Radial glial origin of the adult neural stem cells in the subventricular zone

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Abstract
Adult neurogenesis persists within restricted areas of the mammalian brain, giving rise prevalently to neuronal precursors that integrate inside the hippocampus and olfactory bulb. The source of this continuous cell production consists of neural stem cells which have been identified as elements of the astroglial lineage. This counterintuitive finding overlaps with the recent discovery that embryonic radial glia can themselves act as stem cells, capable of producing both neurons and glia during development. Although radial glia was thought to disappear early postnatally at the end of neurogenesis by transformation into parenchymal astrocytes, it has recently been demonstrated that some radial glial cells somehow persist within the adult forebrain subventricular zone, hidden among astrocytes of the glial tubes. This transformation occurs in parallel with overall morphological and molecular changes within the neurogenic site, whose specific steps, mechanisms, and outcomes are not yet fully understood. The modified radial glia appear to be neural progenitor cells belonging to the astroglial lineage (type B cells) assuring both stem cell self-renewal and production of a differentiated progeny in the adult subventricular zone, and also playing regulatory roles in stem cell niche maintenance.

Keywords: Adult neurogenesis; Astrocyte; Radial glia; Cell proliferation; Stem cell niche

Abbreviations: BMPs, bone morphogenetic proteins; BLBP, brain lipid-binding protein; CNS, central nervous system; EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; GFAP, glial fibrillary acidic protein; GLAST, astrocyte-specific glutamate transporter; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; PSA-NCAM, polysialylated neural cell adhesion molecule; RMS, rostral migratory stream; SVZ, subventricular zone; SGZ, subgranular zone; SCZ, subcallosal zone; VZ, ventricular zone

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1. Introduction

1.1. Adult neurogenesis: what remains of embryogenesis?

The central nervous system (CNS) of mammals is a highly complex structure made up of a huge number of neurons, glial cells, and synapses, all linked by extremely heterogeneous anatomical and functional relationships (Chklovskii et al., 2004). Its elaborate architecture is the result of subsequent cell divisions and precise cell–cell and cell–substrate interactions starting from a small amount of undifferentiated cells in the neural tube, then assembling throughout development, including a relatively short postnatal period (Bayer and Altman, 2004). In parallel with important regional differences under morphological and gene expression pattern profiles, a general rule involves peri-ventricular germinative layers harbouring neural stem cells as a source of most neuronal and glial cell precursors. The stem cell progeny herein generated populate the growing

CNS parenchyma by a combination of centrifugal radial and to a lesser extent, tangential cell migration (Rakic, 1990; Marin and Rubenstein, 2003), later differentiating into specific neuronal and glial fates according to a precise spatial and temporal pattern, finally establishing appropriate connections.

After CNS assembly, besides the functional specificity of its structure and hardwiring, neural plasticity, namely the ability to make adaptive changes related to the architecture and function of the nervous system, remains a complementary attribute (Zilles, 1992). In addition to widespread structural changes reshaping adult neuronal circuits through multiple microscopic modifications mainly affecting synaptic contacts, neurogenesis does persist in the CNS of adult mammals, albeit in restricted domains (Gage, 2000; Gross, 2000). In the brain of adult rodents two consistently active germinative layers are present: the subventricular zone (SVZ), associated with the anterior part of the forebrain lateral ventricles, and the subgranular zone (SGZ), corresponding to the inner layer of the dentate gyrus, within the hippocampal formation (Peretto et al., 1999; Gage, 2000; Alvarez-Buylla and Garcı`a-Verdugo, 2002; Fig. 1). The SVZ more evidently retains embryonic features of primitive germinal layers. Firstly, it maintains direct contact with the ventricles, whereas the SGZ loses such a contact after the 'rollin in' of the hippocampus during development (Smart, 1961). Furthermore, SVZ neuronal precursors undergo long-distance migration to reach their final site of destination in the olfactory bulb (Lois and Alvarez-Buylla, 1994), whereas those generated within the dentate gyrus differentiate locally (Zhao et al., 2006).

Although harbouring all subsequent steps of cell differentiation from stem cell division to cell replacement, persisting neurogenic sites do not faithfully recapitulate development. Indeed, they change pre- and post-natally under their morphological, cellular, and molecular profile in order to adapt to the non-permissive environment of the mature nervous tissue (Alves et al., 2002; Tramontin et al., 2003; Peretto et al., 2005). Apart from anatomical changes relating to a different conformation of cerebral ventricles (Bonfanti and Ponti, in press), the most evident differences concern SVZ glial cell types and their distribution (Fig. 1A). On the other hand, notwithstanding differences in the cytoarchitecture, a common pattern can be found under morphological and functional profiles in the cell composition of different neurogenic sites and at different developmental stages.

1.2. Cell types in embryonic germinative layers and perinatal SVZ

The primitive germinal layers consist of a primary proliferative zone called ventricular zone (VZ) containing direct descendants of the primitive neuroectoderm (neuroepithelium), and a secondary proliferative zone called subventricular zone (SVZ), which later emerges from the VZ. The SVZ contains rapidly proliferating cells and expands greatly during the last third of prenatal development, in parallel with progressive reduction of the VZ (for review see Levison and Goldman, 2006). This trend continues early postnatally (first postnatal week in rodents), followed by the disappearance of the VZ and the persistence of the SVZ exclusively within the forebrain. The embryonic/perinatal SVZ contains cell types that are heterogeneous under the profile of their morphology and molecular expression, and whose exact origin has not yet been fully understood. Different regional specializations such as the dorsolateral neuroepithelium of the telencephalon, the medial (MGE) and lateral (LGE) ganglionic eminences do contribute cells to the forebrain SVZ (Levison and Goldman, 2006). In this context it is likely that cells with different potentials, ranging from stem cells to cell progenitors endowed with various differentiative capabilities, can coexist along the entire SVZ extension, their mutual relationships being modulated at different developmental stages. Beside the first migrating neural cell precursors, one of the earliest cells to differentiate in the developing CNS is radial glia. Radial glial cells are bipolar elements oriented orthogonally to the growing tissue, with a soma in the VZ sending a short cellular process to make contact with the ventricular surface (basal foot), and a long radial fiber reaching the pial surface with two or more enlarged endfeet (glia limitans) (Figs. 1 and 2). These cells can be marked by

several antigens (Hartfuss et al., 2001), such as RC1, RC2 (Misson et al., 1988), vimentin (Pixley and De Vellis, 1984), nestin (Hockfield and McKay, 1985), the calcium-binding protein S-100β, the brain lipid-binding protein BLBP (Feng and Heintz, 1995), the glutamate transporter GLAST (Shibata et al., 1997), and tenascin-C (Peretto et al., 2005), most of which can also be found in astrocytes. Some of these antigens, particularly vimentin and nestin, are not specific for radial glia since they are intermediate filament proteins present in a wide range of cell progenitor cells and immature glia. In addition, the expression and time of appearance of most antigens vary depending on the species and the developmental stages (e.g., in primates radial glial cells express GFAP very early, whereas in rodents they are GFAP-negative until the completion of corticogenesis; Rakic, 2003).

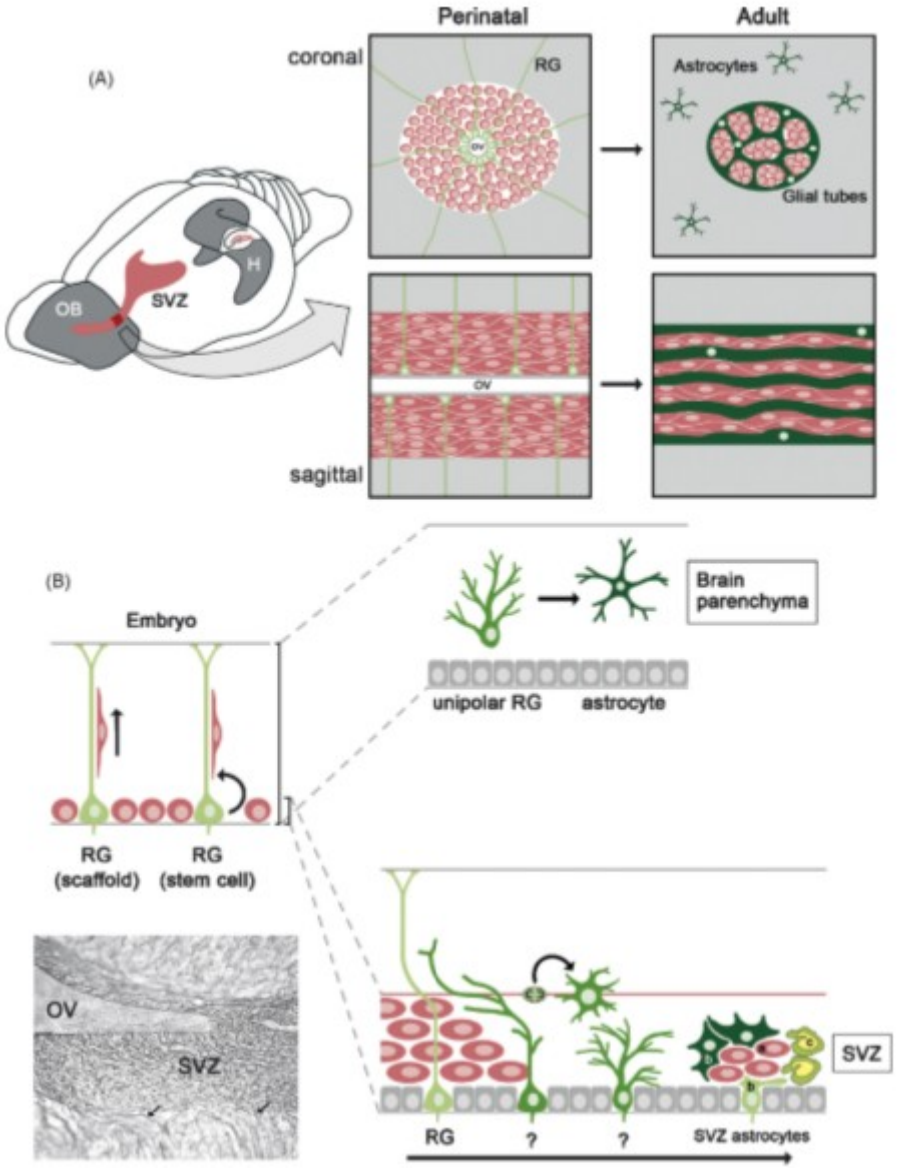

Fig. 1. Origin of adult neural stem cells from radial glia. (A) Postnatal modifications in the subventricular zone (SVZ) primarily affect glial cells (green). On the right: schematic representation of coronal and sagittal visions of the SVZ rostral extension (the area represented is highlighted in red on the left) at perinatal and adult stages. Glial cells shift from radial glia (light green) whose cell bodies are in contact with the olfactory ventricle (OV) to astrocytes (dark green) packed to form the glial tubes in the SVZ or isolated stellate cells in the surrounding parenchyma. Migrating neuroblasts (pink) simply change their relationships, from a homogeneous mass to tangential chains (see also Fig. 3A–F). H, hippocampus (in pink, the SGZ); OB, olfactory bulb. (B) Fate of radial glial cells from development (left) to adulthood (right) in CNS germinative layers (bottom) and in the parenchyma (top). Different shades of green indicate progressive maturation from radial glia to astrocytes. In the embryo, besides the classic role of scaffolding for neuronal

migration, a new role has been established for radial glia as multipotent stem cells. In brain parenchyma they transform into astrocytes through a transient unipolar form which has lost contact with both pia mater and ventricular surface (in grey: ependyma). In the SVZ something similar occurs, with a change in radial process orientation from radial to tangential, and increasing ramifications (see also the micrograph on the left: sagittal section of the SVZ rostral extension at birth, immunostained for vimentin, showing the glial network in the SVZ and the bending of radial glial processes (arrows) at the border; reproduced from J. Comp. Neurol., Peretto et al., 2005). OV, olfactory ventricle, partially closed. Both the subsequent stages of cell transformation which give rise to astrocytes of the glial tubes and the contribution of local cell proliferation mainly occurring at the border between SVZ and mature tissue (pink line) are not known in detail. The degree of heterogeneity of glial cells (type B cells, b) in the adult stem cell niche has not yet been fully elucidated either.

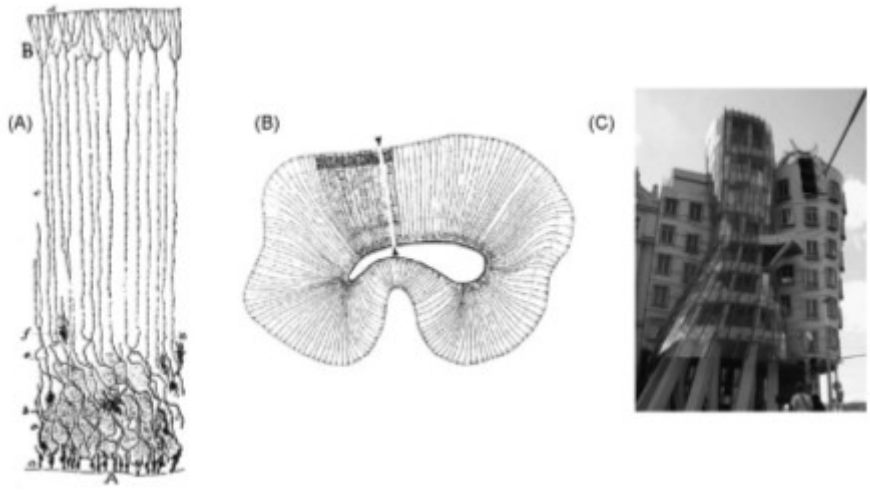

Fig. 2. (A) Ramòn y Cajal's drawing of radial glia in the postnatal rabbit cerebral cortex stained with Golgi method. From 'Histologie du Système Nerveux de l'Homme et des Vertébrés', vol. II. Maloine, Paris, 1911, p. 859. (B) Drawing of a Golgi-impregnated section from the monkey fetal brain showing the bending of radial glia which allows them to follow the heterogeneous growth of the nervous tissue; originally published by Rakic (1972). (C) The National Nederlanden building (dancing house) designed by architect Frank O. Gehry, in Prague, reminiscent of radial glia distorted scaffolding. (A and B). Reproduced from Bentivoglio and Mazzarello, Brain Res. Bull. (1999).

Another cell type appearing in late embryonic germinal layers is ependyma. In mice, most ependymal cells are generated from radial glia between embryonic days 14 and 16 (Spassky et al., 2005), a process that has been proposed might occur both through radial glia division and by its direct transformation into ependymal cells. These cells differentiate later, as revealed by the appearance of cilia during the first postnatal week.
In most CNS regions the germinal layers disappear soon after birth, leaving a non-germinal epithelium composed of multiciliated cells: the ependymal monolayer (Boulder Committee, 1970). On the lateral wall of the lateral ventricle the embryonic SVZ corresponding to the dorsolateral neuroepithelium, MGE, LGE, is more prominent (Anderson et al., 1999; Wichterle et al., 2001; Métin et al., 2006) then persisting throughout adulthood as a forebrain neurogenic site.

1.3. Cell types in the adult subventricular zone

The VZ is thought to disappear in the postnatal and adult brain, replaced by an actively proliferating SVZ which continues to harbour neural stem cells. The anatomical arrangement of adult forebrain SVZ, as well as its extension, depends on the species and their cerebral ventricle conformation (Bonfanti and Ponti, in press). In laboratory rodents, due to the postnatal closure of the olfactory ventricle, only the middle/posterior part of the SVZ remains in contact with the ventricles, the anterior part forming a rostral extension or rostral migratory stream (RMS) through the olfactory peduncle and bulb axes (Peretto et al., 1999). Nevertheless, apart from the absence of the ependymal monolayer in the RMS of these species, the SVZ cytoarchitecture is substantially similar

at all levels (Doetsch et al., 1997; Peretto et al., 1999; Alvarez-Buylla and Garcìa-Verdugo, 2002; Levison and Goldman, 2006). Two main cell compartments are detectable: (i) newly generated neuroblasts, which migrate in the form of tangentially-oriented chains (Lois et al., 1996; Doetsch and Alvarez-Buylla, 1996), and (ii) astrocytes forming a dense meshwork of intermingled cell bodies and processes throughout the SVZ area, thus delineating longitudinally-oriented channels called glial tubes (Jankovski and Sotelo, 1996; Lois et al., 1996; Peretto et al., 1997). Neuroblasts (also referred to as type A cells; Lois et al., 1996) are bipolar cells with a large nucleus and a thin rim of electrondense cytoplasm mostly co-expressing β-tubulin, doublecortin, and PSA-NCAM (Bonfanti and Theodosis, 1994; Menezes and Luskin, 1994; Rousselot et al., 1995; Nacher et al., 2001). Astrocytes (type B cells) are ramified cells with electronlucent, watery cytoplasm, characterized by immunocytochemical staining for GFAP (Jankovski and Sotelo, 1996; Lois et al., 1996; Peretto et al., 1997; Fig. 3G and H). These glial cells have some cytological and molecular features of radial glia, since they contain glycogen granules, the intermediate filaments vimentin and nestin (Jankovski and Sotelo, 1996; Peretto et al., 1997, 1999; Doetsch, 2003a,b), and glutamate transporters (Bolteus and Bordey, 2004). Furthermore, some of them retain a thin cellular process protruding in the ventricle through the ependymal monolayer (Doetsch et al., 1997). In addition to A and B cell types, a third element with intermediate ultrastructural features and high proliferative capacity has been identified as type C cells (Doetsch et al., 1997). Type C cells are considered to function as ‘transit amplifying’ cells in the neural stem cell niche, namely a bridge between the slow proliferating stem cells and their progeny of neuronal precursors (Doetsch, 2003a; see below). Indeed, the SVZ harbours a population of multipotent neural progenitor cells that can

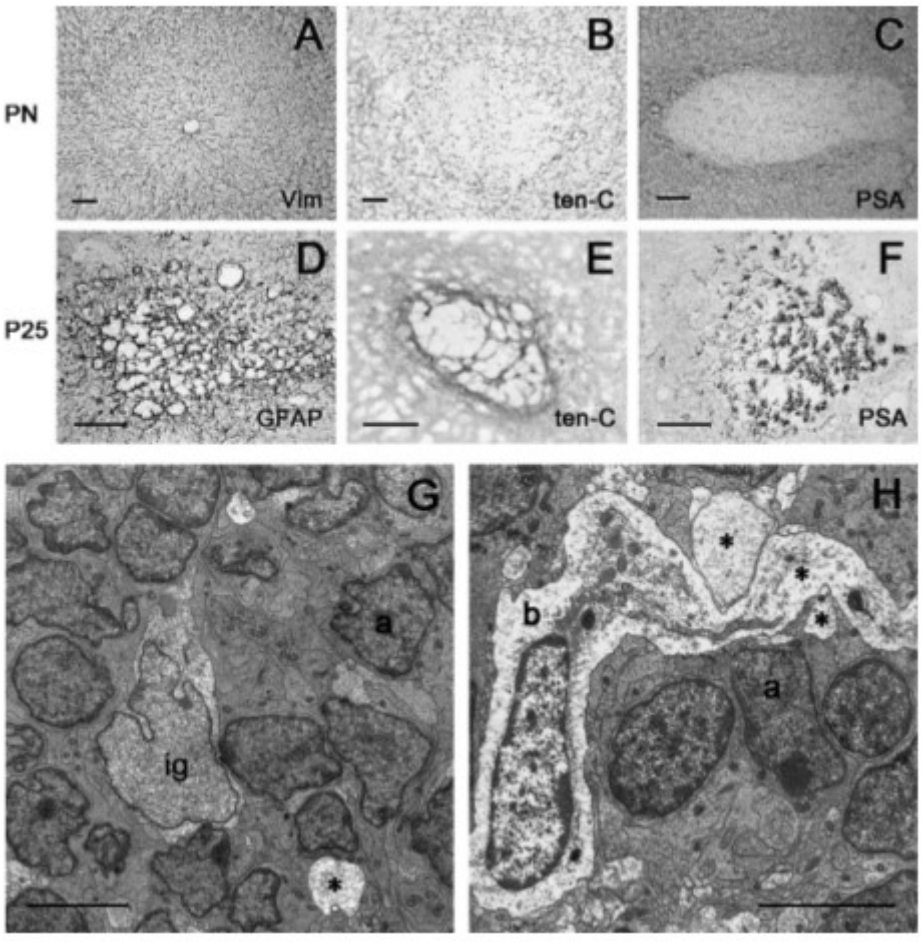

Fig. 3. Morphological and molecular changes in the SVZ of rodents during postnatal brain maturation. PN, perinatal; P25, postnatal day 25. (A and D) Radial glia immunoreactive for vimentin (vim) is replaced by $GFAP^{+}$ astrocytic glial tubes. The glial changes are accompanied by molecular modifications involving astrocyticsecreted molecules of the

extracellular matrix (tenascin-C, in B and E) and associated with the migrating neuroblasts (PSA-NCAM, PSA in C and F). (G and H) Cell types in the postnatal SVZ at the ultrastructural level. Immature glial cells (Ig) whose cell bodies are no longer in contact with the ventricle are immersed in masses of typical neuronal precursors (a) with rare astrocytic processes (asterisks), at postnatal day 6 (G); astrocytes (b) and their processes start to aggregate at subsequent stages (postnatal day 13, H). Reproduced from Peretto et al. (2005). Scale bars: (A–F), 50 μm; (G and H), 3 μm.

be isolated and expanded in culture under the effect of trophic factors such as the epidermal growth factor (EGF), the fibroblast growth factor 2 (FGF-2) or a combination of them, thus giving rise to self-renewing and multipotent neurospheres that can differentiate into neurons, astrocytes and oligodendrocytes (Reynolds and Weiss, 1992; Gage, 2000). Although stem cells can be isolated throughout the SVZ extension (Gritti et al., 2002), transit amplifying cells are rare in the RMS, thus suggesting a prevalent activity of the neural stem cell niche at the ventricular level, wherein ependyma represents a fourth cell type.
Hence, a major difference between embryonic and adult SVZ cell composition concerns the glial compartment, whereby a meshwork of astrocytes replaces radial glial cells in the adult. On the other hand the neuronal precursors seem to change only in their mutual relationships, splitting from a homogeneous mass into discrete chains.

1.4. Glial identity of adult neural stem cells

A consistent number of reports support the hypothesis that adult neural stem cells belong to the astrocytic lineage (Doetsch et al., 1999a,b; Laywell et al., 2000; Skogh et al., 2001; Imura et al., 2003; reviewed in Alvarez-Buylla et al., 2001, 2002; Doetsch, 2003b). Experiments carried out in vitro and in vivo on the mouse SVZ demonstrated that (i) astrocytes and transit amplifying cells (type B and type C cells) can produce neurospheres that contain multipotent progenitor cells (Doetsch et al., 2002); (ii) after targeting SVZ astrocytes in transgenic GFAP-TVA mice which express the receptor of the avian leukosis virus under the control of the GFAP promoter, marked neurons migrating to the olfactory bulb were detected (Doetsch et al., 1999a); (iii) after elimination of transit amplifying cells and neuroblasts with the arabino-furanoside Ara C, the remaining astrocytes within the SVZ were able to regenerate the entire system (Doetsch et al., 1999b).
In addition, GFAP-expressing astrocytes from non-neurogenic CNS regions such as cortex, cerebellum, and spinal cord can produce glial and neuronal cells in vitro only if isolated before the second postnatal week (Laywell et al., 2000). On the other hand, astrocytes of the forebrain SVZ do retain stem cell potential, as observed in cultures from transgenic mice expressing herpes simplex virus thymidine kinase from the GFAP promoter, in which dividing GFAP-expressing cells can be selectively eliminated by anti-viral agents (Imura et al., 2003). The same conclusion has been reached in vivo after observation of depletion of newly generated neurons in the olfactory bulb (Garcia et al., 2004). The first studies aimed at identifying in vivo a cell category coinciding with neural stem cells have led to the conclusion that some SVZ astrocytes undergo a low rate cell division giving rise to type-C cells that proliferate with remarkable frequency (Doetsch et al., 1999a,b). Such a transition coincides with a high expression of the EGF receptor, thought to be implicated in the shift from a relatively quiescent cell to a ‘transit amplifying’ cell capable of expanding the progenitor population (Doetsch et al., 2002).
Thus, substantial evidence indicates GFAP-expressing progenitor cells as a predominant source of constitutive adult neurogenesis, suggesting that neural stem cells could retain cytological aspect and functions of highly specialized cells in the CNS in contrast with the common idea that stem cells are highly undifferentiated elements (Alvarez-Buylla et al., 2001).

2. Radial glia and adult neurogenesis

2.1. Early and recent history of radial glia

The early history of radial glia starts between the end of the 18th and the beginning of the 19th century with a number of observations spanning from Giuseppe Magini to Ramo`n y Cajal (quoted in Bentivoglio and Mazzarello, 1999; Fig. 2A), classifying them as a well defined glial cell morphological phenotype. With the advent of electron microscopy, their role as transient scaffolding for neuronal radial migration from the neuraxis toward the pial surface was confirmed (Rakic, 1971). Particularly in mammals endowed with a thick cerebral cortex, radial glia is considered essential in maintaining the topographical relationships of neuronal precursors ascending from the subventricular zone in spite of unequal growth of the building cortex, according to the 'radial unit hypothesis' (for review, see Rakic, 1990, 2003; Fig. 2B and C). The classic view of a transient cell population destined to disappear parallel to the end of neurogenesis and neuronal migration was supported by the finding of its progressive transformation into parenchymal astrocytes of the mature CNS (Schmechel and Rakic, 1979; Pixley and De Vellis, 1984; Voigt, 1989; Misson et al., 1991). The bipolar radial cell of the embryo, through positional and morphological changes characterized by displacement of the cell soma from the ventricles and retraction of the radial process from the pial surface, shifts into a transitional form known as monopolar radial cell, then becomes a multipolar astrocyte by increased ramification of its cell processes (Takahashi et al., 1990; Fig. 1B). In the mouse, the shift from the radial glial fiber system to a diffuse glial network is mostly achieved in the E17-P2 interval. These changes are accompanied by molecular modifications, mainly affecting the expression of intermediate filament proteins. In general, after being labelled only for vimentin at birth, most transforming radial glial cells are double-labelled for vimentin/GFAP during postnatal stages then gradually lose their vimentin staining and remain GFAP $^{+}$ in the adult (Voigt, 1989; Pixley and De Vellis, 1984). In the complex CNS architecture, the radial glia/astrocyte transition, which can also be viewed as a sort of transdifferentiation (Chanas-Sacre´ et al., 2000), can display different traits depending on regional specializations, with particular reference to neurogenic sites (see below).
More recent studies added new roles for radial glia since it has been shown that embryonic, bipolar radial glia can act as stem cells, being capable of divisions leading to the genesis of astrocytes, neurons (Malatesta et al., 2000; Hartfuss et al., 2001; Noctor et al., 2001, 2002; Tamamaki et al., 2001; Gotz et al., 2002) and oligodendrocytes (Merkle et al., 2004). Two models have been suggested to explain how this might occur in vivo: either newly generated neuroblasts migrate along the radial fiber of their mother cell (Noctor et al., 2001) or the radial glia cell transforms into migrating neurons by translocating the nucleus within the radial process, leaving a stem cell within the VZ (Miyata et al., 2001). Although prevalently assessed for cerebral cortex development, similar properties of radial glia as neural progenitors have been demonstrated to exist within wide areas of the CNS (Anthony et al., 2004). At later developmental stages, radial glial cell division can also produce ependymal cells (Spassky et al., 2005).

2.2. The 'apparent' disappearance of radial glia

Until recently, transformation of radial glia into astrocytes has been interpreted as the disappearance of a transient cell type at the end of development. Although many embryonic radial glia actually disappear, probably by the generation of two postmitotic neurons (Gotz, 2003), a subset of cells continues to proliferate and/or transform into astroglial cells, as described above. Several kinds of radial glia-like cells are known to persist in the mature CNS at specific locations, including cerebellar Bergmann glia, retinal Muller cells, and periventricular tanycytes of the hypothalamus (reviewed in Rakic, 2003). Radial glia-like cells independent from the surface of the third ventricle have also been described in the hypothalamic supraoptic nucleus (Bonfanti et al., 1993). All these

categories share morphological and molecular features with radial glia, yet they do not retain proliferative capacity, but rather represent adaptations to local functional requirements (Rakic, 2003).
Paradoxically, for a long time not much attention has been given to the fate of radial-glia within persistent neurogenic sites. The occurrence of radial glia-like cells in the hippocampus was known, since during the morphogenesis of the hippocampus radial glial cells translocate from the SVZ to the dentate gyrus (Eckenhoff and Rakic, 1984) then persist as radial astrocytes in the SGZ (Seri et al., 2001, 2004). On the other hand, the unique astrocytic meshwork forming the glial tubes in the SVZ-RMS was previously unknown (Jankovski and Sotelo, 1996; Lois et al., 1996; Peretto et al., 1997), and only recently a link between radial glia and the SVZ glial compartment has been established.

2.3. Postnatal modifications in the subventricular zone

The SVZ is much larger and contains more cells in neonates than in adults, then undergoing remarkable reduction in size during the first two weeks of life (Peretto et al., 1999, 2005; Tramontin et al., 2003; Fig. 3A–F). An actively-proliferating VZ characterized by interkinetic nuclear migration is still present in neonatal mice. The VZ cells have a soma which is elongated orthogonally to the ventricular surface with a cilium extending into the ventricular lumen, and a long radial process extended into the parenchyma, thus being identifiable as radial glia (Tramontin et al., 2003). Most of these cells are progressively replaced by immature ependymal cells during the first 2–4 postnatal weeks (Spassky et al., 2005).
In the neonatal SVZ the transition between radial glia and astrocytes is attested by the occurrence of glial cell bodies that have translocated from the ventricular surface, by the increase in ramification of their cell processes, and by the appearance of GFAP immunoreactivity (at the end of the first postnatal week in rats, during the second in mice; Tramontin et al., 2003; Peretto et al., 2005). Nevertheless, what actually characterizes the behaviour of radial glia in the SVZ is a change in the orientation of their main process from radial to tangential on the external border, at the limit with the surrounding parenchyma (Alves et al., 2002; Peretto et al., 2005; Fig. 1B). At the same stage, around the first postnatal week, most glial cell bodies are grouped along this border, some of them still proliferating (Peretto et al., 2005), whereas the inner part of the SVZ is occupied by a huge number of tangentially-migrating neuronal precursors forming a rather homogeneous mass (Fig. 1). The increasingly complex ramifications of SVZ glial cells firstlylead to a partial compartmentalization of the neuroblast cell mass, later aggregating to form thick septa which will ultimately become the glial tubes during the 3rd and 4th postnatal weeks (Figs. 1B and 3). In parallel, the mass of migrating neuroblasts splits into chains following a pattern which seems quite independent from the glial arrangement, and more linked to molecular boundaries (Peretto et al., 2005; Fig. 3A). Hence, the most relevant modifications in the maturing SVZ affect the glial cell population, in which the morphological changes do occur in parallel with changes in their identity.
Recent studies have proposed that radial glia may have multiple roles in olfactory bulb development (Bailey et al., 1999; Puche and Shipley, 2001). In young rodents, radial glia extends from the VZ which lines the olfactory ventricle, to the outer pial surface (Valverde et al., 1992). Detailed analyses performed using immunohistochemistry and application of nanocrystals of DiI to specifically label single radial glial cells revealed two morphologically distinct cell subpopulations referred to as type I and II radial glia. Type I radial glia shows an apical process which extends from the olfactory ventricle to the glomerular layer, forming a restricted tuft or ‘‘glial glomerulus.’’ A tight spatial and temporal organization of these radial glial processes with the olfactory glomeruli and the growing olfactory receptor neuron axons suggests that they participate in the formation and/or stabilization of mammalian olfactory bulb glomeruli. Type II radial glia apical processes do not reach the glomerular layer and ramify deeper in the bulb at the level of the external plexiform layer. This latter type of glial cells may participate to the formation of the olfactory bulb layered

architecture, providing a scaffold for the migration of the main projection neurons, namely the mitral and tufted cells. Indeed, these projection neurons are generated at the level of the olfactory bulb VZ and then migrate radially to their final destination (Hinds, 1968a,b; Blanchart et al., 2006). A direct role for radial glial cells in the migration and genesis of the olfactory bulb projection neurons, as well as their role as primary progenitors of these cells, remains to be established. Olfactory bulb radial glia progressively disappear during the first 3 weeks of postanatal life (Chiu and Greer, 1996), likely by transformation into several classes of astrocytes (Chiu and Greer, 1996; Puche and Shipley, 2001). This situation, similar to that described for the cerebral cortex, indicates that other mechanisms serve the radial migration of interneurons coming from the RMS, through the adult olfactory bulb (Saghatelyan et al., 2004).

2.4. The hidden heterogeneity of the SVZ glial population(s)

On the whole, in spite of an increasing literature regarding the origin of glial-like neural stem cells, little is known about the complex and regionally-heterogeneous radial glia cell transformation in the postnatal SVZ. A precise pattern of adhesion and extracellular matrix molecules seems to parallel the shift from neonatal to adult SVZ (Peretto et al., 2005). For instance, the extracellular matrix protein tenascin-C, which is produced by glial cells, contributes to the SVZ neuronal/glial compartmentalization as a molecular boundary. This molecule shows a different distribution along the entire length of radial glial cells being prevalently vesicular in the cytoplasm of the portion contained within the SVZ, whereas it is largely widespread in the parenchymal tissue surrounding the SVZ (Peretto et al., 2005). This suggests that, besides the heterogeneity of radial glia belonging to different brain regions, different signals could act along the extension of single radial glial elements. Noteworthy is the fact that no evident signs of classic radial glia cell morphology remain in the SVZ at the end of postnatal modifications, at least in the regions characterized by the coexistence of glial tubes and high cell proliferation which are considered to host the neural stem cell niche. Nevertheless, radial glia-like cells retaining an elongated morphology and co-expressing GFAP and vimentin have been described in the ventral part of the lateral ventricle (Sundholm-Peters et al., 2004), thus indicating that the transformation of radial glia into astrocytes within the SVZ can be dorsoventrally heterogeneous. Apart from this example, the remarkable changes affecting SVZ glial cell populations at different postnatal stages and at different rostral-caudal anatomical levels leave a (apparently) rather homogeneous meshwork of astrocytes: the glial tubes. Unlike the hippocampal SGZ, the high density of astrocytic cells packed within the glial tube structure, further complicated by the intermix of their processes, could explain how the existence of a remnant of radial glial cells in this adult neurogenic site was not detected soon after its discovery (Luskin, 1993; Jankovski and Sotelo, 1996; Lois and Alvarez-Buylla, 1994; Lois et al., 1996; Peretto et al., 1997). In addition, due to such a complexity, the exact morphology and spatial organization of individual glial cells therein is at present unknown. Immunocytochemical stainings carried out in the adult SVZ suggest that the glial compartment can be heterogeneous (e.g., a subset of SVZ astrocytes continue to express vimentin and nestin). On the other hand, only slight differences can be observed among these glial cells at the ultrastructural level (Doetsch et al., 1997). Combined morphological and functional studies estimated that about 12% of SVZ cells are $GFAP^{+}$, but only 1% of SVZ cells generate neurospheres (Doetsch et al., 1999b), thus indicating that not all SVZ astrocytes are stem cells. The carbohydrate antigen known as Lewis X (LeX), which is expressed by a subpopulation of astrocytes and tanycytes (Bartsch and Mai, 1991), is also present in 6% of SVZ astrocytes, thus being a putative marker for neural stem cells (Capela and Temple, 2002). Nevertheless, the existence of $Lex^{+}/GFAP^{-}$ cells with stem cell properties, further complicates the picture, leaving unresolved the issue of neural stem cell in vivo identification.

2.5. Origin of adult SVZ neural stem cells

The observations reported in the previous paragraph provide an indirect demonstration that radial glia somehow persist in the SVZ, yet underlining how it is difficult to follow the behaviour of single glial cells throughout their postnatal modifications. More direct proof of a link between embryonic radial glia and adult neural stem cells was obtained by employing a lox-Cre-based technique to specifically and permanently label a restricted population of radial glia in newborn mice (Merkle et al., 2004). Radial glia in the lateral wall of the lateral ventricle genetically tagged with a replication incompetent, EGFP-expressing adenovirus at postnatal day 0, was shown to give rise to GFAP + astrocytes of the SVZ as well as to multiple classes of brain cells including neurons, ependymal cells, and oligodendrocytes. The fact that radial glia become the forebrain neural stem cells is also supported by other observations in the work of Merkle and co-workers, namely: (i) the RMS contained marked migratory neuroblasts produced after the disappearance of radial glia; (ii) marked glial and neuronal cells also labelled for exogenously-administered bromodeoxyuridine were found in the olfactory bulb; (iii) the progeny of marked radial glial cells isolated from adult animals generated self-renewing, multipotent neurospheres.
Radial glia-like cells whose cell bodies translocate from the SVZ to the dentate gyrus (Eckenhoff and Rakic, 1984) are known to persist as ‘radial astrocytes’ of the SGZ (Seri et al., 2004), taking part in the hippocampal stem cell niche. These cells show functional properties similar to those of the SVZ, although some intermediate transit cells can be slightly different (Seri et al., 2001, 2004; Namba et al., 2005). By comparing glial cells of the two main neurogenic sites, SVZ and SGZ, a common pattern in the lineage radial glia-astrocyte-stem cell is shown to exist, although revealing that radial glia transform differently within different tissue environments.
The production of new neuronal precursors from radial glia was known in other vertebrates, such as songbirds (Alvarez-Buylla et al., 1990), in which radial glia are retained during adult life being associated with the production and migration of newly generated cells in wide regions of the telencephalon (Alvarez-Buylla et al., 1987; Alvarez-Buylla and Nottebohm, 1988). Radial glial cells similar to those existing during development also persist in reptiles and fish (reviewed in Garcı`a-Verdugo et al., 2002), whereby they are thought to support adult neurogenesis and regeneration in many CNS regions (Zupanc and Clint, 2003; Zupanc, 2006). Thus, the persistence of this class of cells could be considered as a well conserved feature in vertebrates, although in mammals radial glia persist only within specific regions and after a morphological transformation that makes their identification more difficult. An important difference consists of the fact that in birds and reptiles ependymal cells and germinal/radial glial cells coexist throughout the life of the animal, whereas in mammals they coexist for an undetermined period of development, whereas the adult cerebral ventricle surfaces are largely composed of non-proliferative ependymal cells (Spassky et al., 2005), the contact of the radial glia-derived neural stem cells with the ventricular lumen being assured by their cilium-like process (Doetsch et al., 1997, 1999b; Alvarez-Buylla et al., 2002). In addition, comparative analysis carried out on the SVZ of different mammals pointed out that differences in the cytoarchitecture of the stem cell niche, particularly concerning the arrangement of the astrocytic compartment, do exist even among closely-related species (Rodriguez-Perez et al., 2003; Sanai et al., 2004; Ponti et al., 2006; Quinones-Hinojosa et al., 2006). A further step in this direction would be to better understand how this variability might be related to differences involving the development of radial glial cells (see Rakic, 2003).
Neural stem cell features seem to be retained solely within persistent neurogenic sites since astrocytes from other (non-neurogenic) CNS regions can generate stem cells in vitro only when isolated at early developmental stages, whereas they lose this property after the second postnatal week in mice (Laywell et al., 2000). This confirms that adult neurogenic sites other than harbouring stem/progenitor cells do possess intrinsic cellular/molecular factors capable of favouring the retention of stemness properties as well as of allowing them to extrinsecate. This fact does not

exclude that quiescent stemness properties could possibly be retained elsewhere (see below).

3. Conclusive remarks and future perspectives

An increasing number of data indicate glial cells as active players in neural plasticity in both the developing and adult nervous system. Subsets of astrocytes and radial glia-like cells persisting in the adult brain can be involved in structural modifications allowing a modulation in the number of synapses that control the neuronal activity under physiological stimulation, as it has been well demonstrated in non-neurogenic areas such as the hypothalamo-neurohypophysial system (Theodosis et al., 2006). Since the discovery of an additional role for glial cells as stem/progenitor cells many features of cells belonging to the astrocytic lineage might be implicated in regulating the stem cell niche maintenance and function. Astrocytes refer to the ability to undergo striking changes in their cytoskeleton and consequently cell shape and contact with neurons and blood vessels, as well as their capability to synthesize and secrete a wide variety of cellular matrix and regulatory molecules. On the other hand, taking into account that embryonic radial glia form a rather heterogeneous cell population (reviewed in Hartfuss et al., 2001; Gotz, 2003; Gotz and Barde, 2005, and article by M. Gotz in this issue), a subsequent differential expression of the above mentioned features in different developmental and regional situations can render the extremely wide astrocytic cell populations remarkably heterogeneous under the electrophysiological, molecular, biochemical, and functional profiles (see also D'Ambrosio et al., 1998; Wallraff et al., 2004; Zhou et al., 2006), although such an issue is still a matter of debate (see for example: Walz, 2000).

3.1. The role of astroglial cells in the control of adult neurogenic niches

In addition to a characterization of the location and identity of neural stem cells in the adult CNS, major efforts are directed at addressing the mechanisms that regulate their activity within the so-called ''niches'' (Lim et al., 2000; Spradling et al., 2001; Scadden, 2006). The stem cell niche is the microenvironment made up of cells and extracellular substrates that allow stem cells to express their potential, thus regulating how they participate in tissue generation, maintenance and repair (Spradling et al., 2001; Scadden, 2006). Several molecular components characterizing the adult neurogenic niches include classical developmental signals such as the bone morphogenetic proteins (BMPs), its antagonist noggin, or Notch and sonic hedgehog (Shh) which play an important role in controlling adult neurogenesis (see for review Doetsch, 2003a; Alvarez-Buylla and Lim, 2004; Hagg, 2005). Within the neural stem cell niche, astroglial cells, besides functioning as primary progenitors, also serve as important regulators of neurogenic activity. These features are mostly attributable to their abundance, their extensive cell–cell interactions, direct contact with blood vessels as well as with a specialized basal lamina, and to the secretion of neurogenic regulative molecules (Doetsch, 2003a). For example, two crucial factors for maintenance of neural stem cell pool such as FGF-2 and transforming growth factor-a (TGFa) are produced by CNS astrocytes. In vitro studies indicate that astroglial derived soluble factors can direct the proliferation, as well as the fate and differentiation, of both hippocampal and SVZ stem cells (Lim and Alvarez-Buylla, 1999; Song et al., 2002). Moreover, in vivo studies indicate that SVZ astrocytes express and secrete molecules that are implicated in creating molecular barriers during development (i.e., tenascin-C, Jankovski and Sotelo, 1996; Peretto et al., 2005), or that are strictly related to neurogenic processes, such as EphB receptors and ligands (Conover et al., 2000), BMPs and their receptors (Lim et al., 2000; Coskun et al., 2001; Peretto et al., 2002). Additional strong evidence supporting a direct role for glia in directing adult neurogenesis comes from the demonstration that the protein noggin, expressed by ependymal cells of the lateral ventricles, can regulate the specifications of newly formed cells toward a neuronal fate by antagonizing the BMPs present in the SVZ astrocytes (Lim et al., 2000). By using multiple approaches, including the analysis of noggin LacZ heterozygous mice, consistent expression of noggin and two members of the BMP family, BMP4 and BMP7, has

been recently demonstrated in the anterior forebrain of adult mice (Peretto et al., 2002, 2004). Along the full extent of the SVZ, from the lateral ventricle to the olfactory bulb, noggin, BMP4, and BMP7 are mainly associated with the astrocytic glial compartment. In the olfactory bulb, BMP4 and BMP7 proteins remain primarily associated with SVZ astrocytes, while strong noggin expression was also found in the granule cell layer. Taken together these data indicate that the antagonism between noggin and BMPs, both molecules produced by the glial tubes, acts through autocrine/paracrine mechanisms to maintain a neurogenic environment throughout the SVZ extension, thus further confirming a primary role of astrocytes in the control of adult neurogenesis. Recent data obtained after patch clamp recordings in acute slices from transgenic mice expressing green fluorescent protein (GFP) driven by the promoter of human GFAP (Liu et al., 2006) show that astrocytes of the postnatal SVZ display typical properties of astroglia and radial glia but also possess functional features intermediate between these two cell categories. In concert with ependymal cells, SVZ astrocytes perform K + and glutamate buffering, a property that can be involved in the regulation of proliferation and migration of SVZ progenitors (Bolteus and Bordey, 2004). Thus, also electrophysiolgical observations are consistent with the idea that modified, relatively immature astroglial cells with unique functional features persist within the neurogenic sites, and indicate how difficult is to classify them in commonly accepted cell types.

3.2. Possible heterogeneity of radial glia-derived cell precursors in the brain

As suggested in Sections 2.3 and 2.4, it is not clear if radial glial cell transformation can produce different types of astrocytic stem/progenitor cells. On the other hand, it is clear that during development radial glia can also instruct the neuronal progeny to adopt appropriate region-specific phenotypes (reviewed in Hall et al., 2003), and that the SVZ shows diverse specializations throughout its entire extension. A portion of the caudal part of the adult mouse SVZ squeezed between the hippocampus and the corpus callosum and no longer associated with an open ventricle has been recently described as a distinct germinal zone (subcallosal zone; Seri et al., 2006) containing cell progenitors that are not immunoreactive for b-tubulin and give rise to oligodendrocyte and astrocyte precursors. Together with other recent reports (Hack et al., 2005; Lemasson et al., 2005), this suggests that adult neurogenic sites could be heterogeneous as to the commitment of newly generated progenitor cells, on the basis of regional and/or age-dependent cues. Future research should be addressed to unravel to what degree this heterogeneity either directly involves the astroglial stem cell progenitors or indirectly affects the commitment of the progeny.

In addition, the heterogeneity of glial cells belonging to the astrocytic lineage could be extended to other regions of the adult CNS since cell proliferation is not restricted to the hippocampus and SVZ-olfactory bulb system. In non-neurogenic regions this activity is thought to give rise primarily to glial cells (Horner et al., 2000), nevertheless several ex vivo studies provide evidence that multipotent progenitors can be isolated from very diverse regions of the adult brain including septum, striatum, cortex, spinal cord and optic nerve (Palmer et al., 1995, 1999; Kondo and Raff, 2000). These cells cultured in vitro with FGF-2 or FGF-2/EGF differentiate into astrocytes, oligodendrocytes and neurons, and when transplanted into neurogenic regions, they behave like the SVZ and SGZ progenitors, producing hippocampal and olfactory bulb interneurons (Shihabuddin et al., 1997). On the whole, these observations, besides confirming the role of the local environment in influencing the specification of progenitor cells, suggest the likely existence of a common glial latent progenitor displaced within the mature brain parenchyma. Nevertheless, in vivo, the putative glial identity and the existence of such a precursor, as well as a possible feasible function in brain homeostatic mechanisms, so far, have not been clearly demonstrated. Evidence of the presence and functional activity of local parenchymal progenitors in vivo came from studies of induced neurogenesis (see Emsley et al., 2005; Lie et al., 2004, for an exhaustive review concerning this topic) and comparative analyses. In the rabbit we have recently shown that the adult striatum is able

to maintain a low neurogenic activity under normal conditions (Luzzati et al., 2006). Neurogenesis in this region is attributable to the existence of small clusters of proliferating progenitors settled within the parenchyma of the caudate nucleus. Similar to the SVZ type C cells, striatal parenchymal progenitors give rise to chains of doublecortin $^{+}$ /PSA-NCAM $^{+}$ neuroblasts which partially differentiate into mature striatal interneurons. Notably, adult striatal proliferating progenitors are immunoreactive for BLBP. This protein is exclusively expressed in radial glial cells and astrocytes throughout the developing CNS (Feng et al., 1994; Feng and Heintz, 1995; Kurtz et al., 1994; Hartfuss et al., 2001). Therefore, in accordance with in vitro studies these results indicate that, in addition to SVZ and SGZ neurogenic regions, other areas of the adult mammalian brain can harbour progenitor cells of the astroglial lineage. Considering that glial cells are the most numerous cells in the CNS, and that astrocytes are the most representative glial cells, a lot of research in this direction is expected to be done in the future.

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