the olfactory bulbs. Unfortunately, in this study proliferation was not examined beyond the rostral forebrain. Thus, it is possible that at least some of the neuroblasts recruited into the olfactory bulbs originate from even more caudally located proliferative areas.

Taken together, these findings demonstrate the generation and tangential migration of neuroblasts from distant proliferative zones in the telencephalic VZ to the olfactory bulbs in adult reptiles. As in mammals, the migrating neuroblasts do not seem to penetrate the surrounding parenchyma and migrate instead alongside the ventricular surface, suggesting the presence of guidance cues that spatially restrict migration. Despite the superficial similarity, we do not know whether migrating neuroblasts in the reptilian RMS are surrounded by tubes of glial cells as in mammals.

Cell Types Born in the Adult Telencephalon

Cellular proliferation in the telencephalic VZ could, in principle, give rise to glial cells and/or neurons. The phenotype of cells born in the adult reptilian brain has been determined by electron microscopic analysis of light microscopic autoradiograms or combined [^{3}H]thymidine autoradiography and immunohistochemistry for cell-specific markers. In lizards, the vast majority of [^{3}H]thymidine-labeled cells found outside the proliferative VZ are not immunoreactive for GFAP, a marker of astroglia, and have the ultrastructural characteristics of neurons (fig. 6). This suggests that cellular proliferation in the VZ of adult lizards produces essentially new neurons and no free glial cells [López-García et al., 1988a; Pérez-Cañellas and García-Verdugo, 1996]. This conclusion is supported by reports indicating that, in the intact brain, free glial cells are very scarce in the telencephalon of lizards. In the cerebral cortex, their numbers are estimated at less than 5%. They include microglial cells with a distinctly laminar pattern of distribution and oligodendroglial cells in areas with myelinated fibers [García-Verdugo et al., 1981; Yanes et al., 1992; Font et al., 1995]. No GFAP-positive astrocytes have been observed in the intact telencephalon of lizards [Dahl et al., 1985; Yanes et al., 1990; Pérez-Cañellas and García-Verdugo, 1996; but see Monzón-Mayor et al., 1990]. In fact, GFAP immunohistochemistry has confirmed that, unlike adult mammals and birds, in the adult lacertilian telencephalon radial glial cells are the predominant astroglial element (fig. 5B). The possibility that proliferation in the VZ gives rise to new radial glial cells has not been explored.

Unlike the situation in lizards, adult neurogenesis in the turtle *Trachemys scripta* results in both neurons and

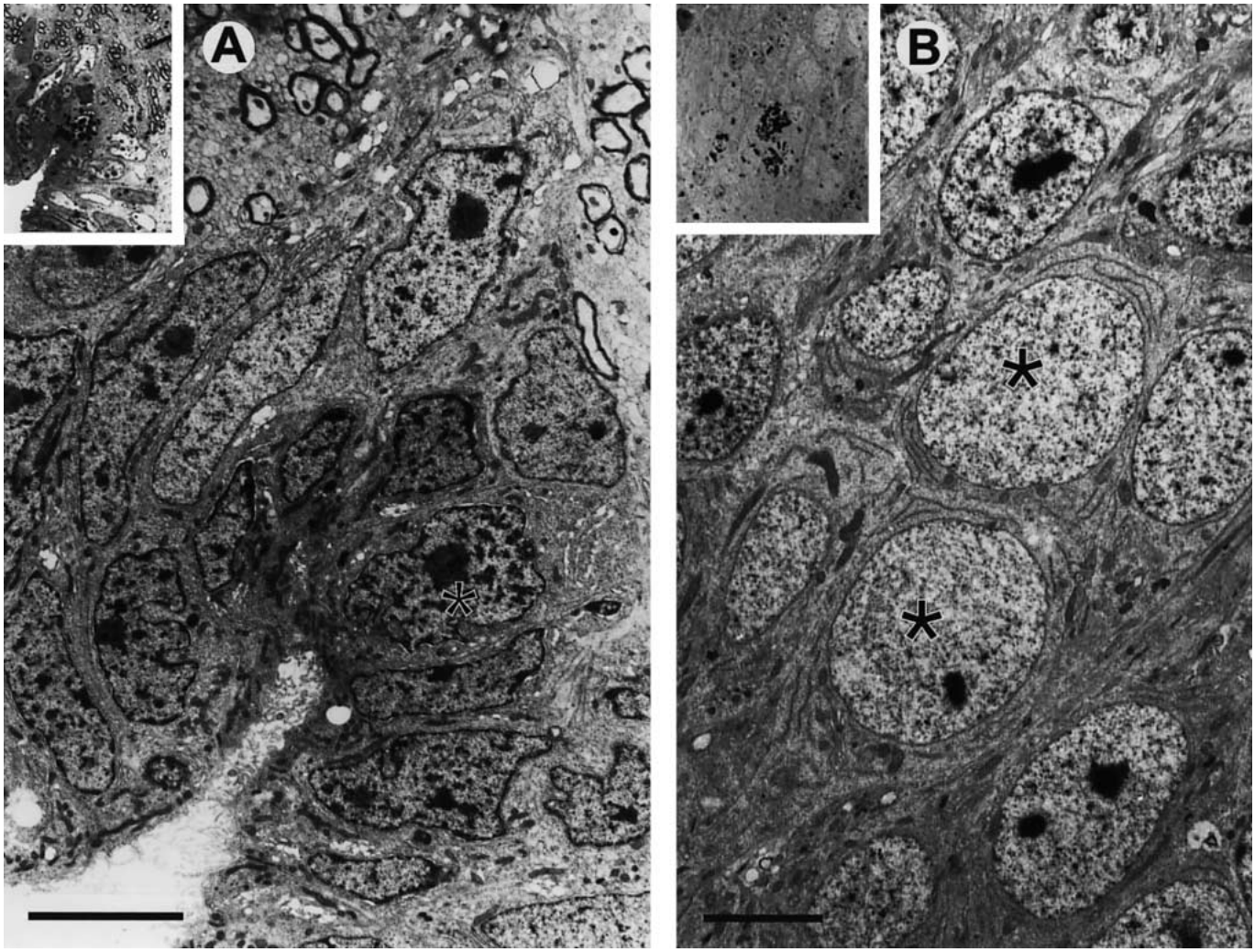

Fig. 6. Electron micrographs of [^{3}H]thymidine-labeled cells in the medial cortex of *P. hispanica*. A Ultrastructure of labeled ependymal cell (asterisk) one day after [^{3}H]thymidine injection. B Ultrastructure of two labeled cells (asterisks) in the cell layer of the medial cortex of a lizard sacrificed five weeks after [^{3}H]thymidine administration. The newly generated cells display ultrastructural features typical of neurons, such as a large cell body and a round or oval nucleus with pale karyoplasm and a prominent nucleolus, and are indistinguishable from the adjacent neurons. The insets show details of the radiolabeled cells in the toluidine-stained light microscopic autoradiograms from which the ultrathin sections were prepared (note the presence of silver grains above the nuclei of [^{3}H]thymidine-labeled cells). Scale bars, 5 μm.

free glial cells. Glial cell production appears to be restricted to the striatum, where [^{3}H]thymidine-labeled astrocytes and oligodendrocytes can be seen free in the parenchyma as well as in satellite association with neurons [Pérez-Cañellas et al., 1997]. This finding apparently contradicts reports that GFAP-positive astrocytes are absent in the telencephalon of adult turtles [Onteniente et al., 1983; Kálmán et al., 1994]. However, Kriegstein et al. [1986] described GFAP-negative astrocytes in the striatum of the turtle, which may correspond to the labeled astrocytes observed by Pérez-Cañellas et al. [1997].

In mammals adult neurogenesis produces essentially microneurons [Altman, 1970], but in adult reptiles microneurons (e.g., in the olfactory bulbs) as well as large neurons (e.g., in the medial and dorsal cortices, anterior dorsal ventricular ridge, and nucleus sphericus) are produced. Moreover, the widespread occurrence of neurogenesis in the telencephalon of adult reptiles suggests that multiple types of neurons are produced, as has been described for the avian telencephalon, where both interneurons and projection neurons are generated [Alvarez-Buylla, 1990; Nottebohm and Alvarez-Buylla, 1993].

At least some of the neurons recruited into the cerebral cortex of adult *Podarcis hispanica* are inhibitory neurons. Using antibodies against GABA and parvalbumin, Martínez-Guijarro et al. [1994] were able to detect a several-fold increase in the number of GABA- and parvalbumin-immunoreactive cells in the cortex of adult lizards, as compared to newborns. From this, the authors infer that a fraction of the neurons generated postnatally in this species are GABA- and/or parvalbumin-containing neurons which participate in inhibitory cortical circuits.

Two neuronal types are found in the cell layer of the medial cortex in lizards. These are distinguished on the basis of cell body size and ultrastructure. Small (type I) neurons are characterized by a small round nucleus with clumped chromatin and scarce cytoplasm containing few organelles. Large (type II) neurons have a large round or oval nucleus with dispersed chromatin and abundant cytoplasm containing numerous organelles. Large neurons predominate in the medial cortex of young lizards, whereas small neurons are the most abundant type in adults [López-García et al., 1984]. Interestingly, all [^{3}H]thymidine-labeled neurons in the cortex of *Podarcis hispanica* and *Tarentola mauritanica* are large. This suggests that large neurons may eventually transform into small ones, possibly due to a maturation process associated with chromatin condensation [Pérez-Cañellas and García-Verdugo, 1996]. This conversion from large to small neurons has also been observed in birds [Kirn et al., 1999].

Continuous Growth, Neuronal Replacement, or Both?

As stated above, the reptilian brain shows ongoing growth beyond the postnatal stage (fig. 1A). However, an increase in brain mass or volume could be due to an increase in neuronal size or number, or to a decrease in packing density. The finding of widespread neurogenesis in adult lizards and turtles suggests that the age-dependent increase in brain size may be partly due to the addition of new neurons. Indeed, cell counts performed in the cerebral cortex of *Podarcis hispanica* of different sizes (reflecting different ages) confirm that recruitment of newly generated neurons is partly responsible for the size increase in this telencephalic area [López-García et al., 1984] (fig. 1B). The population of cortical neurons roughly doubles during the first three years after birth. At the end of this period, individuals of this species reach their largest snout-vent length. The available evidence, however, is inconclusive as to whether the recruitment of new neurons in the cerebral cortex or elsewhere in the reptilian brain is accompanied by neuronal replacement.

As in the cortex of lizards, the number of cells in the brain of adult gymnotiform fish increases with increasing size (age) of the fish [Zupanc and Horschke, 1995]. In the cerebellum, where most of the neurons generated postembryonically are located, approximately 50% of the young cells are eliminated through apoptosis within the first few weeks after their generation [Soutschek and Zupanc, 1996; Zupanc et al., 1996]. The other 50% integrate into pre-existing neural networks and survive for the rest of the fish's life, thus leading to a continuous increase in the volume of the granule cell layer of the cerebellum [Zupanc et al., 1996; Ott et al., 1997].

In the brains of songbirds, seasonal changes in the size of nuclei in the song control system reflect seasonal changes in neuron numbers. The new neurons replace older dying neurons, but the neuronal turnover is seasonally regulated and is greatest during the non-breeding season. During the breeding season, elevated levels of circulating sex steroids decrease the turnover and increase the survival of new neurons, thus increasing the net number of neurons [Kirn and Nottebohm, 1993; Rasika et al., 1994; Hidalgo et al., 1995; Tramontin and Brenowitz, 2000]. In the avian hippocampus, which is also a site of adult neurogenesis, the total number of hippocampal neurons remains constant throughout the year, but neuronal recruitment shows a peak in the fall. The recruitment of new neurons makes up for cell loss with no net gain in neuron numbers [Barnea and Nottebohm, 1994, 1996].

Adult neurogenesis and neuronal death appear to be causally linked in birds and probably also in the mammalian hippocampus. However, routine light and electron microscopic techniques, as well as degeneration-sensitive silver impregnation procedures, have failed to detect neuronal death in the telencephalon of adult lizards and turtles [López-García et al., 1990a; Font et al., 1995, 1997]. This suggests that the neurons born in the brain of adult reptiles represent a net addition to already existing populations, rather than a replacement for older neurons. Further, neurons labeled with [^{3}H]thymidine or BrdU survive for long periods of time in their target areas [Pérez-Cañellas et al., 1997; E. Font, E. Desfilis, M.M. Pérez-Cañellas, J.M. García-Verdugo, unpublished observations]. From this we conclude that the permanent addition of new neurons, together with their long-term survival, forms the basis for the continuous growth of most telencephalic areas in adult reptiles. However, due to the small number of relevant studies, even this conclusion may be premature.

An equally important, but as yet unresolved, question is whether or not the rate of neuronal production and/or recruitment in reptiles remains constant throughout the life of the individual. In chickadees, the hippocampus of juvenile birds recruits more new neurons and has more neurons than that of adults, possibly due to overproduction of hippocampal neurons during early postnatal development [Barnea and Nottebohm, 1996]. Several studies have examined neurogenesis in lizards of different ages [López-García et al., 1988a, b; Pérez-Sánchez et al., 1989]. Unfortunately, the results of these studies were reported as percentages instead of absolute numbers of labeled cells and, therefore, the data cannot be used to compare neurogenesis in lizards of different ages. Recently, Molowny et al. [1995] claimed that the rate of neurogenesis in the cerebral cortex of *Podarcis hispanica* is age-dependent, decreasing as lizards get older. These authors also reported a five-fold increase in the number of proliferating cells in the medial cortex of lizards sacrificed in spring-summer, relative to those sacrificed in fall-winter. This suggests that adult neurogenesis in lizards may be, as in birds, seasonally regulated. However, such conclusions should be interpreted tentatively, because no information on the sample size and the statistical tests used has been provided.

Stem Cells for Adult Neurogenesis

The identity of neural stem cells in the adult vertebrate brain is currently a much debated topic [Barres, 1999; Temple and Alvarez-Buylla, 1999; Alvarez-Buylla et al., 2001; Morshead and van der Kooy, 2001; Temple, 2001]. With few exceptions, neurogenic regions responsible for adult neurogenesis in mammals, birds, and reptiles are located in the ventricular/subventricular zone [Alvarez-Buylla and Lois, 1995; Alvarez-Buylla et al., 2001]. In lizards, the cellular composition of the VZ has been studied in some detail and has been shown to comprise two main cell types [García-Verdugo et al., 2000]. The VZ cells directly in contact with the ventricular lumen are almost exclusively radial glial cells that stain positive for GFAP and extend radial fibers that ascend into the overlying neuropil [García-Verdugo et al., 1981; Yanes-Méndez et al., 1988a, b; Yanes et al., 1990]. A second cell type found immediately adjacent to the VZ consists of cells with the ultrastructural characteristics of young migrating neurons. When mitoses are found in the VZ, the mitotic cells are always exposed to the ventricular lumen and occasionally display typical radial glial processes (fig. 3). These findings confirm that the presence of GFAP in a fiber-containing cell is not incompatible with further replication of the cell and suggest that a homogeneous precursor cell population comprises the proliferative VZ. Further experiments combining GFAP immunohistochemistry and autoradiography have shown that the majority of [^{3}H]thymidine-labeled cells in the VZ of lizards sacrificed at short intervals following a pulse of [^{3}H]thymidine display anti-GFAP immunoreactivity and have ultrastructural features of radial glial cells [Font et al., 1995]. Taken together, these findings lead to the conclusion that radial glial cells, or a subset of them, in the VZ are stem cells for adult neurogenesis in reptiles.

Consistent with this proposal, recently identified neural stem cells in mammals and birds also possess characteristics of glial cells. In mammals, the bulk of the evidence points to a glial origin of the neurons that comprise the RMS, although there is disagreement as to whether the stem cells are ependymal cells [Johansson et al., 1999] or subventricular zone astrocytes [Chiasson et al., 1999; Doetsch et al., 1999]. In adult birds, the neural stem cells have been identified as radial glial cells that contact the ventricular lumen and extend a characteristic single cilium into the cerebrospinal fluid [Alvarez-Buylla et al., 1998]. The conclusion that glial cells are neural stem cells may seem extraordinary in light of the prevailing view of stem cells as largely undifferentiated cells, and is forcing a revision of previous theories regarding the lineage relationships of neurons and glial cells [Barres, 1999; Laywell et al., 2000; Alvarez-Buylla et al., 2001].

Factors Affecting Adult Neurogenesis

Research on birds and mammals has identified a number of environmental and physiological factors that modulate the production, migration, and/or survival of neurons born in adulthood. In seasonally breeding birds, for example, exposure to long spring-like days leads to growth of several song control nuclei. This growth is thought to be controlled primarily by a photo-induced increase in circulating testosterone rescuing neurons that would otherwise die [Tramontin and Brenowitz, 2000]. In temperate-zone lizards, temperature and photoperiod are the primary environmental cues that stimulate seasonal reproductive activity [Licht et al., 1969; Duvall et al., 1982]. In spring, rising temperatures and increasing day length stimulate gonadal recrudescence, development of secondary sexual characteristics, and increases in circulating blood levels of sex steroids [Moore and Lindzey, 1992; Whittier and

Tokarz, 1992]. The effects of temperature and photoperiod on adult neurogenesis in the cerebral cortex of *Podarcis hispanica* have been examined experimentally by maintaining lizards over 23 days under all possible combinations of short-long photoperiod and cool-warm temperature [Ramirez et al., 1997]. Although PCNA- and [^{3}H]thymidine-labeled cells were present in the VZ in all treatment conditions, no [^{3}H]thymidine-labeled cells were found in the inner plexiform layer or in the cell layer of cold-acclimated lizards. This suggests that neuroblast migration, but not proliferation, is inhibited by low temperatures (10°C). The authors also report a reduction in the number of VZ cells labeled with PCNA in three lizards kept on warm, short days. The latter result is difficult to interpret, as it was not accompanied by a corresponding reduction in the number of VZ cells labeled with [^{3}H]thymidine and may be an artifact due to the small sample size used. Moreover, the conclusions of this experiment may be flawed because no overall analysis of variance was performed to establish a treatment effect among the four experimental conditions, and the authors conducted many pairwise significance tests with no mentioning of any multiple comparison procedures.

Reactive Neurogenesis and Neuronal Regeneration in the Telencephalon of Lizards

Although neurons are sometimes capable of re-establishing lost connections, examples of true regeneration of whole neurons in the central nervous system of vertebrates are rare. Studies of the effects of the neurotoxin 3-acetylpyridine (3AP) in lizards have exposed what is arguably the most striking case of neuronal regeneration in the forebrain of any vertebrate studied thus far [Font et al., 1991, 1995, 1997; López-García et al., 1992; Desfilis et al., 1993; López-García, 1993; Molowny et al., 1995]. This research has convincingly demonstrated that intoxication with 3AP, which causes severe neuronal loss in several telencephalic areas, triggers a cascade of events, including reactive proliferation and migration of replacement neurons. These processes ultimately result in successful regeneration of the lesioned areas in lizards of all ages. Interestingly, reactive proliferation has also been reported following surgical ablation of a wedge of cortical tissue in the lizard *Lacerta viridis*, but regeneration of the lesioned areas was still incomplete after 260 days [Minelli et al., 1978].

A single intraperitoneal injection of 3AP at a dose of 150 mg/kg body weight causes degeneration in several telencephalic areas of the lizard *Podarcis hispanica* [Font et al., 1991, 1997]. In the early phase following 3AP treatment, the most frequent neuropathic changes are clumping of the nuclear chromatin with formation of pyknotic nuclei and intense neuropil vacuolization. These changes resemble those taking place during apoptotic cell death in the adult brain of other vertebrates, such as fish. In the latter taxonomic group, apoptosis occurs in both the intact and the injured brain [Soutschek and Zupanc, 1995, 1996; Zupanc et al., 1998; for review, see Zupanc, 1999]. The neuronal damage produced by 3AP in lizards is most evident in, but not restricted to, the cell layer of the medial cerebral cortex. In a sample of eight lizards sacrificed 7–15 days after a 3AP injection, up to 97% of the neurons in this area displayed pyknotic nuclei. Pyknotic nuclei are also found in the dorsal and lateral cortices, anterior dorsal ventricular ridge, nucleus sphericus, and lateral amygdaloid nucleus. However, damage to these areas is highly variable and generally affects less than 50% of their neuronal populations [Font et al., 1997]. Preliminary data indicate a similar distribution of areas of 3AP damage in other lizards (e.g., *Anolis carolinensis*), but not in snakes [E. Font, E. Desfilis, M.M. Pérez-Cañellas, J.M. García-Verdugo, unpublished observations].

During the four weeks following 3AP treatment, the neuropil gradually returns to normal, and pyknotic nuclei progressively disappear, although some are still detectable two months after the lesion. Removal of the degenerating axonal and cellular debris takes place through the combined action of microglia and radial glial cells [López-García et al., 1994; Font et al., 1995; Nacher et al., 1999a, b]. As early as one week after the lesion, the number of microglial cells in the lesioned areas undergoes a several-fold increase relative to controls, while radial glial fibers swell and display conspicuous pseudopodial extensions which surround pyknotic nuclei and other degenerating debris. One month after the lesion, the somata of radial glial cells contain dense bodies. This indicates transport of degenerating debris from the neuron-depleted areas toward the ependyma and, possibly, the ventricular system [Font et al., 1995].

Concurrent with these changes, there is an increase in the rate of adult neurogenesis which leads to regeneration of the damaged areas within approximately 6–8 weeks post lesion [Font et al., 1991]. One to two weeks after 3AP administration, a surge of reactive neurogenesis supplies new neurons to the neuron-depleted areas (fig. 7). Replacement neurons originate in the same persistent pool of mitotically active cells that is normally responsible for

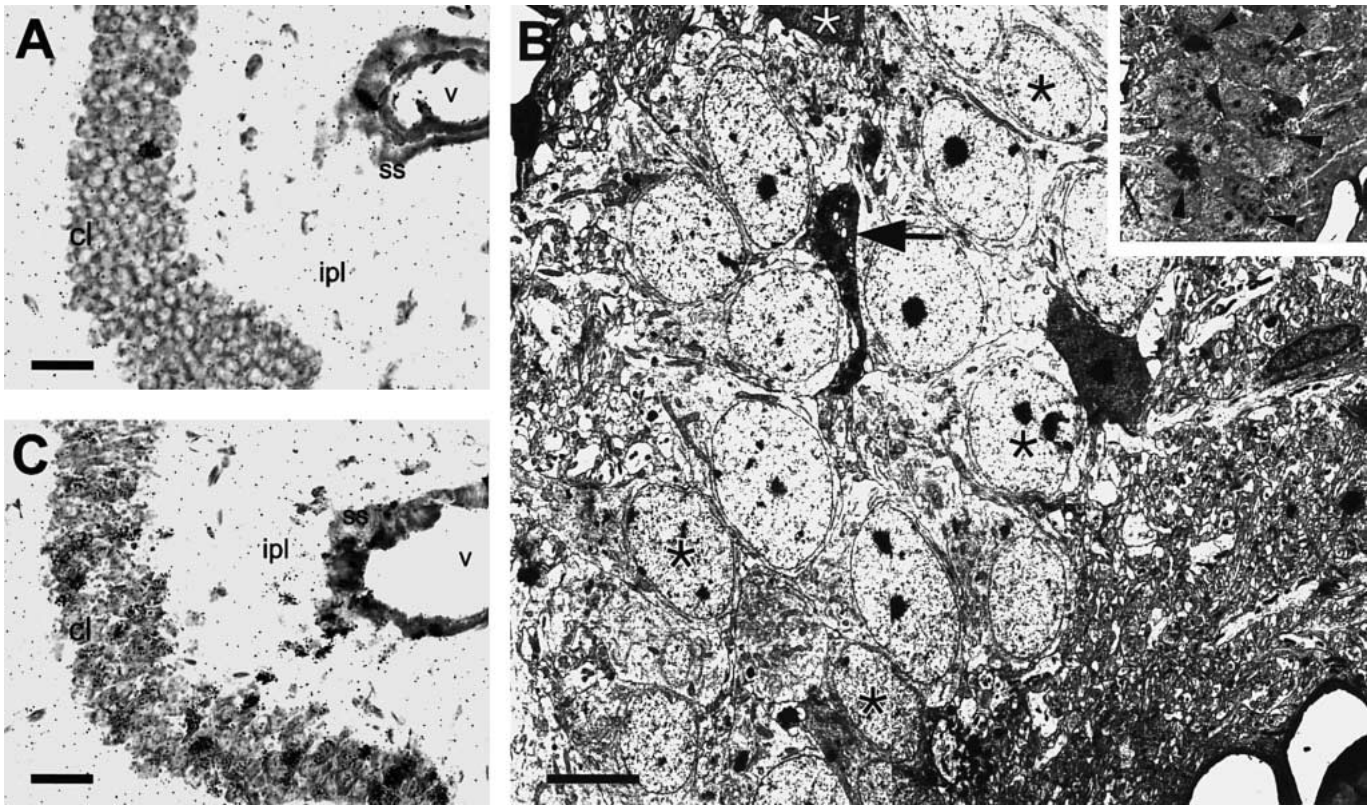

Fig. 7. Light microscopic autoradiograms from the medial cortex of control (A) and 3AP-treated (C) lizards *(P. hispanica)* allowed to survive five weeks after [^{3}H]thymidine administration. Labeled cells are more abundant in the medial cortex of lizards previously injected with 3AP than in that of untreated controls. B Electron micrograph of the medial cortex of a 3AP-treated lizard exposed to [^{3}H]thymidine five weeks prior to sacrifice. The ultrastructure of [^{3}H]thymidine-labeled cells (asterisks) is similar to that of the adjacent neurons. Note the presence of degenerating neuronal debris (arrow) that confirms that the cell layer was effectively damaged by 3AP. Upper right inset shows radiolabeled cells in the light microscopic autoradiogram from which the ultrathin section in B was obtained. Arrowheads in the inset point to the same cells as indicated in the electron micrograph by the asterisks. cl, cell layer; ipl, inner plexiform layer; ss, sulcus septomedialis; v , ventricle. Scale bars in A and C, 25 μm; in B, 5 μm.

adult neurogenesis [Font et al., 1997]. In addition to large numbers of cells of unmistakable neuronal phenotype, the cerebral cortex of lizards receiving a pulse of [^{3}H]thymidine after the 3AP injection also contains radioactively labeled stellate cells. These cells stain positive for GFAP, have ultrastructural features of astrocytes, and apparently contribute to the removal of debris. This suggests that, in contrast to normal adult neurogenesis, reactive proliferation in the telencephalon of lizards produces both neurons and free glial cells [Font et al., 1995].

By two months after the lesion, the damaged areas, including the medial cortex, seem fully recovered and are almost indistinguishable from the corresponding areas in control, non-lesioned lizards [Font et al., 1991, 1997]. Further, there is evidence that some regenerated neurons establish synaptic contacts and are incorporated into functional circuits [Molowny et al., 1995]. One can begin to appreciate the sheer magnitude of this regenerative process by noting that in the medial cortex alone there may be well in excess of 300,000 neurons, most of which are replaced in a lizard that has received 3AP. Experimentally induced reactive neurogenesis has been reported in fish [Zupanc and Ott, 1999], amphibians [Kirsche, 1983; Minelli et al., 1987; Del Grande et al., 1990; Margotta and Morelli, 1996], songbirds [Scharff et al., 2000], and mammals [e.g., Gould and Tanapat, 1997], but lizards are the only known tetrapods capable of regenerating entire portions of their cerebral cortex bilaterally as adults. It is possible that the ability of lizards to replace damaged neurons is causally linked to their capacity to generate new ones in the uninjured brain [Font et al., 1991]. Indeed, the persistence of neurogenesis beyond postembryonic develop-

ment has been proposed as one possible reason why regeneration is successful in some taxa, but not in others [Hulsebosch and Bittner, 1980; Kirsche, 1983].

The regenerative process induced by 3AP confirms the pivotal role of radial glial cells in relationship to neurogenesis and regeneration [Font et al., 1995; see also Margotta and Morelli, 1997]. In the telencephalon of 3AP-treated lizards, radial glial cells participate in the removal of degenerating debris, act as multipotent stem cells that produce neuronal and glial progeny, and serve as guides along which replacement neurons migrate to their final destination. Radial glial cells are also critical for the ability of adult lizards and newts to successfully regenerate their spinal cord after injury. As in the telencephalon of 3AP-treated lizards, radial glial cells in this location divide and give rise to new neurons and glia [Alibardi, 1994; Chernoff, 1996]. Because radial glial cells are complex and fully differentiated cells (i.e., they have radial processes, an elaborate cytoskeleton, and pial endfeet), these observations raise questions about the cellular and molecular mechanisms by which these cells accomplish such diverse functions.

Function and Evolution of Adult Neurogenesis in Vertebrates

The functional significance of adult neurogenesis in reptiles remains a critical but elusive problem. Adult neurogenesis has been described in representatives of most vertebrate classes. Consequently, caution must be exercised in ascribing any hypothesis regarding the function of adult neurogenesis exclusively to reptiles. It is also important to remember the distinction between original function and current fitness effects in discussions of the adaptive significance of adult neurogenesis. Given its phylogenetic distribution, continuous addition of neurons in the adult brain may be the ancestral (plesiomorphic) condition for tetrapods and perhaps all vertebrates. Thus, we should not look for factors specific to reptilian ecology or life history to find selective pressures favoring the initial evolution of adult neurogenesis.

Because the proximal mechanisms that come into play during adult neurogenesis closely resemble those in embryonic neurogenesis, it is conceivable that the capacity for adult neurogenesis is an inherent attribute related to embryonic development, rather than an adaptation to specific selective pressures in adult life [Goss, 1992]. It is also possible that the ability to add neurons throughout life is a vestigial character that reptiles, birds, and mammals inherited from their Paleozoic ancestors, but which currently has no adaptive value. However, the possibility that adult neurogenesis is not an adaptation has rarely been entertained, and most discussions of this phenomenon include some speculation about its functional significance in living vertebrates.

Assuming a link between adult neurogenesis and neuronal regeneration, one obvious advantage of adult neurogenesis is the potential for self-repair of brain areas that may be lost due to disease or injury. However, it is difficult to imagine that reptiles or other vertebrates might endure sublethal injuries to specific portions of their brain with such a high frequency that selection for neuronal regeneration plays a significant role [see also Goss, 1992].

A more plausible hypothesis relates adult neurogenesis to plasticity, including learning and memory. Processes of learning and memory in vertebrates are thought to be accompanied by considerable structural modification [Alvarez-Buylla, 1992; Kolb and Whishaw, 1998]. It has been proposed that these processes could take advantage of adult neurogenesis [Alvarez-Buylla et al., 1990b; Nottebohm and Alvarez-Buylla, 1993; Barnea and Nottebohm, 1996; Gould et al., 1999b]. Although focused on the mammalian brain, many of the arguments put forward by Gross [2000] in support of the importance of adult neurogenesis for learning and memory are applicable to other vertebrates including reptiles.

In mammals, newly generated neurons in the adult olfactory bulb have been shown to play a role in odor discrimination [Gheusi et al., 2000]. Many structures undergoing adult neurogenesis in the reptilian brain are likewise functionally related to chemoreception. These include the olfactory bulbs as well as their main target structures in the telencephalon, namely the lateral cortex and the nucleus sphericus. It is possible that addition of new neurons in these areas is important for the formation of olfactory memories.

In reptiles, the medial, dorsomedial, and dorsal cortices, all of which undergo adult neurogenesis, are thought to be homologous, at least as a field, to the medial (hippocampal) pallium of other vertebrates [Butler, 1994; Butler and Hodos, 1996]. Adult neurogenesis has also been reported in the hippocampus of birds and mammals [Nottebohm and Alvarez-Buylla, 1993; Eriksson et al., 1998; Kornack and Rakic, 1999; Hastings et al., 2000]. Hence, adult neurogenesis in the hippocampus appears to be a general feature of amniotic vertebrates. In birds and mammals, the hippocampus is critical for encoding complex spatial information to form map-like cognitive repre-

sentations of the environment [Nadel, 1991; Squire, 1993; Bingman et al., 1995; Clayton and Krebs, 1995; Healy and Krebs, 1996; Sherry and Healy, 1998]. Reptiles are able to solve spatial tasks using allocentric frames of reference and build complex spatial cognitive representations of their environment that allow them a flexible spatial behavior [e.g., Holtzman et al., 1999; Zuri and Bull, 2000]. Further, work done in our laboratory has shown that the hippocampus of lizards is implicated in the control of such spatially organized behavior and, thus, shares some functions with the hippocampus of birds and mammals [Font and Desfilis, 1993]. Recent findings have established a contribution of adult neurogenesis to memory function in the mammalian hippocampus [Shors et al., 2001]. It seems reasonable to hypothesize that proliferating cells in the reptilian hippocampus may similarly be required for the updating of hippocampal nerve circuits involved in spatial learning. Perhaps as reptiles grow older they range over a larger area and encounter greater environmental diversity, which necessitates addition of neurons for the acquisition of new spatial memories.

However, adult neurogenesis in reptiles also takes place in brain areas such as the anterior dorsal ventricular ridge or the striatum for which a functional role in learning and memory has not been established. The challenge for future research is to identify the functional consequences of the permanent generation and integration of new neurons into existing circuits in these brain areas.

Final Remarks

Despite large gaps in our knowledge, the findings summarized in this review demonstrate that, in all of the few reptiles examined thus far, new neurons are added during adulthood at high rates and in many regions of the brain. The reptilian brain has figured prominently in studies of vertebrate brain evolution and brain-behavior relationships [Greenberg and MacLean, 1978; Schwerdtfeger and Smeets, 1988; Northcutt and Kaas, 1995; Striedter, 1997; Shimizu, 2001]. Theories of reptilian brain structure and function that overlook the fact that the telencephalon of reptiles constantly adds new neurons are likely to be seriously misleading. However, our understanding of adult neurogenesis and regeneration in the reptilian brain remains at an early stage. Very little is known, for example, of the factors that control or modulate the proliferation and migration of new neurons. Elucidation of these factors may have practical implications, as it has been suggested that in mammals adult neurogenesis may be restricted by the loss of permissive signals for daughter cell migration or survival in the adult brain parenchyma [Goldman and Luskin, 1998]. Another unresolved issue concerns the relative contributions of cell birth and programmed cell death to reptilian brain growth.

Living reptiles are an extremely diverse group. Future studies should take advantage of this diversity and explore adult neurogenesis and regeneration in a wider range of reptilian species, including crocodiles and snakes. Such a comparative approach is badly needed if research is to advance from a collection of isolated findings to a truly comparative database that allows the identification of broadly meaningful patterns in adult neurogenesis.

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