

Editorial

Adult neural stem cells: fate and function

Cellules souches neurales de l'adulte : destin et fonction

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1. Discovering neuronal adult stem cells

It has long been thought that neurogenesis in the vertebrate brain is a phenomenon exclusive to embryonic and early postnatal development. Today, however, postnatal neurogenesis has been reported in fish, amphibians, reptiles, birds, rodents and primates, including humans [1]. This finding represents one of the major recent advances in neuroscience. Elucidating the chain of events involved in cell production, in the migration of neuroblasts and their maturation in the targeted tissue will provide new fuel for understanding the fundamental processes employed by both healthy and diseased adult brains. In addition, discovery of determinants regulating neuronal fate and endogenous factors controlling the mechanisms of migration and maturation may highlight alternative possibilities for cell replacement therapies, namely the use of endogenous stem cell capabilities following introduction of fate determinants into neuroblasts and changing their molecular microenvironment to re-direct them into damaged tissues.

2. Distinct neurogenic sites in the adult brain

Although some debates still persist [2,3], it is now definitively established that there are at least three sites of neuronal production in the adult mammalian brain: the subgranular zone of the dentate gyrus of the hippocampus [4], the subventricular zone (SVZ) which lines the lateral walls of the ventricles [5] and the subependymal zone of the spinal cord [6]. These neurogenic niches are considered as vestiges of the developmental program of the central nervous system. They appear to be more active than others (e.g., the overall daily production of neurons is 10 times lower in the hippocampus than in the SVZ). Recent findings indicate that the SVZ may also contribute to the genesis of either astrocytes in response to trauma, neurons in re-

sponse to cortico-thalamic denervation, or even oligodendrocytes in response to inflammatory-induced demyelination.

3. Stem cells and neuronal progenitors

The recent infatuation with adult neurogenesis has led to a promiscuous use of the term 'stem cell' leading thus to a broadening of its definition. Based on their functional properties, 'stem' cells should be distinguished from 'progenitor' or 'precursor' cells. The former is currently defined as an undifferentiated cell that exhibits the ability to *proliferate*, to *self-renew*, and to *differentiate* into multiple yet distinct lineages. In the adult brain, most stem-like cells are in a quiescent stage, except in few neurogenic zones considered here, the SVZ and the dentate gyrus, where they have a very slow dividing turnover (few weeks). In contrast, *progenitor* cells are mitotic cells with a faster dividing cell cycle (few hours) that retain the ability to proliferate and to give rise to terminally differentiated cells but are not capable of indefinite self-renewal; they are more committed than stem cells and their multi-potentiality is still a matter of debate. Finally, when the cell type being studied is not clear, as is the case in vivo, both stem and progenitor cells are referred to as *precursor* cells. For an overview of the characteristics that can be used to differentiate neural stem from progenitor cells, and of the controversies that still persist in definitions, we refer the reader to previous reviews [4,6–8].

3.1. The subventricular zone of the forebrain

The SVZ harbors the largest population of proliferating cells in the adult brain of rodents, monkeys, and humans, and it has been estimated that 30,000 cells per day are generated bilaterally in the mouse SVZ. This region exhibits a rostro-caudal gradient of proliferative activity: proliferation is higher in the dorso-lateral corner of the rostral ventricle and falls caudally,

moving from the striatum towards the hippocampus. This gradient has been correlated with the capability of the constitutively proliferative cells to divide and to form neurospheres and certainly reflects the existence of two populations of dividing cells, one of quiescent stem-like cells and one of rapidly proliferating progenitor cells.

Four cell types have been described in the SVZ:

- the ependymal ciliated cells (type-E) facing the lumen of the ventricle, to circulate the cerebrospinal fluid;
- the proliferating type-A neuroblasts, expressing neuronal markers, and migrating in ‘chains’ toward the olfactory bulb;
- the slowly proliferating type-B cells expressing nestin and GFAP, and ensheathing migrating neuroblasts;
- the actively proliferating type-C cells or ‘transit amplifying progenitor’ expressing nestin, and forming clusters interspaced among chains throughout the SVZ.

Following the targeted introduction of a retrovirus into the dividing cells, which include type-B and -C cells, labeled cells migrate towards the olfactory bulb within few days where they give rise to new neurons [9]. From this data, the lineage progression: B → C → A has been proposed. Remarkably, a similar lineage has been described in the dentate gyrus [4].

Due to space constraint, we will comment exclusively how neural stem cells produce newborn neurons in the SVZ of adult mammals. Early work in this field has provided the framework for our current understanding of how the maturing of newborn neurons takes place in the adult forebrain. At first glance, the adult neurogenesis may be seen as a putative response to the death and turnover of neurons that might occur throughout life. We will see that in fact adult neurogenesis endows mature microcircuits with unique properties that can be used as well for brain repair.

4. Becoming a newborn neuron in the adult forebrain

Unlike other immature neurons, precursor cells of the SVZ migrate tangentially without the aid of radial glial cells and continue dividing during their migration to populate the olfactory bulb, where they differentiate into local inhibitory interneurons. When the neuroblasts reach the bulb, they migrate radially and of those that survive, approximately 95% and 3% differentiate into GABAergic and dopaminergic interneurons, respectively.

It has been shown that neurogenesis in the adult, as in the immature, brain includes cell division, differentiation and migration, as well as synapse formation and programmed cell death. Until recently, however, only morphological and immunohistochemical studies supported the idea that cells generated in the SVZ in adulthood become true bulbar interneurons. Lack of direct functional evidence left open the possibility that the newly generated neurons never fully integrate into the bulbar network. Information concerning maturation of SVZ neuroblasts is essential to appreciate how newly formed neurons be-

come functionally integrated into adult neural networks. To obtain this type of information, we characterized the electrophysiological properties of newborn cells during their migration and differentiation using a replication-defective retrovirus that carries GFP [10]. GFP-labeled neurons were visualized in acute olfactory bulb slices prepared following different times after viral injection. We demonstrated that integration of functional neurons in adult neural networks is achieved through sequential steps in a highly regulated manner. These steps include proliferation of the neural stem cell, differentiation into an immature neuron, migration to the final location, growth of dendrites and formation of synapses with other neurons in the circuit, and ultimately maturation into a fully functional neuron.

As early as 7 days after birth, some of the new neurons have reached the olfactory bulb and migrate radially to their final position. A few days later, they already display dendritic spines, which suggests that they are receiving synaptic inputs. Indeed, soon after new cells enter the layers of the olfactory bulb, they express functional GABA and glutamate receptor-channels and display voltage-dependent potassium currents typical of more mature neurons. Spontaneous synaptic activity then emerges soon after radial migration is completed. Interestingly, GABAergic and glutamatergic synapses impinging onto newborn neurons were established sequentially, GABAergic synapses being established first. In contrast to the sequential acquisition of synapses during embryogenesis, we found NMDA glutamate responses in neurons that were unable to fire and had started to receive GABA_A and AMPA glutamate inputs. The rules that govern the incorporation of adult-generated neurons into mature neuronal circuits may thus differ from those previously described in the developing brain [9]. In the adult brain, the correlated maturation of intrinsic electrical properties with synaptic activity of newborn cells may influence both maturation and integration processes and therefore represents a mechanism by which neuronal activity may regulate neurogenesis.

Future studies will need to determine if the newborn neurons make functional synapses with their downstream target neurons and release appropriate neurotransmitters in order to unequivocally demonstrate their integration into adult circuitries. However, it is now certain that adult-generated neurons, derived from neural stem cells, could become physiologically mature in the olfactory bulb circuitry. Surprisingly, the spiking activity of newly generated neurons does not occur until late in the maturation process [10], and this delay in excitability may prevent their disrupting the function of the preexisting circuitry. This characteristic further illustrates that maturation of adult-generated neurons does not recapitulate embryogenesis, partly due to the high neuronal activity already occurring in the adult mature circuitry. In central sensory pathways, this activity results from external stimuli that lead to experience-dependent changes in anatomical connectivity, both during late development and in adulthood. Since these anatomical changes involve synaptic reorganization, understanding the maturation of synaptic transmission at newly formed synapses may provide important insight into how experience modifies already

functioning neural circuits. The late occurrence of NMDA receptors and the maturation sequence of glutamatergic synapses we have reported may indicate unique maturation sequences adapted to experience-dependent maturation of adult neural circuits. Do newly generated bulbar neurons differ from older granule cells? Functionally, are there two types of granule cells, i.e. a larger population that is generated during development and early postnatal life and another one generated during adulthood? Answering these questions will clearly influence the final interpretation of the role of adult neurogenesis. Finally, it should be kept in mind that only about half of the newly generated bulbar interneurons survive more than several days after having reached their mature state. We do not yet know if this survival time is sufficient for these cells to play a significant role in olfactory bulb function. Obviously, understanding the role of neuronal replacement in the adult brain implies that one has to take into account that half of the newborn neurons are transient and will be replaced through a process still to be discovered.

In summary, following a century of doubt and controversy [1], there is now a consensus that neurogenesis occurs in the adult brain at least in two regions, the olfactory bulb and the hippocampus. In both structures, neuroblasts migrate and differentiate mainly into granule neurons that will synthesize GABA in the olfactory bulb and glutamate in the dentate gyrus. A smaller proportion of adult-born cells differentiate into other types of interneurons (the periglomerular in the olfactory bulb and the basket cells in the dentate gyrus). Altogether this adult neurogenesis leads to the birth of ~ 30,000 new neurons per day in the SVZ and around 3000 in the dentate gyrus of adult rats. The reasons for which: 1) the SVZ and the dentate gyrus harbor adult neurogenesis, 2) neurogenesis is curtailed in the dentate gyrus in comparison to the olfactory bulb, and 3) genesis rates are much lower in primates, are currently unknown. Furthermore, since these structures do not grow in size, a homeostatic compensatory equilibrium should be attained through an increase in cell death that should be equivalent to the initial addition of neurons. This phenomenon, poorly understood, in particular in the dentate gyrus, deserves more attention for it is an important partner of neurogenesis. Finally, recent evidence indicates that the adult-born neurons of the olfactory bulb and the dentate gyrus are functional and have thus a physiological role. Although these findings suggest a relevant contribution of these newly generated neurons to the bulbar or hippocampal function, further studies are needed to confirm these reports and fully unravel their fundamental consequences on the animals' behavior. Of great interest are the recent findings supporting functional implications of adult neurogenesis in production and integration of newborn neurons in the diseased brain. By understanding the mechanisms involved, it may be possible to harness the plasticity of the adult brain to recruit endogenous neural stem cells or to graft stem cells to achieve structural brain repair. Thus, the mature brain has the capacity for a greater degree of structural plasticity than previously thought.

5. Adult neuronal stem cells and brain repair

The discovery of the ability of the adult brain to produce newborn neurons has been particularly exciting since utilization of adult-derived stem cells will overcome most ethical and scientific problems brought into play by the use of embryonic stem cells. While non-autologous tissue grafting can give rise to the immunological rejection and will require long-term, if not life-long, use of immunosuppressive agents, employment of adult-derived autologous stem cells would avoid this caveat. In addition, one of the most obvious limitations in the use of embryonic stem cell therapies comes from the fact that grafted pluripotent embryonic stem cells can give rise to teratomas and teratocarcinomas. Thus, these serious drawbacks in combination with the reduced viability and purity of embryonic stem cells, has led to consider using more defined populations of stem cells for cell-replacement therapies of neurological diseases. Adult neural stem cells have the advantage of being readily amenable to manipulation, enrichment and expansion; however, there are certain limitations which currently render their use quite difficult. In adulthood little is known about the regulation of the signaling pathways, which are critical in cell fate decisions and progenitor differentiation and as yet, there are no *in vivo* or *in vitro* functional assays to easily evaluate the potential of individual cells.

A key step for application of adult-derived neural stem cells in therapeutic cell transplantation is their controlled transition into defined somatic cell populations, able to survive, generate multiple phenotypes in appropriate relative numbers and restore lost connectivity within the local microenvironment. Efforts towards these goals focus on two strategies: targeted differentiation, which aims at a stepwise transition of multipotent cells into a defined phenotype based on extrinsic instructive cues, and lineage selection, which involves genetic modification of the donor cells to a specific phenotype or characterization of distinct adult neurogenic niches able to provide the brain with different neuronal subtypes [11]. Consequently two major prerequisites for the development of successful brain repair strategies are:

- the in depth understanding and control of the developmental potential and biological properties of neural stem cells;
- the genetic manipulation of stem cells to (over) express survival and/or differentiation-promoting genes in order to generate cells with enhanced survival and/or differentiation properties.

Adult neurogenesis within each neurogenic site is regulated differently by a growing list of 'epigenetic factors'. Although most of these factors modulate cell proliferation through unknown mechanisms, a regulation of Cyclin-D1 and p27^{Kip1} expressions may constitute a common pathway [12]. The specificities of each neurogenic site may be related to differences in the intrinsic properties of the dividing cells (for example their intrinsic ability to sense neural activity) and/or in their microenvironment, which corresponds to the summation of local

neurogenic signals expressed or synthesized ‘locally’ by healthy neighboring cells or by dying cells. These signals may also be synthesized in the ‘periphery’ and released in neurogenic sites by neuronal afferences or by blood vessels [13]. In this context, the role of the cerebral vasculature has gained importance as adult neurogenesis occurs within an ‘angiogenic niche’ and an alteration of the vascular microenvironment—or its ability to respond to changes in metabolic demands—may be responsible for a disruption of neurogenesis.

In conclusion, we have discussed the role of adult neurogenesis in the context of the brain plasticity. Under the assumption that the olfactory bulb processes odor information before relaying it to the olfactory cortex, we hypothesize that adult neurogenesis allows the bulb to adjust the degree of processing appropriately. Second, the existence of a pool of juvenile neurons, that enables the system to adapt to future similar situations, raises the possibility that adult neurogenesis acts *post hoc* to provide a structural basis for brain plasticity. Overall, we believe that research on adult neurogenesis is not only interesting in itself but provides a new avenue to the understanding of adult brain plasticity and its potential reparation [14].

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