eJBio

Special Issue

Electronic Journal of Biology, 2008. Vol. 4(2): 68-78

Typical and Atypical Neural Stem Cell Niches

Stefano Pluchino[1,*], Luca Bonfanti[2,**]

1 DIBIT and Institute of Experimental Neurology (InSpe)-San Raffaele Scientific Institute, via Olgettina 58, I-20132, Milan, Italy

2 Department of Veterinary Morphophysiology, University of Turin, via Leonardo da Vinci 44, I-10095, Grugliasco, and Istituto Nazionale di Neuroscienze (INN), Italy

* Corresponding authors. Tel: +39 02 26432791; Fax: +39 02 2643 4855; E-mail: pluchino.stefano@hsr.it;
** Corresponding authors. Tel: +39 011 6709115; Fax: +39 011 6709138; E-mail: luca.bonfanti@unito.it;

Abstract

The adult central nervous system (CNS) is a tissue with a low rate of renewal and can be seriously affected by injuries and diseases. The generation of new neural cells, such as neurons and glia, is prevalently restricted to specific CNS areas (*niches*) deriving from embryonic germinative layers. Continual neurogenesis is sustained by multipotent astro/radial glia-like neural stem/precursor cells (NPCs), which persist within adult CNS *niches* endowed with molecular/cellular signals capable of regulating their biological features. Multipotent NPCs have less typically been identified in the more spread mammalian CNS parenchyma. Due to their inherent plasticity, NPCs from *typical* and *non-typical CNS* germinal areas might therefore concur to nervous system repair upon injury and/or disease. In parallel, the transplantation of NPCs promotes remarkable CNS repair via both cell replacement as well as intrinsic bystander neuroprotective capacities. Strictly depending on when injected into a live host suffering from a CNS disease (e.g., inflammatory vs. degenerative), transplanted NPCs display an extraordinary capacity of finding in vivo the proper way(s) towards certain favourable perivascular sites (*atypical niches)*, where they survive and act as therapeutic weapons trough the interaction with the (micro)environment. The next challenge for the future of (endogenous vs transplanted) stem cell-based therapies will be the development of new protocols for carefully weighting and tightly regulating the different therapeutic alternative mechanisms NPCs may instruct in vivo.

Keywords: neural stem cells; neurogenesis; germinal niches; atypical perivascular niches.

1. Introduction

In agreement with the dogma by Ramon y Cajal that '*everything may die, nothing may be regenerated*', at the end of the 19th century the Italian histologist Giulio Bizzozero classified the nervous system in the group of '*perennial*' tissues, in contrast with '*labile*' and '*stable*' tissues capable of renewal and self-repair [1]. Indeed, the cells of those bodily tissues which are frequently exposed to stressful conditions, are very short living and undergo continuous replacement [2]. Also, tissues with less physiological cell turnover can activate regeneration programmes in response to different types of injury [3]. The highly complex structure of the mammalian central nervous system (CNS) – encountering a huge number of neurons, glial cells, and synapses, all linked by extremely heterogeneous anatomical/functional relationships [4] – does not follow the same rules.

The extremely composite CNS architecture is the result of a number of subsequent cell divisions and precise cell-to-cell and cell-to-substrate interactions starting from a small amount of undifferentiated cells in the developing neural tube, that assemble during the whole embryonic and a relatively short postnatal life [5].

This process, called neurogenesis, principally involves the highly heterogeneous population of neural stem and precursor cells (NPCs) located in major CNS germinal regions. Within these CNS regions, NPCs undergo self-renewal and give rise to most neuronal and glial cell precursors that populate the growing CNS by a combination of centrifugal radial and tangential cell migration [6,7]. Upon CNS assembly and generation of neuronal glial progeny, the functional specificity of the CNS hardwiring is granted by various molecular/cellular cues which are also responsible for its highly static properties that might both limit cell renewal and hamper brain repair following tissue damage (reviewed in [8]).

In addition to the widespread structural changes reshaping adult neuronal circuits through microscopic modifications of the synaptic contacts [9], neurogenesis persists in restricted domains of the adult mammalian CNS, such as the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus [1,10-12]. These

two major forebrain germinal areas harbour discrete numbers of NPCs capable of sustaining neurogenesis throughout life. In this review, we will first summarize the present knowledge and future directions concerning both the typical NPC germinal niches as well as new research trends dealing with the identification of widespread progenitor cells spontaneously cycling within the post-natal CNS. Then, we will devote additional efforts to the new concept of atypical perivascular niches as prototypical favourable (micro)environments where transplanted NPCs survive and act as therapeutic weapons in experimental models of human CNS disease.

2. Origin and fate of stem cells in the CNS

One of the major findings in neuroscience concerns the understanding that radial glia play a dual role in neurogenesis (Figure 1). Although classically considered as a transient scaffold cells for neuronal migration [13], radial glial have been recently re-considered as stem-like cells during embryogenesis (reviewed in [13,14]). As such, some radial glia are described to be endowed with self-renewal and multipotentiality [15,16]. This might occur in vivo in two different ways: i) newly born neuroblasts are generated by asymmetric division and then migrate along the radial fiber of their mother cell [17]; or ii) radial glia differentiate into migrating neurons by translocating the nucleus along the radial process, leaving a stem cell within the ventricular zone [17]. Although prevalently assessed in cerebral cortex development, similar properties of radial glia as multipotent NPCs have been demonstrated to exist within wide areas of the adult rodent brain [14,18]. Most of radial glial cells disappear at the end of neurogenesis by progressive transformation into mature parenchymal astrocytes [13]. The postnatal modifications of the embryonic radial glia involve both morphological as well as molecular changes (reviewed in [14,19]). Further, while in most of CNS parenchyma radial glial cells transform into stellate astrocytes, in restricted regions (e.g. the cerebellum and the retina) they retain some features displayed at the precursor cell state (e.g., Bergmann fibers and Muller cells, respectively). The radial glia of the SVZ change its orientation and degree of ramification thus forming the unique astrocytic meshwork of the glial tubes [20] (Figure 1). Finally, during the morphogenesis of the hippocampus, radial glial cells of the SVZ translocate to the SGZ of the DG [21], where they later persist as radial astrocytes [22]. In the adult SVZ and SGZ, glial cells have cytological and molecular features of radial glia, as they contain glycogen granules, the intermediate filaments vimentin and nestin, and glutamate transporters [14,19], thus disguising the subpopulation that retains stem-like cell properties.

Therefore, a subset of radial glial cells which transform into astrocytes within major CNS germinal areas might retain some morphological, molecular, and functional features of primary (multipotent) NPCs, which can be eventually isolated and expanded in culture [12]. Combined morphological and functional studies estimated that about 12% of the total SVZ cells express the astroglial marker glia fibrillary acidic protein (GFAP), while only 1% of SVZ cells is able to give rise to neurospheres in vitro [23], thus suggesting that only a small subset of SVZ astrocytes can be considered as *bona fide* stem cells. Although some of the modified radial glial cells retain a thin cellular process protruding in the ventricle through the ependymal monolayer [24,25], the identification of NPCs as well as the mechanisms – either cell autonomous vs cell non-autonomous – underlying the morphological, molecular and functional transition of putative neurogenic glial cells remain largely unknown.

More direct proof on the link between embryonic radial glia and adult NPCs has been obtained by employing a Cre-lox-based transgenic approach allowing the specific and permanent fating of a highly restricted population of radial glia in newborn mice [16]. Within this precise experimental context, radial glia of the lateral wall of the lateral ventricles – genetically tagged with a replication incompetent eGFP-expressing adenovirus at postnatal day 0 – gave rise to $GFAP^+$ astrocytes of the SVZ as well as to multiple classes of post-mitotic neural cells including neurons, ependymal cells, and oligodendrocytes [16]. All this represented the functional proof that radial glial cells not only serve as progenitors for neurons and glial cells soon after birth, but also behave as SVZ stem-like cells that continue to produce neurons throughout adult life. Yet, NPC features seem to be retained solely within persistent neurogenic sites, as astrocytes from other (*non-germinal and non-neurogenic*) CNS regions (e.g. cerebral cortex, cerebellum, spinal cord) can show multipotency in vitro only when isolated at early developmental stages, whereas the very same cells lose this property after the second postnatal week in mice [26]. These latter findings confirm that adult CNS regions other than prototypical germinal areas may possess certain intrinsic cellular/molecular factors capable of regulating (*cell non-autonomous regulation*) the maintenance of stem-like cells in specific (micro)environments in vivo.

A further understanding of the (single vs multiple) NPC origin, and that of mechanisms allowing the retention of stem properties across pre- and post-natal development, will be necessary for the design of new strategies for brain repair, aimed at modulating in situ the endogenous sources of neuronal and glial progenitor cells. Current research reveals that different types of neurogenic events, in addition to the one(s) occurring more canonically within prototypical germinal regions, are being characterized.

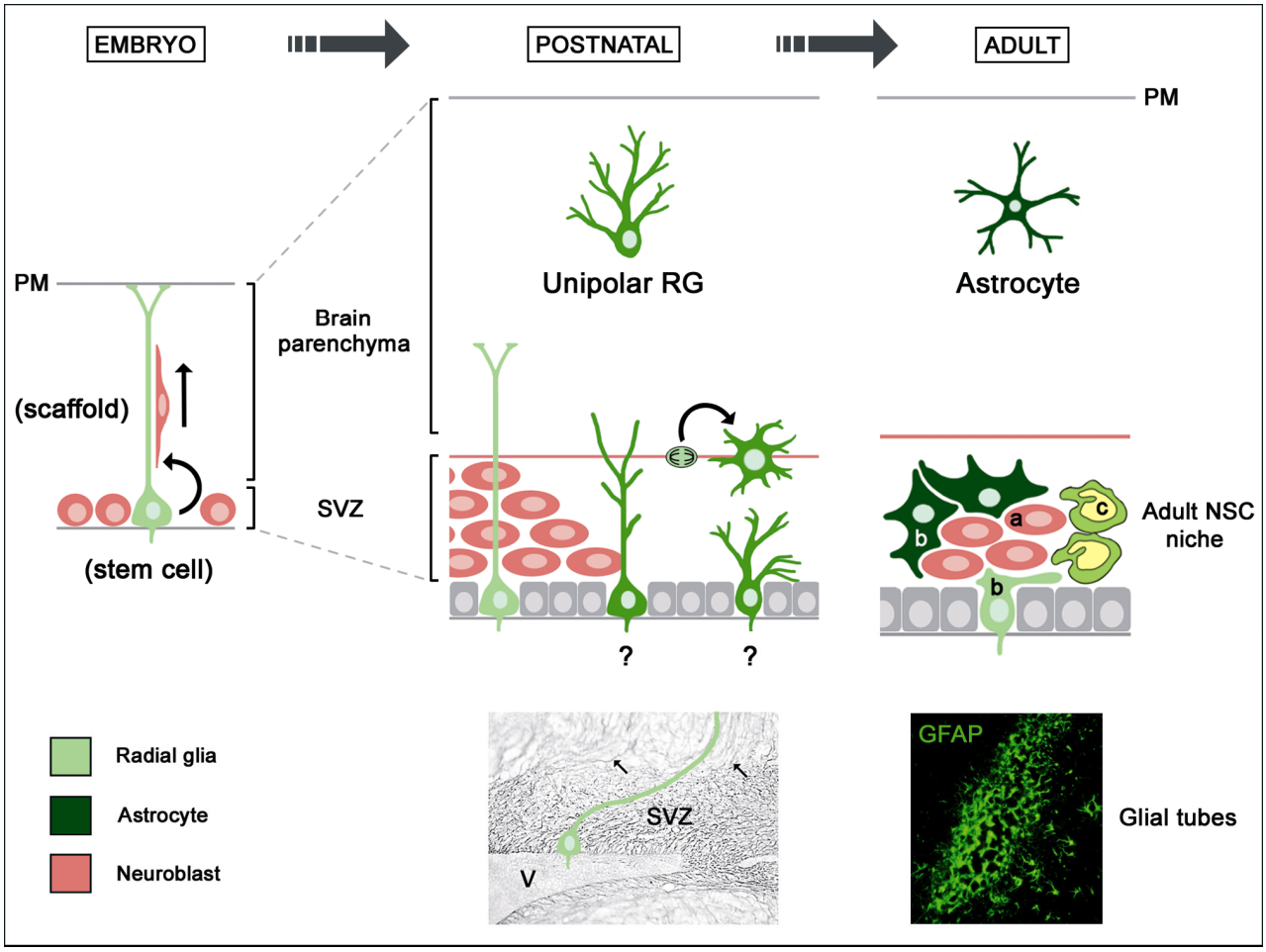

Figure 1. Developmental origin of adult NPCs and CNS typical neurogenic niches. A, Postnatal modifications in the subventricular zone (SVZ) primarily affect glial cells (green). Glial cells shift from radial glia (light green) in contact with the ventricle (V) to astrocytes (dark green) packed to form the glial tubes in the SVZ or isolated stellate cells in the brain parenchyma. Migrating neuroblasts (pink) simply change their relationships, from a homogeneous mass to tangential chains. PM, pia mater.

3. Adult neurogenesis and typical stem cell niches

The SVZ of the brain of adult rodents more evidently retains embryonic features of primitive germinal layers, as it maintains direct contact with the ventricles and its neuronal precursors undergo long-distance migration to reach their final site of destination in the olfactory bulb [27].

The SVZ of the lateral ventricles is made up of two main cell compartments: i) newly generated, migrating neuroblasts, which form tangentially oriented chains towards the olfactory bulb (OB) [28,29] and ii) protoplasmic astrocytes organized to form longitudinally-oriented channels (*glial tubes*) [28,30].

The neuroblasts, also referred to as type A cells [28], co-express β-tubulin, doublecortin, PSA-NCAM, and have an electrondense cytoplasm [31-33] (Figure 2). The astrocytes (type B cells) are $GFAP^+$, ramified cells with an electron lucent, watery cytoplasm. A subpopulation of type B cells has been indicated as the true NPCs, which would divide at a very slow rate in vivo [23]. A third element with high proliferative capacity and ultrastructural features intermediate between A and B cell types has been identified as type C cells, considered as 'transit amplifying' progenitor cells at bridge between slow proliferating stem cells and their progeny [34] (Figure 2).

The DG of the hippocampus is a three-layered cortex (*allocortex*) made up of small neurons (*granules*), which form a 4-10 cell thick layer comprised between two fiber layers (Figure 2). Hippocampal progenitor cells divide in the SGZ, and generate a progeny which gives rise to mature granule cells extending an axon within the mossy fiber pathway and reaching the Ammon's horn [35] (Figure 2). In the SGZ, both radial and horizontal astrocytes are present, the former being a special type of radial glia-like cell, similar to the type B cells of the SVZ (Figure 2). These glial cells can divide and give rise to the granule cell precursors [22]. Nevertheless, transit amplifying cells – reminiscent of SVZ type C progenitors – have not been identified

in the hippocampus, the intermediate-like cells (type D cells) being equivalent to SVZ type A cells (Figure 2). This partially addresses the apparent complexity in growing neurospheres from SGZ tissue, unless it is contaminated with portions of the dorsal-lateral wall of the lateral ventricle. Indeed, in comparison with the SVZ, the rate of adult hippocampal neurogenesis more evidently decreases with age [36], thus suggesting that the SGZ contains progenitors with limited self-renewal potential rather than true stem cells.

Other cell types of adult neurogenic areas, including the ependymal cells (Figure 2) and endothelial cells both in the ventricular SVZ [37] are considered to be part of the adult stem cell niche of the forebrain and to originate from a modification of the primitive germinative layers.

A portion of the caudal extension of the adult mouse SVZ no longer associated with an open ventricle but squeezed between the hippocampus and the corpus callosum, has been recently described as a distinct 'subcallosal' zone [38]. This region mainly produces oligodendrocyte precursors, thus confirming that adult neurogenic areas can generate different types of cells other than neurons.

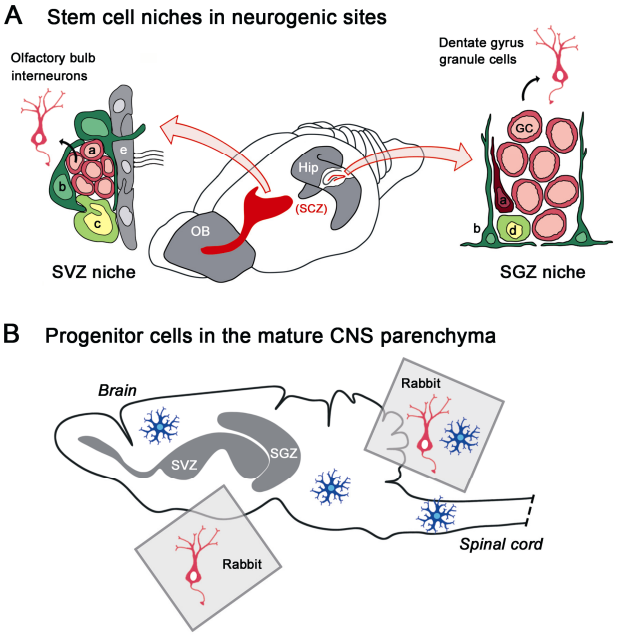

Figure 2. Typical stem cell niches and parenchymal progenitors in the CNS. A, Common features shared by the subventricular zone (SVZ) and subgranular zone (SGZ). a, neuroblasts; b, astrocytes; c and d, transit amplifying cells; e, ependyma. SCZ, subcallosal zone. B, Glia-like progenitor cells (blue) are widespread in the CNS. In adult rabbits, neurons are also produced outside the classic neurogenic sites (insets: striatum and cerebellum).

4. Cell genesis in the mature CNS parenchyma

In addition to *typical neurogenic* CNS germinal areas, some other *non-typical neurogenic* germinal regions, including the neocortex, the striatum, the substantia nigra and the amygdala, have been proposed to exist, though variability in the sensitivity of methods used to reveal the local generation of glial vs neuronal daughter cells has made it difficult to replicate most of the findings [39]. Although several in vitro data indicate that neuron-competent neural precursors naturally exist in widely divergent tissues of the adult brain [40,41], the neural precursors residing in *non-typical neurogenic* germinal regions are thought to give rise primarily to glial cells.

4.1 Adult gliogenesis

A generally accepted concept states that adult gliogenesis is more widespread than neurogenesis [42]. The presence of mitotically active glial cells has been observed since long time [43]. In recent years it was confirmed that glial elements are highly plastic cells under many profiles, both as part of homeostatic mechanisms and in response to injury and disease (reviewed in [42]). A continuous production of glial progenitors proliferating in situ and differentiating into astrocytes and oligodendrocytes has been shown to occur in the white matter of the corpus callosum [44], spinal cord [45] and substantia nigra [46] of adult rodents.

In the adult rat spinal cord, which is not endowed with spontaneous neurogenic capacities, a process of slow but widespread and persistent gliogenesis has been described to occur [47]. Newly generated cells of the spinal cord were found to express the nerve/glial (NG)2 proteoglycan, a marker of glial progenitors [48,49]. Even in other CNS areas, NG2-expressing cells are a widespread yet heterogeneous population of glial progenitor cells most of which retain the capability to divide during adulthood [48,49]. They are also called synantocytes [50,51] or polydendrocytes [52], and are morphologically, antigenically, and functionally distinct from mature astrocytes, oligodendrocytes, and microglia, being at present the best example of parenchymal progenitors [47,52]. The *cell autonomous vs cell non-autonomous* factors that create a gliogenic environment in the adult CNS are not well understood. In addition, the $NG2^+$ cells resident in both the SVZ and the SGZ are intrinsically multipotent in vitro and can generate neurons in vivo [49,53], whereas the multipotency of parenchymal NG2-expressing cells remains to be evaluated [52]. An appealing hypothesis about the origin and dispersion of multipotent progenitors in the mature brain parenchyma has been raised by following the fate of a traceable clone of human NPCs grafted into the developing monkey brain [54]. These authors observe that dispersed undifferentiated cells provide a local resident pool of dividing, multipotent progenitors, thus representing the stem-like cells extracted by several investigators.

Yet, it has not been resolved if the stem-like pools of progenitors isolated from various parenchymal regions at post-developmental periods are of physiologically relevance or artefacts of experimental manipulation [54].

4.2 Spontaneous parenchymal neurogenesis in non-rodent mammals

Spontaneous in vivo parenchymal-derived neurogenesis has been described to occur in some mammals [8,10,55-57]. A small rate of local neurogenesis has been shown to occur in the rat neocortex [56,58], whereas the migration of new neuroblasts from the SVZ to the brain parenchyma remains quite controversial [56,59-62].

Neurogenesis has also been described in the rabbit caudate nucleus, though this region of the corpus striatum is generally considered as non-neurogenic under physiological condition [55]. Phenotypic analyses of these striatal cell precursors indicate that most of them express the astroglial marker brain lipid binding protein (BLBP), a protein abundant in radial glia. Interestingly enough, some of the newly generated cells differentiate into Calretinin$^+$ striatal interneurons, reaching non-negligible values with respect to the whole population [55,63]. Thus, the parenchyma of the rabbit striatum is capable of maintaining adult neurogenesis under physiological conditions.

The hypothesis that the adult rabbit CNS parenchyma could be particularly supportive for neurogenesis is further strengthened by the recent demonstration that prolonged neurogenesis persists in the cerebellar cortex at peripuberal and adult ages [64]. Neuroblasts generated within a proliferative subpial layer replacing the external granule layer (EGL), are arranged to form thousands of tangential chains reminiscent of those responsible for cell migration in the forebrain subventricular zone [65]. Although subpial chains disappear from the cerebellar surface after the 5th month of life, the newly-generated cells continue to be detectable within the rabbit cerebellar cortex, giving rise to GABA-ergic, Pax2$^+$ neurons as well as Sox2$^+$/Olig2$^+$ cell precursors reminiscent of synantocytes or polydendrocytes [64].

Thus, even in the (*expected*) highly static mammalian cerebellum new neuronal and glial progenitors are generated within the mature parenchyma, in the absence of a clearly identifiable germinal layer.

In conclusion, a number of recent studies focusing on different CNS regions and/or in different mammalian species have challenged the concept of 'non-neurogenic' parenchyma. Fifteen years after the demonstration of adult neurogenesis in mammals [66,67], research is now shifting from the study of spatially restricted *typical neurogenic* CNS regions to the potentialities hidden within the spread brain parenchyma. Among the issues remaining to be tackled is the heterogeneity of parenchymal cell progenitors and to what extent they can be activated in order to contribute to brain repair upon different types of injury (e.g., focal vs multifocal, acute vs chronic).

5. Endogenous neural stem cells and CNS diseases

Stem and progenitor cells residing within CNS germinal areas might concur to nervous system repair due to their capacity to drive neurogenesis and gliogenesis during post-natal life [68]. Nonetheless, basic biological features of NPCs, such as self-renewal, proliferation, migration and differentiation may significantly vary following CNS injuries [69-71].

Increased numbers of proliferating NPCs expressing nestin as well as of doublecortin-reactive type C-like transit amplifying progenitors are detected at the boundaries of the injury site as early as one week after experimental acute focal inflammatory CNS disorders, such as spinal cord injury (SCI) and stroke [71-74]. Experimental acute stroke in rodents triggers neurogenesis and migration of newborn neurons from their sites of origin into ischemic brain regions [75].

The transient occlusion of the middle cerebral artery in the rat increases the incorporation of BrdU into neural cells in the SGZ of the DG, the effect correlating with activation of the cAMP-response-element-binding protein (CREB) [76]. Neural cells labelled with BrdU co-express the immature neuronal markers doublecortin and proliferating cell nuclear antigen (PCNA), while do not express the more mature cell markers neuronal nuclear (NeuN) and Hu, thus suggesting that they are nascent neurons [77]. The acute phase of experimental stroke is associated to a significant shortening of the cell cycle and a decreased G1 phase of SVZ-resident neural progenitors [78] regulating a transient increase in both (terminal) symmetric cell division as well as generation of neuronal progenitors migrating through the ischemic striatum towards the damage, closely associated with blood vessels [73,79]. Also in patients with stroke, neural cells that express markers associated with newborn neurons are present in the ischemic penumbra surrounding cerebral cortical infarcts and preferentially localize in the vicinity of blood vessels [80].

By the other hand, following acute SCI in the mouse, neural progenitors in the ependymal zone (EZ) of the central canal mobilize and migrate vigorously toward the direction where the contusion injury is generated – the most favourable migration occurring in the adjacent region close to the epicentre of the lesion – and differentiate optimally into NeuN-immunoreactive neurons, but not into astrocytes or oligodendrocytes [81]. After SCI in the adult rhesus monkey, BrdU-based analysis of cell proliferation in vivo reveals an increase of ≥ 80-fold

in the number of newly divided cells in the spinal cord. By 7 months after injury, 15% of these newly generated neural cells express markers of mature oligodendrocytes while 12% express astrocytic markers. These newly born oligodendrocytes are present in zones of injury-induced demyelination and appear to ensheath or fully remyelinate host axons [82].

In experimental autoimmune encephalomyelitis (EAE), the animal model of MS, mitotically active neural progenitor cells, which reside either in the SVZ of the brain or in the EZ of central canal of the spinal cord, subvert their physiological rostral migration to the OB (or the radial migration to the lateral columns of the spinal cord) and migrate into areas of demyelination where they differentiate into glial cells [69,70].

Though accumulating evidence suggests that endogenous neurogenesis and gliogenesis might occur as part of an 'intrinsic' self-repair process during inflammatory CNS disorders, there are no convincing explanations about the overall incapacity of the endogenous CNS stem cell compartment to promote full and long-lasting CNS repair. Recent data suggest that chronic brain inflammation, induced by myelin-specific immune cells, irreversibly alters the proliferative and migratory properties of SVZ-resident endogenous NPCs in vivo. This effect is generally sustained by a pro-inflammatory cytokine-dependent inhibition of cell cycle progression leading to significant accumulation of non-migratory neuroblasts within the SVZ germinal niche. In parallel, quantitative reduction of the putative brain stem cells is also observed. Extensive in vitro culturing of neurospheres from mice with chronic brain inflammation completely reverses the impairment, thus suggesting that the hostile chronically inflamed brain microenvironment may sustain a non cell-autonomous dysfunction of the endogenous NPCs. Furthermore, during sub-acute lipopolysaccharide (LPS)-induced brain inflammation, interleukin (IL)-6 released by microglia significantly impairs neurogenesis in the hippocampus in vivo, the impairment being fully restored when non-steroidal anti-inflammatory drugs (such as indomethacin) are used [83]. In vitro generation of new neurons and oligodendrocyte from NPCs is induced and supported by mouse microglia that have encountered T cell-associated cytokines (such as interferon-γ and IL-4), but blocked by those that have encountered endotoxins (such as LPS) [84]. More recently, hippocampal neurogenesis induced by an enriched environment has been associated with the recruitment of brain-derived neurotrophic factor (BDNF)-releasing T cells and the activation of microglia in the DG. When studied in immune-deficient mice, hippocampal neurogenesis has been found markedly impaired and not enhanced by environmental enrichment, while restored and boosted by T cells recognizing a specific CNS antigen, such as myelin basic protein (MBP) [85].

Therefore, certain common immune-associated mechanism(s) are likely to underlie different aspects of structural plasticity and cell renewal in the adult CNS [86,87]. Further, we cannot exclude that (at least) in certain chronic CNS inflammatory disorders (such as MS), some regional tropism of blood-borne inflammatory cells for major germinal niches might occur as a consequence of the capacity of the different cell components of the niches to secrete molecules preferentially attracting inflammatory cells. Hence, the idea that some CNS diseases might occur as the consequence of a dysfunction of stem cells rather than the upshot of an uncontrolled, and still undiscovered, pathogenic alien(s), may not sound that provocative.

6. Neural stem cell transplantation and CNS diseases

Soon after the in vivo identification of stem cells from the CNS, different procedures have been developed in order to safely expand and maintain these cells in chemically defined media for years [88]. Protocols to obtain in vitro a large number of NPCs have then been established, thus supporting the proof that these cells might represent a renewable source of uncommitted ready-to-use cells for transplants [89]. Successful NPC-based therapies for nervous system disorders — for example, stroke, Parkinson's disease (PD), Huntington's disease (HD), MS, acute SCI — have been developed. However, there are still important issues that need to be solved before envisaging any potential human applications of such promising therapies. Not only the ideal cell source for transplantation and the best route of cell administration have to be determined. Also, it is unclear the putative mechanism(s) sustaining both repair capabilities as well as long-term functional integration of NPCs upon transplantation. Although indications that transplanted cells can reach the target organ and differentiate into the appropriate lineage exist, there is still scarce evidence that transplanted NPCs can reconstruct a replica of the former brain architecture and give rise to large numbers of properly functioning cells integrating into the new brain circuitries.

The route of cell administration represents a major issue for NPC transplantation and appears to be very much depending on the CNS lesion site(s) (focal vs. multifocal). The anatomo-pathological features of focal CNS disorders, might suggest that direct local (intralesional) cell transplantation would facilitate tissue regeneration, while the multifocality of certain others CNS disorders - such as MS and epilepsy - would represent a major limitation for intralesional cell-transplantation approaches. Following the first observation in experimental brain tumours, in multifocal CNS disorders, systemic (e.g.

intravenous, intrathecal) transplantation of NPCs can be therapeutically efficacious owing to the ability of transplanted cells to follow, once travelling into either the blood stream or the cerebrospinal fluid, a gradient of chemoattractants (e.g. pro-inflammatory cytokines and chemokines) occurring at the site of inflammatory lesions [86,90]. Specific homing of transplanted neural stem cells has been shown, so far, in SCI, epilepsy, and stroke. However, the exact molecular mechanism sustaining this phenomenon has been detailed, so far, only in EAE. Tethering, rolling, and firm adhesion to inflamed endothelial cells and transendothelial migration into inflamed CNS areas are sequentially mediated by the constitutive expression of functional cell adhesion molecules (CAM) (e.g. CD44), integrins (e.g. α4, β1), and chemokine receptors (e.g. CCR1, CCR2, CCR5, CXCR3, CXCR4) on neural stem cell surface [86,90]. Irrespective from the characteristics of the experimental disease (e.g. disease course [acute vs. chronic], neuropathological features [focal vs. multifocal] and type of inflammation [primary vs. reactive]), functional recovery obtained by neural stem cell transplantation scarcely correlates with absolute numbers of transplant-derived newly generated terminally differentiated neuronal cells. Transplantation of NPCs into rodents with experimental PD or HD, very scarcely differentiate into tyrosine hydroxylase (TH)-immunoreactive neurons despite significant behavioural improvement. Similarly, mice with SCI, acute stroke and intracerebral hemorrage recover despite pathological evidence of preferential astroglial fate of transplanted NPCs. The large majority of NPCs injected into mice with experimental cerebral hemorrage or with acute ischemic stroke, express markers of undifferentiation, such as nestin, when surrounding damaged CNS areas. In EAE, very low differentiation of transplanted NPCs into myelin forming oligodendrocytes is accompanied by neurophysiological evidence of axonal protection and remyelination. In the very same context, more than 20% of transplanted NPCs accumulate within inflammatory demyelinated CNS areas while not expressing differentiation markers. This scarce and inappropriate terminal differentiation and the propensity of maintaining an undifferentiated phenotype within the host tissue, might suggest that transplanted NPCs are therapeutic efficacious via a number of bystander mechanism(s) alternative to the expected cell replacement. Transplanted NPCs reduce the scar formation and/or increase survival and function(s) of endogenous glial and neuronal progenitors surviving to the pathological insult. This neuroprotective effect is accompanied by increased in vivo bioavailability of major neurotrophins. Moreover, transplanted NPCs promote bystander immunomodulation as they release soluble molecules (e.g. cytokines and chemokines), express immune-relevant receptors (e.g. chemokine receptors, CAMs), capable of profoundly altering the inflammatory environment [90]. More recent evidence suggest that transplanted NPCs contribute to down-regulate the effector functions of inflammatory T cells and macrophages also into bodily sites alternative to the CNS (e.g., within draining lymph nodes) [91].

All together these results challenge the concept that (somatic) stem cell transplantation has to therapeutically work throughout cell replacement. As a matter of fact, stem cell transplantation may also promote CNS repair via intrinsic neuroprotective bystander capacities, mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of neuroprotective molecules once temporally and spatially orchestrated by environmental needs. The intrinsic nature of most of these molecules as well as their 'constitutive' characteristics, represent a stem cell signature that also reconcile data showing that other sources of somatic stem cells (e.g. bone marrow-derived stem cells [BMSC] and mesenchymal stem cells [MSC]), with very low capabilities of neural differentiation, may efficiently promote CNS repair (therapeutic plasticity) [92].

The exact knowledge and the potential impact of non-conventional stem cell-mediated therapeutic mechanisms might result, in certain circumstances, in more efficacious curative alternatives.

7. NPC transplantation in inflammatory CNS diseases: the atypical ectopic niche

Some recent experiments have shown that in multifocal inflammatory CNS disorders, therapeutic somatic stem cells [e.g. BMSCs, umbilical cord blood stem cells (UCBSC), MSCs, NPCs] - injected trough the blood stream or into the cerebrospinal fluid circulation - can specifically reach inflamed CNS areas where they persist for months and promote recovery [93-97].

Once within inflamed CNS areas, systemically-injected NPCs accumulate (and persist) around the perivascular space where reactive astrocytes, inflamed endothelial cells and blood-borne infiltrating T cells co-reside. In these areas, named '*CNS atypical ectopic niches*' (Figure 3), a molecular cross talk takes place between the different cells of the atypical niche. This can bona fide be postulated as the functional requisite for the therapeutic activity of transplanted NPCs.

On one hand, the great majority of transplanted NPCs survive long term, while displaying undifferentiated features (e.g., round-shaped morphology and lack of major antigens of differentiation), owing to the focal release of stem cell regulators by immune cells and reactive astrocytes. On the other hand, NPCs promote neuroprotection by in situ releasing immunomodulatory molecules (e.g. anti-inflammamtory cytokines) and neurotrophic factors

[e.g., nerve growth factor (NGF), fibroblast growth factor (FGF)-II, ciliary neurotriphic factor (CNTF) and brain-derived neurotrophic factor (BDNF)] [86,90]. Via the release/expression of immunomodulatory molecule (e.g. FasL, Apo3L, TRAIL) NPCs promote apoptosis of effector cells expressing death receptors (e.g. encephalitogenic Th1 cells). Via the release of neurotrophic growth factor (e.g., TGF β, FGF-II), NPCs also contribute to significant reduction of glial scarring [86,90,98]. Last but not least, transplanted NPCs may also differentiate into myelin forming cells [98]. As a net effect of these different mechanisms of neuroprotection, five-fold increase in demyelinating areas of the number of 'remyelinating' endogenous OPCs is obtained [98].

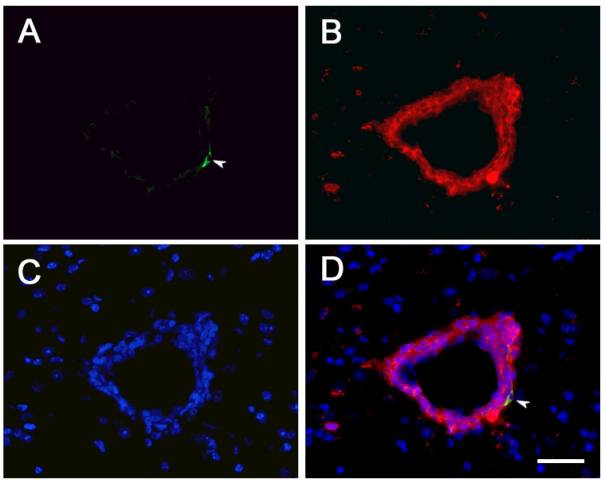

Figure 3. A single GFP-labelled (A, green) NPC is found in close contact to numerous blood-derived $CD45^+$ leukocytes (B, red) infiltrating a CNS perivascular areas of a representative mouse affected by proteolipid protein (PLP)-induced EAE as early as 100 days after intravenous NPC injection. Nuclei have been counterstained with Dapi (C, blue). The picture in D is a merged image of A-C. Scale bar: 75 μm.

8. Conclusions

Research carried out during the last decades revealed that NPCs persist within the adult brain, providing a source of neuronal and glial cell precursors throughout life. Besides restricted neurogenic sites harbouring bona fide stem cells which undergo regulation within their typical niches, multipotent progenitor cells capable of proliferation and self renewal are likely to be present also in widespread areas of the mature CNS parenchyma. Emerging evidence indicates that these progenitor cells form a rather heterogeneous population with multifaceted properties that can be re-expressed under experimental conditions in vitro although remaining mostly unknown in vivo.

Further, while the replacement of lost/damaged cells was until few years ago assumed as the prime therapeutic mechanism of stem cells, it is now clear that transplanted somatic stem cells may simultaneously instruct several therapeutic mechanisms among which the solely cell replacement does not prevail.

The 'therapeutic plasticity' can therefore be viewed as a true functional signature of somatic stem cells. Strictly depending on when injected into a live host suffering from a tissue specific disease (inflammatory vs. degenerative), transplanted stem cells are likely to display some unique therapeutic adaptive functions. This is due to the fact that stem cells display an extraordinarily capacity of finding in vivo the proper way(s) towards certain favourable atypical niches, where they survive and act as therapeutic weapons trough the interaction with the different cell types of the (micro)environment [80,90,91,99-101]. Nonetheless, the recent demonstration that also other sources of somatic stem cells (e.g. mesenchymal, haematopoietic) may display equally significant bystander capacities and promote CNS repair [102,103], further proves the relevance of stem cell-dependent therapeutic mechanisms alternative to cell replacement. The next challenge for the future of stem cell-based therapies would be that of finding the way to carefully weight and tightly regulate the different therapeutic alternative mechanisms such cells may instruct in vivo.

Acknowledgements

This work was supported by the Italian Multiple Sclerosis Foundation (FISM, grants 2004/R/15 to S.P.) the National Multiple Sclerosis Society (NMSS, grants RG-4001-A1 to S.P.), Banca Agricola Popolare di Ragusa (BAPR, unrestricted grant to S.P.), Compagnia di San Paolo (Progetto NEUROTRANSPLANT 2004.2019 to L.B.), Regione Piemonte (CIPE 2004 - A14 to L.B.), M.U.R.S.T. (PRIN), University of Turin (to LB).

References

[1] Bonfanti L. (2008) Neurogenesis during mammalian postnatal brain development: anatomical and functional considerations. In: *Postnatal and Adult Neurogenesis* (Bonfanti L, ed.), Research Signpost, Trivandrum, India (in press).

[2] Potten C.S., Loeffler M. (1990) Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development*, **110**(4): 1001-1020.

[3] Laird, D.J., von Andrian U.H., A.J. Wagers A.J. (2008) Stem cell trafficking in tissue development, growth, and disease. *Cell*, **132**(4): 612-630.

[4] Chklovskii D.B., Mel B.W., Svoboda K. (2004) Cortical rewiring and information storage. *Nature*, **431**(7010): 782-788.

[5] Bayer S.A., Altman J. (2004) *Development of the telencephalon: neural stem cells, neurogenesis, and neuronal migration*. G. Paxinos (Ed.), Academic Press, San Diego, USA, 27.

[6] Marin O., Rubenstein J.L. (2003) Cell migration in the forebrain. *Annu Rev Neurosci*, **26**: 441-483.

[7] Rakic P. (1990) Principles of neural cell migration. *Experientia*, **46**(9): 882-891.

[8] Bonfanti L. (2006) PSA-NCAM in mammalian structural plasticity and neurogenesis. *Prog Neurobiol*, **80**(3): 129-164.

[9] Ito M. (2006) Cerebellar circuitry as a neuronal machine. *Prog Neurobiol*, **78**(3-5): 272-303.

[10] Bonfanti L., Ponti G. (2008) Adult mammalian neurogenesis and the New Zealand white rabbit. *Vet J*, **175**(3): 310-331.

[11] Alvarez-Buylla A., Garcia-Verdugo J.M. (2002) Neurogenesis in adult subventricular zone. *J Neurosci*, **22**(3): 629-634.

[12] Gage F.H. (2000) Mammalian neural stem cells. *Science*, **287**(5457): 1433-1438.

[13] Rakic P. (2003) Elusive radial glial cells: historical and evolutionary perspective. *Glia*, **43**(1): 19-32.

[14] Pinto L., Gotz M. (2007) Radial glial cell heterogeneity--the source of diverse progeny in the CNS. *Prog Neurobiol*, **83**(1): 2-23.

[15] Noctor S.C., et al. (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature*, **409**(6821): 714-720.

[16] Merkle F.T., et al. (2004) Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci USA*, **101**(50): 17528-17532.

[17] Miyata T., et al. (2001) Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron*, **31**(5): 727-741.

[18] Merkle F.T., Mirzadeh Z., Alvarez-Buylla A. (2007) Mosaic organization of neural stem cells in the adult brain. *Science*, **317**(5836): 381-384.

[19] Bonfanti L., Peretto P. (2007) Radial glial origin of the adult neural stem cells in the subventricular zone. *Prog Neurobiol*, **83**(1): 24-36.

[20] Peretto, P., et al. (2005) Chain formation and glial tube assembly in the shift from neonatal to adult subventricular zone of the rodent forebrain. *J Comp Neurol*, **487**(4): 407-427.

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

[22] Seri B., et al. (2004) Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol*, **478**(4): 359-378.

[23] Doetsch F., et al., (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*, **97**(6): 703-716.

[24] Han Y.G., et al. (2008) Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat Neurosci*, **11**(3): 277-284.

[25] Doetsch F., et al. (2002) EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron*, **36**(6): 1021-1034.

[26] Laywell E.D., et al. (2000) Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA*, **97**(25): 13883-13888.

[27] Lois C., Alvarez-Buylla A. (1994) Long-distance neuronal migration in the adult mammalian brain. *Science*, **264**(5162): 1145-1148.

[28] Lois C., Garcia-Verdugo J.M., Alvarez-Buylla A. (1996) Chain migration of neuronal precursors. *Science*, **271**(5251): 978-981.

[29] Doetsch F., Alvarez-Buylla A. (1996) Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc Natl Acad Sci USA*, **93**(25): 14895-14900.

[30] Peretto P., et al. (1997) Glial tubes in the rostral migratory stream of the adult rat. *Brain Res Bull*, **42**(1): 9-21.

[31] Bonfanti L., Theodosis D.T. (1994) Expression of polysialylated neural cell adhesion molecule by proliferating cells in the subependymal layer of the adult rat, in its rostral extension and in the olfactory bulb. *Neuroscience*, **62**(1): 291-305.

[32] Nacher J., Crespo C., McEwen B.S. (2001) Doublecortin expression in the adult rat telencephalon. *Eur J Neurosci*, **14**(4): 629-644.

[33] Menezes J.R., Luskin M.B. (1994) Expression of neuron-specific tubulin defines a novel population in the proliferative layers of the developing telencephalon. *J Neurosci*, **14**(9): 5399-5416.

[34] Doetsch F., Garcia-Verdugo J.M., Alvarez-Buylla A. (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci*, **17**(13): 5046-5061.

[35] Zhao C., et al. (2006) Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci*, **26**(1): 3-11.

[36] Kuhn H.G., Dickinson-Anson H., Gage F.H. (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci*, **16**(6): 2027-2033.

[37] Shen Q., et al. (2004) Endothelial Cells Stimulate Self-Renewal and Expand Neurogenesis of Neural Stem Cells. *Science*, **304**: 1338-1340.

[38] Seri B., et al. (2006) Composition and organization of the SCZ: a large germinal layer containing neural stem cells in the adult mammalian brain. *Cereb Cortex*, **16** Suppl 1: i103-11.

[39] Gould, E. (2007) How widespread is adult neurogenesis in mammals? *Nat Rev Neurosci*, **8**(6): 481-488.

[40] Kondo T., Raff M. (2000) Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science*, **289**(5485): 1754-1757.

[41] Palmer T.D., et al. (1999) Fibroblast growth factor-2 activates a latent neurogenic program in neural stem

cells from diverse regions of the adult CNS. *J Neurosci*, **19**(19): 8487-8497.

[42] Horner P.J., Jacobson B.E. (2008) Gliogenesis in the adult spinal cord. In: *Postnatal and Adult Neurogenesis* (Bonfanti L, ed.), Research Signpost, Trivandrum, India (in press).

[43] Walker B.E., Leblond C.P. (1958) Sites of nucleic acid synthesis in the mouse visualized by radioautography after administration of C14-labelled adenine and thymidine. *Exp Cell Res*, **14**(3): 510-531.

[44] Gensert J.M., Goldman J.E. (2001) Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. *J Neurobiol*, **48**(2): 75-86.

[45] Horner P.J., et al. (2000) Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J Neurosci*, **20**(6): 2218-2228.

[46] Lie D.C., et al. (2002) The adult substantia nigra contains progenitor cells with neurogenic potential. *J Neurosci*, **22**(15): 6639-6649.

[47] Horner P.J., Thallmair M., Gage F.H. (2002) Defining the NG2-expressing cell of the adult CNS. *J Neurocytol*, **31**(6-7): 469-480.

[48] Dawson M.R., et al. (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci*, **24**(2): 476-488.

[49] Belachew S., et al. (2003) Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol*, **161**(1): 169-186.

[50] Butt A.M., et al. (2002) Synantocytes: new functions for novel NG2 expressing glia. *J Neurocytol*, **31**(6-7): 551-565.

[51] Berry M., Hubbard P., Butt A.M. (2002) Cytology and lineage of NG2-positive glia. *J Neurocytol*, **31**(6-7): 457-467.

[52] Nishiyama, A. (2007) Polydendrocytes: NG2 cells with many roles in development and repair of the CNS. *Neuroscientist*, **13**(1): 62-76.

[53] Aguirre A., Gallo V. (2004) Postnatal neurogenesis and gliogenesis in the olfactory bulb from NG2-expressing progenitors of the subventricular zone. *J Neurosci*, **24**(46): 10530-10541.

[54] Ourednik V., et al. (2001) Segregation of human neural stem cells in the developing primate forebrain. *Science*, **293**(5536): 1820-1824.

[55] Luzzati F., et al. (2006) Neurogenesis in the caudate nucleus of the adult rabbit. *J Neurosci*, **26**(2): 609-621.

[56] Dayer A.G., et al. (2005) New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. *J Cell Biol*, **168**(3): 415-427.

[57] Bedard A., et al. (2002) Proliferating cells can differentiate into neurons in the striatum of normal adult monkey. *Neurosci Lett*, **328**(3): 213-216.

[58] Tamura Y., et al. (2007) Multi-directional differentiation of doublecortin- and NG2-immunopositive progenitor cells in the adult rat neocortex in vivo. *Eur J Neurosci*, **25**(12): 3489-3498.

[59] Koketsu D., et al. (2003) Nonrenewal of neurons in the cerebral neocortex of adult macaque monkeys. *J Neurosci*, **23**(3): 937-942.

[60] Gould, E., et al. (2001) Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. *Proc Natl Acad Sci USA*, **98**(19): 10910-10917.

[61] Kornack D.R., Rakic P. (2001) Cell proliferation without neurogenesis in adult primate neocortex. *Science*, **294**(5549): 2127-2130.

[62] Gould E., et al. (1999) Neurogenesis in the neocortex of adult primates. *Science*, **286**(5439): 548-552.

[63] Luzzati F., et al. (2007) Adult neurogenesis and local neuronal progenitors in the striatum. *Neurodegener Dis*, **4**(4): 322-327.

[64] Ponti G., Peretto P., Bonfanti L. (2008) Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. *PLoS ONE,* in press.

[65] Ponti G., Peretto P., Bonfanti L. (2006) A subpial, transitory germinal zone forms chains of neuronal precursors in the rabbit cerebellum. *Dev Biol*, **294**(1): 168-180.

[66] Lois C., Alvarez-Buylla A. (1994) long-distance neuronal migration in the adult mammalian brain. *Science* **264**: 1145-1148.

[67] Reynolds B.A., Weiss S. (1992) generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science **255**: 1707-1710.

[68] Ming G.L., Song H. (2005) Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci*, **28**: 223-250.

[69] Brundin L., et al. (2003) Neural stem cells: a potential source for remyelination in neuroinflammatory disease. *Brain Pathol*, **13**(3): 322-328.

[70] Picard-Riera N., et al. (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci USA*, **99**(20): 13211-13216.

[71] Yagita Y., et al. (2001) Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. *Stroke*, **32**(8): 1890-1896.

[72] Tureyen K., et al. (2004) Transient focal cerebral ischemia-induced neurogenesis in the dentate gyrus of the adult mouse. *J Neurosurg*, **101**(5): 799-805.

[73] Zhang R., et al. (2004) Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. *J Neurosci*, **24**(25): 5810-5815.

[74] Carmichael S.T. (2003) Gene expression changes after focal stroke, traumatic brain and spinal cord injuries. *Curr Opin Neurol*, **16**(6): 699-704.

[75] Zhang R., et al. (2004) Activated neural stem cells contribute to stroke-induced neurogenesis and

neuroblast migration toward the infarct boundary in adult rats. *J Cereb Blood Flow Metab*, **24**(4): 441-448.

[76] Zhu D.Y., et al. (2004) Activation of cAMP-response-element-binding protein (CREB) after focal cerebral ischemia stimulates neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA*, **101**(25): 9453-9457.

[77] Jin K., et al. (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proc Natl Acad Sci USA*, **98**(8): 4710-4715.

[78] Zhang R.L., et al. (2006) Reduction of the cell cycle length by decreasing G1 phase and cell cycle reentry expand neuronal progenitor cells in the subventricular zone of adult rat after stroke. *J Cereb Blood Flow Metab*, **26**(6): 857-863.

[79] Thored P., et al. (2007) Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. *Stroke*, **38**(11): 3032-3039.

[80] Jin K., et al. (2006) Evidence for stroke-induced neurogenesis in the human brain. *Proc Natl Acad Sci USA*, **103**(35): 13198-13202.

[81] Ke Y., et al. (2006) Early response of endogenous adult neural progenitor cells to acute spinal cord injury in mice. *Stem Cells*, **24**(4): 1011-1019.

[82] Yang H., et al. (2006) Endogenous neurogenesis replaces oligodendrocytes and astrocytes after primate spinal cord injury. *J Neurosci*, **26**(8): 2157-2166.

[83] Monje M.L., Toda H., Palmer T.D. (2003) Inflammatory blockade restores adult hippocampal neurogenesis. *Science*, **302**(5651): 1760-1765.

[84] Butovsky O., et al. (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol Cell Neurosci*, **31**(1): 149-160.

[85] Ziv Y., et al. (2006) Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci*, **9**(2): 268-275.

[86] Martino G., Pluchino S. (2006) The therapeutic potential of neural stem cells. *Nat Rev Neurosci*, **7**(5): 395-406.

[87] Martino G., Pluchino S. (2007) Neural stem cells: guardians of the brain. *Nat Cell Biol*, **9**(9): 1031-1034.

[88] Pluchino S., et al. (2005) Neural stem cells and their use as therapeutic tool in neurological disorders. *Brain Res Brain Res Rev*, **48**(2): 211-219.

[89] Vescovi A.L., Snyder E.Y. (1999) Establishment and properties of neural stem cell clones: plasticity in vitro and in vivo. *Brain Pathol*, **9**(3): 569-598.

[90] Pluchino S., et al. (2005) Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature*, **436**(7048): 266-271.

[91] Einstein O., et al. (2007) Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. *Ann Neurol*, **61**(3): 209-218.

[92] Pluchino S., Martino G. (2008) Neural stem cell-mediated immunomodulation: repairing the haemorrhagic brain. *Brain*, **131**(Pt 3): 604-605.

[93] Garbuzova-Davis S., et al. (2003) Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J Hematother Stem Cell Res*, **12**(3): 255-270.

[94] Chu K., et al. (2004) Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. *Brain Res*, **1016**(2): 145-153.

[95] Xiao J., et al. (2005) Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury. *Stem Cells Dev*, **14**(6): 722-733.

[96] Liu H., et al. (2006) Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. *Brain*, **129**(Pt 10): 2734-2745.

[97] Ziv Y., et al. (2006) Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. *Proc Natl Acad Sci USA*, **103**(35): 13174-13179.

[98] Pluchino S., et al. (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*, **422**(6933): 688-694.

[99] Calabrese C., et al. (2007) A perivascular niche for brain tumor stem cells. *Cancer Cell*, **11**(1): 69-82.

[100] Thored P., et al. (2007) Long-Term Neuroblast Migration Along Blood Vessels in an Area With Transient Angiogenesis and Increased Vascularization After Stroke. *Stroke*, **38**(11): 3032-3039.

[101] Lee S.T., et al. (2008) Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. *Brain*, **131**(pt3): 616-629.

[102] Zappia E., et al. (2005) Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T cell anergy. *Blood*, **106**(5): 1755-1761.

[103] Escolar M.L., et al. (2005) Transplantation of Umbilical-Cord Blood in Babies with Infantile Krabbe's Disease. *N Engl J Med*, **352**(20): 2069-2081.