Adult neurogenesis without germinal layers: the "atypical" cerebellum of rabbits

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ABSTRACT

Unlike non mammalian vertebrates, adult neurogenesis in mammals is detectable in highly restricted brain sites. Persistent neurogenesis is thought to depend on stem cells residing in neural stem cell niches which are remnants of the embryonic germinal layers. Local progenitors which retain some proliferative capacity have been identified in the mature brain parenchyma, yet they do not support a constitutive, 'actual' neurogenesis, but rather a 'potential' neurogenesis which does not extrinsecate fully and spontaneously in vivo. In contrast with such a view, genesis of neuronal and glial cells from local progenitors does occur in the peripuberal and adult rabbit cerebellum. This process is independent from persisting germinal layers and involves different cell populations.

Key words
Comparative neurogenesis • Stem cells • Parenchymal progenitors • Plasticity • CNS

Introduction

The dogma of the central nervous system (CNS) as a static, non renewable tissue has been challenged by the discovery of adult neurogenesis (Gage, 2000). In the animal world the capacity of generating new nerve and glial cells throughout life is a phylogenetically highly conserved feature (Lindsey and Tropepe, 2006). Persistent neurogenesis is thought to depend on neural stem cells (NSC) residing in niches, as a remnant of embryonic germinal layers (Kriegstein and Alvarez-Buylla, 2009).

In invertebrates and non mammalian vertebrates, persistent neurogenesis is detectable in wide regions of the CNS (Zupanc, 2006; Kaslin et al., 2008; Zhang et al., 2009). By contrast, in adult mammals the neurogenic processes are highly restricted within two specific brain sites: the forebrain subventricular zone (SVZ) and the hippocampal dentate gyrus (reviewed in Bonfanti and Ponti, 2008). As a consequence, the vast majority of the adult mammalian CNS, including

brain, cerebellum and spinal cord, can be considered as a non renewable tissue. Yet, most neurodegenerative diseases and ischemic/traumatic damage actually occur within this 'perennial' tissue.

Partially in contrast with such a view, in the last few years local progenitors which retain some proliferative capacity have been identified in the mature CNS parenchyma (Horner et al., 2002; Butt et al., 2005; Luzzati et al., 2006; Nishiyama, 2007; Ponti et al., 2008). In the adult mouse, the largest class of these cells do express the nerve/glial antigen 2 proteoglycan (Ng2, Horner et al., 2002; Dawson et al., 2003). They are also called *synantocytes* (Butt et al., 2005) or *polydendrocytes* (Nishiyama, 2007), and are morphologically, antigenically, functionally distinct from mature astrocytes, oligodendrocytes, and microglia. Yet, in spite of their proliferative capacity, the Ng2+ cells do not perform neurogenesis *in vivo*, although they retain potentialities in vitro with remarkable heterogeneity according to their regional location (Nishiyama et al., 2009; Boda and Buffo,

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this special issue). Also other sources of parenchymal neurogenesis remain controversial (e.g. in the cerebral cortex: Gould et al., 1999; Kornack and Rakic, 2001; Ohira et al., 2009), indicating that stem/progenitor cells could persist within the mature brain parenchyma, yet giving rise to neurons only in particular pathological/experimental conditions (Ohira et al., 2009; Pierce and Xu, 2010). Thus, in mammals, a constitutive neurogenesis intended as a spontaneous, genesis of neurons and glia (see Emsley et al., 2005) seems to occur exclusively in the restricted germinal layer-derived neurogenic sites. Besides this 'actual' neurogenesis, in the remaining CNS parenchyma local progenitor cells do support a 'potential' neurogenesis, which does not extrinsecate fully and spontaneously *in vivo* (Fig. 1). In summary, the persistence of widespread, constitutive parenchymal neurogenesis in the mature CNS appears to be a feature of non mammalian vertebrates and invertebrates, whereas in mammals NSCs are concentrated within restricted niches and only progenitor cells with moderate or 'quiescent' potentiality populate the mature parenchyma.

Recent findings obtained in rabbits, provided hints for substantial inter-species differences even in mammals (Bonfanti and Ponti, 2008). Despite the

orders Lagomorpha and Rodentia being quite similar, unexpected examples of structural plasticity and neurogenesis consisting of parenchymal chains of neuroblasts both dependent (Luzzati et al., 2003; Ponti et al., 2006b) and independent from the SVZ (Luzzati et al., 2006; Ponti et al., 2006a; Ponti et al., 2008) have been found in the rabbit brain. These results indicate that in young and adult lagomorphs both SVZ-derived and SVZ-independent newly generated cells can be seen in areas of the mature parenchyma that are considered non-neurogenic in rodents. By extending the search to the rest of the CNS, similar properties have been found in another region considered devoid of cell genesis in mammals: the cerebellum. In the present review we will focus on such an issue.

Postnatal and adult neurogenesis in the rabbit cerebellum

Brief summary of the neurogenic process in the mammalian cerebellum

The current knowledge about the adult mammalian cerebellum is mainly based on studies carried out in rodents, where plasticity is limited to synaptic
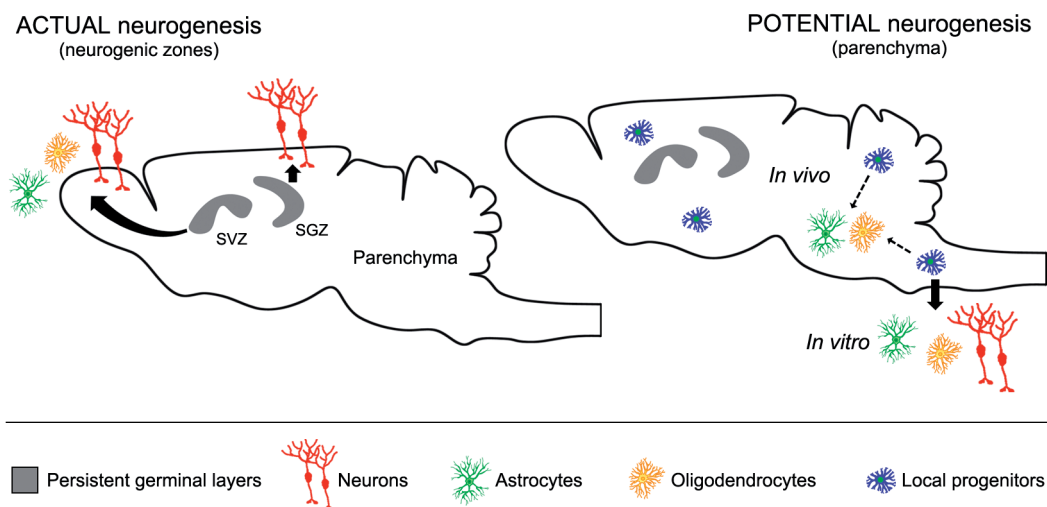

Fig. 1. - Schematic representation of adult neurogenesis in mammals, based on studies carried out in rodents. Constitutive, *actual* neurogenesis persists within restricted neurogenic areas (the forebrain subventricular zone – SVZ, and the hippocampal subgranular zone – SGZ) which are remnants of embryonic germinative layers. Parenchymal, *potential* neurogenesis does not produce neurons *in vivo*, although progenitors either isolated and cultured *in vitro* or stimulated *in vivo* by experimental conditions can extrinsecate some neurogenic properties.

rearrangement of pre-existing circuits (Ito, 2006). The genesis of most cerebellar neuronal and glial cell types occurs very early, from the periventricular neuroepithelium (Fig. 2A). Projection neurons are the first to be generated, whereas interneurons and some astrocytic glial cells complete their specification postnatally (Maricich and Herrup, 1999; Leto et al., 2008; Grimaldi et al., 2009). Unlike the rest of the CNS in which neuronal cell populations are substantially assembled at birth, the mammalian cerebellum undergoes a protracted genesis of granule cells during the early postnatal period. Such cells originate from a transitory, secondary germinative

layer localized on its surface: the external germinal layer (EGL; Altman, 1972; Fig. 2A). The EGL is formed by tangential displacement of cell precursors from the germinal trigone of the fourth ventricle, then leading to protracted genesis of granule cells by radial, centripetal migration of cell precursors that continue to proliferate within the EGL (Leto et al., 2008). In the postnatal developing cerebellar cortex, this transitory germinal zone progressively reduces its thickness as the granule cell precursors migrate deep into the cortex, then disappearing at specific ages in different species, always before puberty (Fig. 2B). The delayed proliferation/migration/dif-
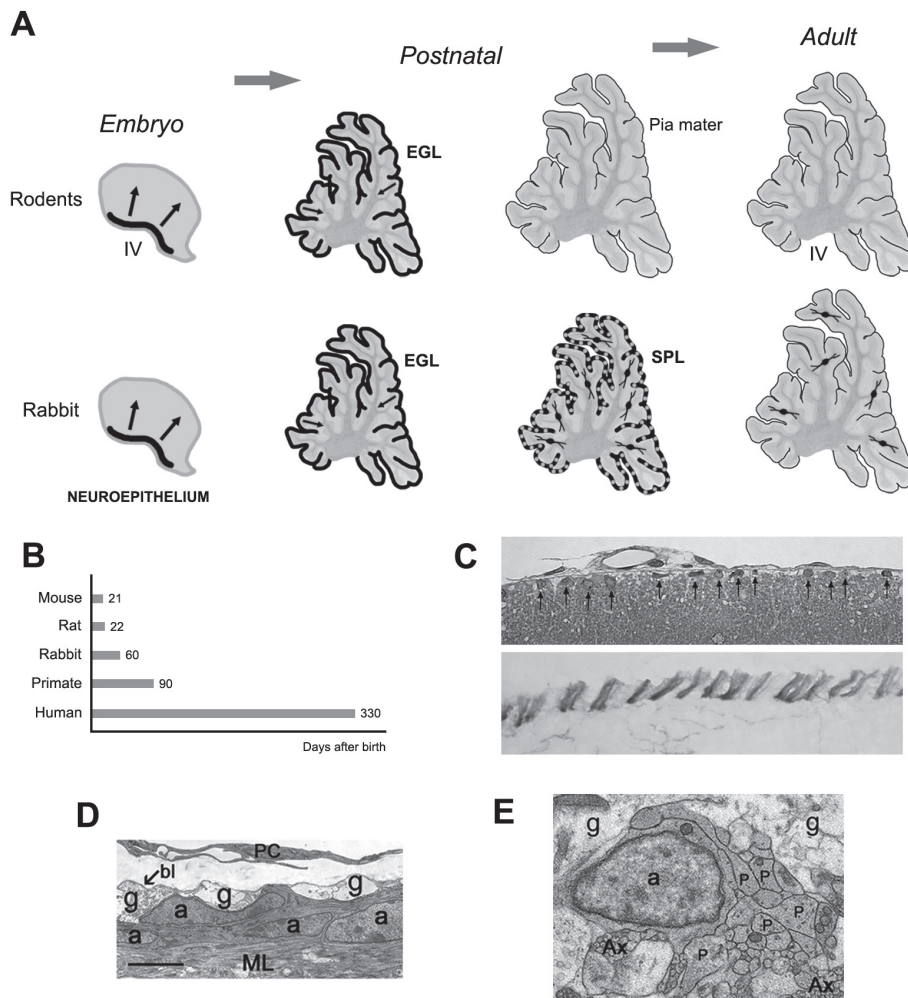

Fig. 2. - Actual neurogenesis in the cerebellum of young/adult rabbits, compared with rodents. A, Exhaustion of germinative layers (thick black) in the pre- and post-natal cerebellum of rodents and rabbits. B, End of granule cell genesis estimated in different mammals. C, Subpial cells of the SPL in the peripuberal rabbit, visualized with cresylviolet staining (arrows; top) and chains of PSA-NCAM+ neuroblasts (bottom). D,E, Ultrastrucural evidence for SPL chains of neuroblasts (D, longitudinal; E, transversal). g, Glial processes; a, neuroblasts; ML, molecular layer; PC, pial cell; P, neuroblast processes; Ax, axons (parallel fibers).

ferentiation of some cerebellar cell precursors can be followed by their expression of specific markers: e.g. the transcription factor *Pax2* for interneuronal precursors of neuroepithelial origin ascending through the prospective white matter (Maricich and Herrup, 1999; Weisheit et al., 2006), and *Pax6* for EGL-derived granule cell precursors (Engelkamp et al., 1999; Yamasaki et al., 2001). In rodents, the delayed proliferation of glial cells and interneurons is concluded before the end of granule cell genesis (Leto et al., 2006; Grimaldi et al., 2009; Leto et al., 2009). As concerns cerebellar neurogenesis in mammals, it was generally accepted that no more cell genesis is detectable starting from species-specific stages which coincide with the end of granule cell genesis, since no germinal layers remain active. Under the functional profile, the postnatal genesis of granule cells and interneurons shares a logic with the role of cerebellar circuits in learning/adapting motor skills to the environmental cues the animal is dealing with during postnatal/young stages of its life. After this stage and throughout life cerebellar plasticity is granted solely by synaptic plasticity.

In contrast with such a view, recent work carried out on the cerebellum of New Zealand white rabbits revealed a remarkable neurogenesis taking place around puberty (Ponti et al., 2006a), and persisting, to a lesser extent, during adulthood (Ponti et al., 2008).

The subpial layer: an extension of the EGL in peripuberal rabbits

Unlike the situation described in most mammals studied, cell proliferation on the rabbit cerebellar surface is detectable up to the fifth month of age, thus extending beyond puberty (Ponti et al., 2006a). Starting from the second month of life, the germinal layer in which such proliferation occurs is quite different from the EGL (*subpial layer*, SPL). Analyses of the rabbit cerebellum at different postnatal/peripuberal stages by combination of different techniques (i.e. detection of Ki67 and BrdU at different survival times in association with antigens of cell specification/differentiation, and electron microscopy), revealed that proliferating elements in the SPL form a single, non-continuous layer independent from the meninges (Ponti et al., 2006a).

Unlike the EGL, the SPL contains *tangential chains* of neuroblasts which express molecules strictly associated to newly generated neuronal precursors

and required for their migration, such as PSA-NCAM and DCX (reviewed in Bonfanti, 2006). The occurrence of these chains was confirmed by electron microscopy serial reconstruction (Ponti et al., 2006a; Fig. 2D, E). They are formed by small subpial cells sharing the same cytology of SVZ neuroblasts in mice (type A cells; Doetsch et al., 1997) and rabbits (Ponti et al., 2006a), and localized at the basal lamina/molecular layer interface. Thousands of these chains regularly arranged with a medial-lateral orientation and spaced about 10-20 µm from one another (in 3 month old animals) cover the entire cerebellar surface. Studies of the cell cicle kinetics in the SPL by employing subsequent pulses of BrdU show that dividing cells and chains of neuroblasts are part of the same population of newly generated cells (Ponti et al., 2006a). Such neurogenic process occurs according to a homogeneous topographical distribution throughout the cerebellar cortical extension, thus excluding that a restricted site could be a source of the newly generated cells. Indeed, as in rodents, cell proliferation in rabbits is absent in the ependymal regions of the IVth ventricle at any postnatal periods.

The rabbit SPL is not just an 'extension in time' of the EGL. Indeed, markers linked to the granule cell precursors, such as *Pax6* and NeuN, disappear in the rabbit cerebellum starting from the third postnatal month (Ponti et al., 2008). The descent of granule cell precursors being exhausted before puberty, the SPL is a persistent structure independent from granule cell genesis. The transition from rabbit EGL to SPL occurs at the end of the first month of life, when the EGL becomes a monolayer. After P40, no distinction between proliferative and pre-migratory layers is detectable, and a clear pattern of chain fragmentation is maintained up to 4-5 months of age, with a progressive increase in the distance among chains and decrease in their number (Fig. 3C). This process is remarkable from the 2nd month to puberty (4th month), then disappearing between the 5th and 6th month. Thus, the SPL spans from 1 to 5 months of age, persisting shortly beyond puberty (Fig. 3C). It shares features with the forebrain SVZ (see Bonfanti and Ponti, 2008), since it originates from structural modification of a secondary germinal layer and generates neuronal precursors which assemble into tangential chains sharing antigenic/ultrastructural features with SVZ chains (Doetsch et al., 1997; Peretto

et al., 1997). This indicates that neurogenic systems which occur in different regional locations (but similar temporal windows) share common changes in the behaviour of newly generated cells. A fact that could be linked to a sort of adaptation to the progressive maturation of surrounding parenchymal tissue (see Peretto et al., 2005; Ponti et al., 2006a).

In order to clarify the dynamics of cerebellar postnatal neurogenesis in mammals after the discovery of the SPL, we re-examined the last phases of EGL exhaustion in the mouse cerebellum using the same technical approaches employed in rabbits (Ponti et al., 2006a). Although a fragmentation of the EGL pre-migratory layer in small chain-like aggregates does occur in mice just before the EGL disappearance, the occurrence of these structures is limited in time (4-5 days) and followed by complete disappearance after P21 (Leto et al., 2008). At present, the role of rabbit SPL chains remains unresolved. They could be involved in the tangential displacement of neuronal precursors, as shown in the mouse EGL pre-migratory layer prior to engagement in radial migration (Komuro et al., 2001).

Genesis of new cells in the cerebellum of peripuberal and adult rabbits

In spite of both the exhaustion of granule cell descent through cortical layers and the progressive reduction in SPL proliferative activity, a remarkable genesis of cells continues to be detectable in the cerebellar cortex of peripuberal rabbits (Fig. 3). Systemically-administered BrdU detected at different post-injection survival times revealed that a substantial amount of these cells is still alive two weeks after their birth (Ponti et al., 2006a), and to a lesser extent, after two months (Ponti et al., 2008). Their morphology can be visualized by double labellings with antibodies raised against markers of structural plasticity implicated in dynamic cellular events of developmental and adult neurogenesis (e.g. PSA-NCAM, DCX), and the cytoskeletal protein Map5 (microtubule-associated protein 5, or Map1B; Wu et al., 2001). Newly generated cortical cells fall into three main morphological types: *bipolar*, *polarized neuronal-like* and *multipolar* (Fig. 3A), which are homogeneously distributed in all lamellae. Immunocytochemical markers and cell morphologies are strictly associated: PSA-NCAM and DCX are detectable in bipolar and polarized, but

not in multipolar cells, whereas only the latter are immunoreactive for Map5. All these cell types are also detectable in adult rabbits (at least up to three years of age; Ponti et al., 2008).

The co-expression of the microtubule binding protein DCX in virtually all the PSA-NCAM+ cells confirms their neuronal nature. The distinction of polarized neuronal cells and multipolar cells into two populations is confirmed by their selective expression of different nuclear transcription factors: the PSA-NCAM+/DCX+ cells are immunoreactive for *Pax2* (Maricich and Herrup, 1999; Weisheit et al., 2006), and consistently negative for both *Sox2* (Graham et al., 2003) and *Olig2* (Zhou and Anderson, 2002). By contrast, all MAP5+ cells are *Olig2+*, *Sox2+*, and *Pax2*-negative, thus suggesting a glial progenitor antigenic profile (Ponti et al., 2008).

Neuronal precursors

The newly generated bipolar and neuronal-like cells which are immunoreactive for PSA-NCAM are reminiscent of neuroblasts and young neurons of the two brain neurogenic sites (Bonfanti, 2006). Bipolar cells show the typical morphology of migrating elements and can be found in all cortical layers, whereas neuronal-like cells are confined in the molecular layer (Fig. 3). They have a piriform cell body, a thin axonal-like process with a U-shaped direction remaining within the molecular layer, and one to three dendritic processes (Ponti et al., 2008; Fig. 3). BrdU/PSA-NCAM double stainings carried out at different post-injection survival times support the existence of a two week-long neurogenic sequence leading to a young neuronal cell type in the molecular layer. Their expression of *Pax2* and γ-aminobutyrric acid do support their identity of GABAergic cerebellar interneurons of neuroepithelial origin (Ponti et al., 2008). Among the two populations of newly generated cells, neurons are more numerous at the peri-puberal stage (9/1 rate with respect to glial-like cells), whereas they decrease dramatically in the adult, reaching a 1/1 rate.

In spite of the presence of an active SPL, the genesis of granule cells in the cerebellum of peripuberal rabbits can be excluded since the newly generated neurons, other than having a specific morphology, are present exclusively in the molecular layer. In addition, the existence of PSA-NCAM+/DCX+ newlyborn bipolar cells with antigenic features of

granule cell precursors (NeuN, *Pax6*) was restricted to the third-fourth postnatal month (Ponti et al., 2008; Fig. 3).

By following these neuronal precursors at different BrdU post-injection survival times, it appears that the cytoplasmic and cytoskeletal markers used to visualize the neuronal cells during the first three-four weeks (PSA-NCAM, DCX), subsequently fade and disappear, thus hampering the identification of specific cell shapes in more mature cells (Fig. 3B). This gradual disappearance of early developmental markers can be interpreted as a normal process of cell differentiation in postnatal/adult neurogenic systems (Rao and Shetty, 2004; Bonfanti, 2006). Indeed, by extending the countings of BrdU+ nuclei up to 60 days after the last injection we showed that most of the cells that remain in the cortex after the first wave of death actually survive for two months (Ponti et al., 2008). In BrdU/PSA-NCAM double stainings, only traces of PSA-NCAM can be detected up to 60 days post-injection survival, thus not providing information about the morphology of the cells. In some BrdU+ cells detected at this long term survival by electron microscopy, healthy cells showing the morphology of both neurons and syn-antocytes were still in place (Ponti et al., 2008). The occurrence of rare synaptic profiles onto the soma of these newly generated cells suggests that they are young neurons available for new contacts. Yet, their morphology does not match with any of the neuronal types described in the mammalian cerebellum, thus leaving open the issue whether these cells are specific to rabbit.

Glial-like cell precursors

The Map5+ multipolar cells have a spheric cell body and a stellate, highly ramified morphology. BrdU/Map5 double stainings indicate that most of
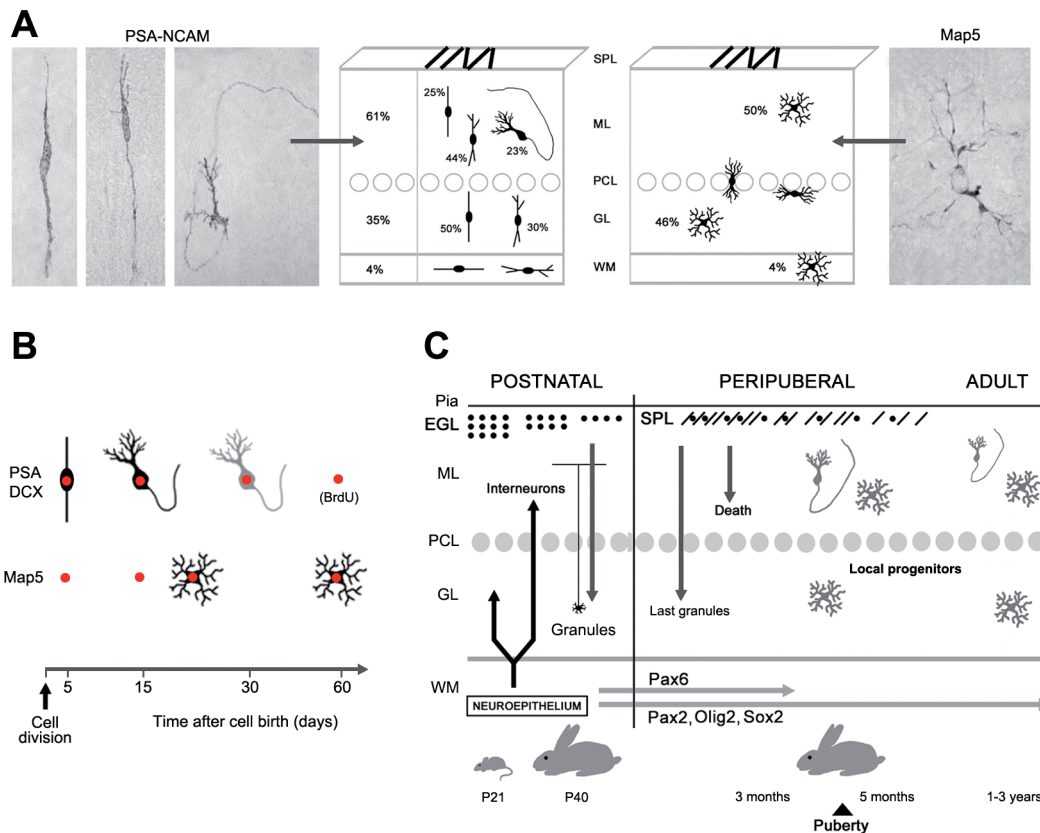

Fig. 3. - A, Two populations of newly generated cells in the peri-puberal rabbit cerebellum (left, neuronal-shaped cells; right, multipolar cells). B, After their birth, the two cell populations acquire and lose cellular markers at different stages of their life. Neurons (top) can be identified with cytoplasmic markers very early, whereas glial cells (bottom) only starting from the third week. C, Time course of germinative layer-dependent and independent cell genesis in the rabbit cerebellum.

them are cycling (Ponti et al., 2008). Unlike the neuronal cells, they are homogeneously distributed in all cerebellar layers. In the three-month old rabbits multipolar cells represent a small population with respect to the neuronal cells, whereas their relative amount increases in the adult, reaching almost half of the total. In spite of their stellate shape, they are GFAP-negative. They express the Map5 antigen starting from the second week from their birth, thus hampering characterization in the first phases of differentiation. Then, they maintain the Map5 staining for at least two months (Ponti et al., 2008; Fig. 3B).

The morphology, antigenic profile, and regional distribution of the multipolar cells is reminiscent of Ng2+ progenitor cells referred in rodents to as *synantocytes* (glial elements that form multiple contact with neurons, astrocytes and oligodendrocytes (Butt et al., 2005), or to as *polydendrocytes* (related to oligodendrocytes but with more processes and functions; Nishiyama, 2007). All anti-Ng2 antibodies available failed to detect this molecule in the rabbit, suggesting that a different epitope is present in lagomorphs, and thus leaving undetermined if the rabbit Map5+ cells represent either mice Ng2+ progenitors or a subpopulation of them (Ponti et al., 2008).

In literature, the microtubule-associated protein Map5 (MAP1B) has been described in cells of both the neuronal and glial lineage. It is present in proliferating neuroblasts of the embryonic mouse telencephalic ventricular zone (Cheng et al., 1999) but not in astrocytes (Fischer et al., 1990; Vouyiouklis and Brophy, 1993). Its expression precedes the development of the mature oligodendrocyte phenotype, and interactions between microtubules and Map5 could have a role in the stabilization of myelin-forming processes (Vouyiouklis and Brophy, 1993). According to the limited literature available on the expression of Map5 *in vivo*, we know that it remains at relatively high level in neurogenic/structurally plastic regions of the adult brain (Nothias et al., 1996). Yet, the distribution of Map5 in rodents does not match that observed in rabbits, thus suggesting that in the cerebellum of lagomorphs anti-Map5 antibodies could function as a marker for a reservoir of progenitor cells related to the synantocyte/polydendrocyte lineage which are actively dividing. Cells of the same type are detectable in the rest of the rabbit CNS, although we still lack definitive data

regarding their proliferative rates (Paola Crociara, unpublished data).

Interestingly enough, the Map5+ cells are consistently double-stained for both the transcription factors *Sox2* and *Olig2*. *Sox2* is implicated in the proliferation/maintenance of NSCs and in adult neurogenesis (Graham et al., 2003; Episkopou, 2005; Pevny and Nicolis, 2009). *Olig2* continues to be expressed in multipotent progenitor cells of the adult CNS, although its role remains obscure (Ligon et al., 2006). The presence of these transcription factors in multipolar Map5+ cells suggests they could be multipotent progenitors instead of simple glial progenitors.

In conclusion, rabbit Map5+ multipolar cells display morphological features of astrocytes, molecular profiles of oligodendrocyte precursors (Butt et al., 2005; Nishiyama, 2007), and nuclear transcription factors that are usually expressed by multipotent progenitor cells (Avilion et al., 2003; Graham et al., 2003; Episkopou, 2005). Putting together these data, the rabbit multipolar cells could represent a specific population of parenchymal progenitor cells that are similar but not identical to rodent' synantocytes, or possibly a subpopulation of them.

Dynamics and significance of neurogenic processes in the rabbit cerebellum

The dynamics of neurogenic processes in the peripuberal and adult rabbit cerebellum are remarkably different from those described in other mammalian species studied so far. The production of new cell progenitors, including neuronal precursors, does not cease after the end of granule cell genesis, but continues at high rates up to and beyond puberty, then progressively decreasing with age (Ponti et al., 2006a; 2008). During a first period (peripuberally), such a production is localized both in a persistent SPL on the rabbit cerebellar surface (limitedly to the 2nd-5th month of life) and within the cortex. The SPL is largely independent from granule cell genesis, and its transient existence could be linked to the protracted proliferation and tangential migration of cell precursors in subpial position, which have been described in the EGL of rodents (Komuro et al., 2001). Such a process, in rodents, is consistent with the brief appearance of chain-like structures in the late EGL (Ponti et al., 2006a), yet is restricted to a short temporal window, whereas in lagomorphs it appears to find a permissive environment for longer persistence.

SPL cell genesis is paralleled by a widespread production of both GABAergic interneuronal precursors and synantocyte-like cells within the cerebellar cortical parenchyma. These cells continue to be generated, although in a smaller proportion, throughout a second period entering post-puberal and adult stages (Ponti et al., 2008). Such parenchymal genesis occurs in the absence of an SPL, and independently from any other residual germinal layer. The expression of transcription factor *Pax2* in the population of neuronal progenitors explains their identity as neuroepithelial-derived progenitors ascending through the white matter postnatally (Maricich and Herrup, 1999; Weisheit et al., 2006), then continuing to proliferate in rabbit during peripuberal and adult stages. In rodents, the division of this cell population is spatially restricted to the prospective white matter (Leto et al., 2009), then ceasing as the cells enter the cerebellar cortex. This fact underlyes the qualitative difference of postnatal neurogenesis in two closely related mammalian species.

The antigenic properties of the newly generated cells (both neuronal and glial), along with cell fate and positional information provided by transcription factors *Olig2*, and *Sox2*, indicate the rabbit cerebellar neurogenic process as qualitatively similar at different postnatal, peripuberal and adult stages. The real difference is quantitative, consisting of a progressive decrease in the number of newly generated cells, affecting both cell populations but at different rates, so that in the three year old rabbits a lower, rather equilibrated genesis of the two cell populations is present. Such a progressive reduction of rabbit cerebellar cell genesis with age (prolonged far beyond the sexual maturation of the animals with respect to its sharp end characteristic of rodents), could be related to a protracted growth of cerebellar circuits in young lagomorphs, whose life span is longer than in rodents. Different patterns of growth, maturation, and senescence may have an impact on the extent and plasticity of neurogenesis throughout life (see Lindsey and Tropepe, 2006). Tissue growth may be a critical feature for the regulation of adult neurogenesis, what could explain why teleost fish species that have indeterminate growth also have continual addition of new neurons throughout life and striking capacity for brain repair and regeneration (Zupanc, 2006). In this context, it is known that the brains of mammalian species that live relatively long, progressively and consistently grow postnatally (Purves, 1988). Although most of such increase in volume actually consists of neurite branching and consequent increasing complexity of neuronal circuits formed by pre-existing cells, one cannot exclude that the addition of new cells (e.g. interneurons) is required in this ongoing process.

Does rabbit Bergmann glia divide?

In addition to newly generated cells in the molecular and granule layers of the rabbit cerebellar cortex, some proliferating cells are also detectable in the Purkinje cell layer. Although some of them do correspond to PSA-NCAM+/DCX+ neuroblasts and Map5+ synantocyte-like cells that are generated (or possibly migrate) herein, other BrdU+ and Ki67+ nuclei can be found to be double stained with brain lipid binding protein (BLBP) in cell bodies located within the same layer and showing the morphology and topographical location of Bergmann glia (Ponti et al., 2008). Since BLBP is a marker for undifferentiated progenitor cells (Feng and Heintz, 1995; Luzzati et al., 2006), these preliminary results open the possibility that also Bergman glia could be involved in the protracted neurogenesis of rabbit cerebellum.

Bergmann glial cells are unipolar astrocytes located around the soma of Purkinje cells, and extending long processes (Bergmann fibers) that traverse the molecular layer to terminate with their endfeet at the pial surface. Multiple roles have been attributed to these glial cells. During development and early postnatally, Bergmann fibers are known to associate with migrating granule cells (Rakic, 1971). In the young/adult, they provide a substrate which directs dendritic growth, in correlation with synaptogenesis, thus influencing the shape of Purkinje dendritic tree (Lordkipanidze and Dunaevsky, 2005). In addition, the active mitosis of Bergmann glia cells in the early postnatal period would contribute to the supply of the glial framework and substrates to new expanding regions (Yamada and Watanabe, 2002). Yet, we know that no cell genesis at all is detectable after P21 in mice (Ponti et al., 2006a), and studies carried out in rats with $_3$H-thymidine labeling showed that proliferation of Bergmann glial cells ceases in the third week (Das et al., 1974; Shiga et al., 1983).

On the basis of our BrdU injection studies, at present we are investigating the possibility of rabbit Bergmann

glia proliferation at peripuberal stages and during adulthood. Following the hypothesis that in lagomorphs a protracted neurogenesis could be linked to progressive growth in young animals (see above), Bergmann glia proliferation would fit in adapting the glia framework to the expanding cerebellum. However, another interest resides in the analysis of a radial glia-derived, astrocytic like cell type that divides within a mature parenchyma instead of a germinal layer-derived NSC niche. Something similar occurs for radial astrocytes in the hippocampal dentate gyrus (that in this case is partially a remnant of a germinal layer; see (Bonfanti and Ponti, 2008), which are considered part of a stem cell niche (Seri et al., 2004). Accordingly, Bergmann glia express *Sox2* both in mouse and rabbit (Alcock et al., 2007; Ponti et al., 2008), a feature that could be related with potential NSC properties. The aim of future work is to unravel if a subpopulation of Bergman glia could retain some stem cell properties, and if an atypical stem cell niche could possibly regulate such a process in the rabbit cerebellum (Crociara et al., in preparation).

Conclusions

We know that local progenitor cells do exist in the CNS parenchyma of mammals. Nevertheless, these cells do not extrinsecate their potentialities in physiological conditions, thus not providing a 'constitutive' or 'actual' neurogenesis, which remains a feature of germinal layer-derived neurogenic sites (reviewed in Emsley et al., 2005; Bonfanti and Ponti, 2008; Kriegstein and Alvarez-Buylla, 2009). Experimental/pathological states, other than modulate constitutive neurogenesis (see for example Kempermann et al., 1997; Gould et al., 1999; van Praag et al., 2002; Kokaia and Lindvall, 2003), can induce the activation of reactive pathways in parenchymal progenitors (Buffo et al., 2008; Jiao and Chen, 2008; Ohira et al., 2009), yet not leading to substantial brain repair.

Unlike the generally accepted view, the parenchyma of the cerebellar cortex in rabbits does allow neurogenesis to occur in the adult. One possibility is that the parenchymal tissue of young/adult lagomorphs could be more permissive to structural plasticity than in rodents. The fact that rabbit cerebellar neurogenesis is high at young (peripuberal) stages,

then decreasing as the age of the animal progresses, does suggest that such processes could be linked to a longer lifespan and a slower, temporally diluted growth/maturation in lagomorphs with respect to rodents (Ponti et al., 2008).

Apart from specific issues of comparative neurobiology, the rabbit cerebellar cortex could represent a permissive environment for widespread parenchymal neurogenesis, and thus a model of spontaneous, constitutive neurogenesis, independent from the persistence of germinal layers. Local, parenchymal cell progenitors capable of generating neurons have also been described within the rabbit caudate nucleus (Luzzati et al., 2006). In that perspective, the rabbit striatum and cerebellum could be considered atypical in mammals, and the comparative approach should move to young/adult primates and humans, in order to check if a parenchymal spontaneous neurogenesis does occur in organisms that are characterized by even longer lifespan.

What it is not yet clear, at present, is whether an 'atypical' niche is required for the activation of resident (quiescent) parenchymal progenitors. Hence, in parallel with investigating stem/progenitor cells in the perspective of their transplant or induced local activation, maybe should also be important to understand more about the local tissue environment in which they live (see for example, Jiao and Chen, 2008). In this context, the rabbit cerebellum might be a natural model for studying parenchymal progenitor cells/mature tissue environment relationships in physiological, experimental, or pathological conditions.

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