

Neural Progenitor and Stem Cells in the Adult Central Nervous System

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Abstract

Neurogenesis occurs in the adult brain, and neural stem cells (NSCs) reside in the adult central nervous system (CNS). In the adult brain, newly generated neuronal cells would originate from a population of glial cells with stem cells properties, and be involved in processes such as learning and memory, depression, and in regenerative attempts in the diseased brain and after injuries. In human, a recent study reported no evidence of migrating neural progenitor cells along the subventricular zone (SVZ) to the olfactory bulb (OB), contrary to other species, highlighting the particularity of adult neurogenesis in human. Though the origin and contribution of newly generated neuronal cells to CNS pathophysiology remain to be fully understood, the discovery that NSCs reside in the adult CNS force us to re-evaluate our knowledge and understanding of brain functioning, and suggest that the adult CNS may be amenable to repair. In this manuscript, we will review the recent data, debates and controversies on the identification, origin and function of newly generated neuronal cells in the adult brain, in human and in other species. We will discuss their contribution and significance to CNS pathophysiology, and for cellular therapy.

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Introduction

Seminal studies in the 1960s, using [3H]-thymidine autoradiography, reported that neurogenesis occurs in discrete areas of the adult mammalian brain, in rodents.¹⁻³ Studies in the 1970s and 1980s confirmed that neurogenesis occurs in hippocampus and subventricular zone (SVZ) of rodents.^{4,5} Adult neurogenesis was thought to be an inconsequential peculiarity of restricted regions of these mammals, as it was thought not to occur in primates.⁶ In the 1990s, new protocols for labelling dividing cells in the central nervous system (CNS), like retroviral and bromodeoxyuridine (BrdU) labellings, contributed to confirm that neurogenesis occurs in the adult mammalian brain,⁷⁻⁹ and to the first evidence that neurogenesis occurs in primates, human and non-human.^{10,11}

Neurogenesis Occurs in the Adult Mammalian Brain

Neurogenesis occurs primarily in 2 areas of the adult brain in the mammals, as in rodents and non-human primates: the dentate gyrus (DG) of the hippocampus, and the SVZ (Fig. 1).¹² In the DG, newly generated neuronal cells in the

subgranular zone (SGZ) migrate to the granule cell layer, where they project to the CA3 area of Ammon's horn.^{11,13-16} Newly generated neuronal cells in the SVZ migrate to the olfactory bulb (OB), through the rostro-migratory stream (RMS), where they differentiate into interneurons of the OB.¹⁷⁻²⁰ It is estimated that as many as 9000 new neuronal cells are generated per day in the rodent DG, contributing to about 3.3% per month or about 0.1% per day of the granule cell population.^{11,21} Neurogenesis has been reported to occur in other areas of the adult brain – albeit at lower levels – like the CA1 area, striatum and 3rd ventricle in rodents, and neocortex in non-human primates.²²⁻²⁵ However, some of these data have been the source of debates and controversies, and remain to be further confirmed.²⁶⁻²⁸

In the adult human brain, Eriksson et al¹⁰ reported the presence of dividing cells in the DG, co-labelled with neuronal markers, from tissue samples obtained *postmortem*, providing the first evidence that neurogenesis occurs in the adult human brain. Sanai et al²⁹ reported the existence of a ribbon of astrocytes lining the lateral ventricles

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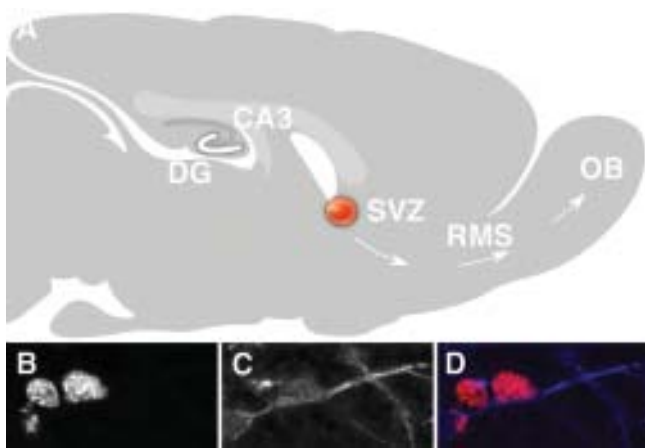


Fig. 1. Neurogenesis in the adult mammalian brain. Neurogenesis occurs primarily in 2 areas of the adult brain, the subgranular zone (SGZ) of the dentate gyrus (DG) and the subventricular zone (SVZ). Newly generated neuronal cells in the SGZ migrate to the granule layer, where they extend axonal projections to the CA3 area. Newly generated neuronal cells in the SVZ migrate to the olfactory bulb (OB), through the rostro-migratory stream (RMS), where they differentiate into interneurons of the OB (A). In human, Sanai et al reported no evidence of chains of migrating neuroblasts in the SVZ. Co-labelling of a BrdU-positive cell (B, red channel) with class III β -tubulin isotype (Tuj1, C, blue channel) in the SGZ. The merge picture shows BrdU- and Tuj1-positive cell (D). BrdU- and Tuj1-positive cells are representatives of newly generated neuronal cells in the CNS.

of adult human brain tissue samples that proliferate *in vivo* and behave as clonal precursor cells of self-renewing, multipotent neurospheres *in vitro*, suggesting that a substantial number of neural stem cells (NSCs) exist in the adult human brain throughout life and identifying SVZ astrocytes as NSCs in the adult human brain. Sanai et al²⁹ further reported no evidence of chains of migrating neuroblasts in the SVZ or in the pathway to the OB. These findings raise the unexpected possibility that migration from the SVZ to the OB does not take place in adult humans or, if it does, precursors migrate as individual cells, highlighting the particularity of neurogenesis in the adult human brain.

Neural Stem and Progenitor Cells Reside in the Adult CNS

It is hypothesised that newly generated neuronal cells originate from NSCs in the adult brain. NSCs are the self-renewing, multipotent cells that generate the neuronal and glial cells of the nervous system.³⁰ To identify the existence of NSCs in the adult brain, investigators have aimed at isolating and characterising *in vitro*, cells with self-renewing and multipotent properties. The demonstration that putative NSCs are multipotent relies on showing that the 3 main phenotypes of the CNS, neurons, astrocytes and oligodendrocytes, can be generated from single cells. The demonstration that putative NSCs can self-renew relies on showing that cells maintain their multipotentiality over

time.¹² However these criteria, although well accepted, are not absolute to demonstrate the existence of NSCs. The main criticisms reside in the number of subcloning steps that one must show to qualify a cell as self-renewing. Since stem cells have the ability to give rise to a large number of progeny, it is proposed to at least demonstrate self-renewal over an extended period of time (more than 5 passages) coincident with the generation of a large number of progeny, several orders of magnitude more numerous than the starting population.³¹ Undifferentiated cells with limited proliferative capacity that cannot self-renew are qualified as neural progenitor cells (NPCs).

In 1992, Reynolds and Weiss³² reported the first isolation and characterisation *in vitro* of NPCs from the adult brain. The investigators isolated, from the adult striatal area containing the SVZ of adult mice, a population of undifferentiated cells expressing nestin, that differentiate into the main phenotypes of the nervous system, neuronal, astrocytic and oligodendrocytic. Isolated cells grew as neurospheres, in defined medium in the presence of epidermal growth factor (EGF). Nestin is an intermediate filament that has been characterised as a marker for neuroepithelial and CNS stem cell during development,³² and so considered as a marker for adult neural progenitor and stem cells.³³ In 1995, Gage et al³⁴ isolated and characterised *in vitro* a population of cells with similar properties from the adult rat hippocampus. Isolated cells were grown as monolayers, in defined medium in the presence of basic fibroblast growth factor (FGF-2). Further *in vitro* studies characterised these populations of cells as containing self-renewing, multipotent NSCs.^{35,36} Since then, self-renewing, multipotent neural progenitor and stem cells have been isolated and characterised from various areas of the adult CNS, including from non-neurogenic areas, like the spinal cord, from various species, including humans.¹² These studies suggest that self-renewing multipotent NSCs reside in the adult CNS, particularly in the SVZ and the hippocampus.

Recent studies have challenged the isolation and characterisation of self-renewing, multipotent NSCs from the adult hippocampus. Seaberg and van der Kooy³⁷ reported the isolation and characterisation of NPCs with limited proliferative capacity, but not cells with self-renewal and multipotent properties from microdissected adult mice DGs. The isolated cells were grown as neurospheres in the presence of EGF and FGF-2. The investigators concluded that the resident cells underlying rodent hippocampal neurogenesis are progenitor cells. Bull and Bartlett³⁸ reported the isolation and characterisation of NPCs with limited proliferative capacity, but not cells with self-renewal and multipotent properties from adult mice hippocampus, supporting the observations and conclusions made by

Seaberg and van der Kooy,³⁷ Bull and Bartlett³⁸ further reported the isolation and characterisation of self-renewing, multipotent NSCs from the subependymal zone of the lateral wall of the lateral ventricle nearby the hippocampus, the posterior lateral ventricle (pLV), leading the investigators to hypothesise that the stem cells responsible for adult hippocampal neurogenesis reside outside the hippocampus, in the pLV. Stem cells in the pLV would produce progenitor cells that migrate into the neurogenic zones and proliferate to produce new neurons and glia in the DG.³⁸ Differences in tissue culture handling, culture conditions, as well as origin of the tissue may underlie the discrepancies between the studies. Particularly, the studies reporting the isolation and characterisation of self-renewing, multipotent NSCs from the adult hippocampus were performed from rat tissue,^{34,36,39} whereas studies reported by Seaberg and van der Kooy³⁷ and Bull and Bartlett³⁸ were performed from mice tissue. Responsiveness of trophic factors, the requirement of autocrine/paracrine factors, and the characteristic of cell growth of neural progenitor and stem cells from these tissues may be different.

Origin of Newly Generated Neuronal Cells in the Adult Brain

Though neural progenitor and stem cells have been isolated and characterised from adult brain tissues, the identity of NSCs in the adult brain remains the source of debates and controversies. Investigators have aimed at identifying the origin of the newly generated neuronal cells in the adult brain. There are currently 3 theories with regard to the identity of the cell type at the origin of newly generated neuronal cells in the adult rodent brain. The first theory contends that newly generated neuronal cells originate from a population of ependymal cells in the SVZ that express the intermediate filament protein nestin. In 1999, Johansson et al⁴⁰ reported that isolated ciliated ependymal cells elicit self-renewing, multipotent properties *in vitro*, and that DiI-labelled ependymal cells labels neuroblast in the SVZ in rodent. An ependymal cell origin for newly generated neuronal cells has also been reported more recently in the 3rd ventricle, as Xu et al²⁴ reported that neurogenesis may also occur in this area of the adult brain in rodent. The second theory identify them as astrocyte-like cells expressing glial fibrillary acidic protein (GFAP) in the SVZ and SGZ. Cell lineage analyses *in vivo* with markers of cell division, electron microscopy, and retroviral markers showed that newly generated neuronal cells in the adult SVZ and DG originate from a population of cells expressing GFAP and nestin that exhibit ultrastructural characteristics of astroglia.⁴¹⁻⁴³ Imura et al⁴⁴ used tissue culture techniques and transgenic mice expressing herpes simplex virus thymidine kinase (HSV-TK) from the mouse GFAP promoter to test the hypothesis that certain NSCs

express GFAP. In this transgenic model, dividing GFAP-expressing cells are ablated selectively by treatment with the antiviral agent ganciclovir (GCV). GCV applied *in vitro* eliminated growth of multipotent neurospheres isolated from SVZ area of adult transgenic mice, indicating that the predominant multipotent NSCs isolated from adult SVZ express GFAP. Garcia et al⁴⁵ developed 2 transgenic targeting strategies to further determine the relative contribution of GFAP-expressing progenitor cells to constitutive neurogenesis in the adult brain. One strategy combined the targeted expression of HSV-TK with delivery of the antiviral agent GCV to achieve the specific and inducible ablation of dividing GFAP-expressing cells *in vivo*. The other strategy allowed fate mapping of progeny cells derived from GFAP-expressing cells by using the targeted expression of Cre recombinase (Cre) to excise a loxP-flanked stop signal and activate reporter gene expression from an independent ubiquitous promoter. Transgenically targeted ablation of dividing GFAP-expressing cells in the adult mouse subependymal and subgranular zones stopped the generation of immunohistochemically identified new neuronal cells in the OB and the hippocampal DG, an observation also reported by Morshead et al.⁴⁶ Transgenically targeted cell fate mapping showed that essentially all new neuronal cells generated in the adult mouse forebrain *in vivo*, and in adult multipotent neurospheres *in vitro*, derived from progenitor cells that expressed GFAP. Constitutively dividing GFAP-expressing progenitors showed predominantly bipolar or unipolar morphologies with significantly fewer processes than non-neurogenic multipolar astrocytes.⁴⁵ These findings identify morphologically distinctive GFAP-expressing progenitor cells as the predominant sources of constitutive adult neurogenesis, further supporting a glial origin for newly generated neuronal cells in the adult brain. The third theory identifies NSCs in the adult brain, as population of cells non-expressing GFAP. Two reports based on flow cytometry show no expression or only partial expression of immunohistologically detectable GFAP in partially purified populations of adult cells from the SVZ area that show neurosphere-forming potential *in vitro*. In one study, homogenous population of neural progenitor and stem cells have been isolated and characterised *in vitro* by negative selection using lectin peanut agglutinin.⁴⁷ In the second study neural progenitor and stem cells were isolated using the carbohydrate moiety Lewis X.⁴⁸ Among these 3 theories, the claim that ependymal cells include a population of NSCs has not been confirmed by various *in vivo* and *in vitro* investigations. In contrast, the glial-origin for NSCs in the adult brain has received much support, though reports by Rietze et al⁴⁷ and Capela et al,⁴⁸ also suggest the existence of a population of NSCs that may not express GFAP. There are several issues that need to be further

investigated to confirm the identification and origin of NSCs in the adult brain. Particularly, the relationship between neurosphere-forming cells *in vitro* and NSCs *in vivo* need to be understood, and the expression pattern and function of GFAP, an intermediate neurofilament, by the NSCs *in vivo* and *in vitro* remain to be elucidated.

In the adult human brain, Sanai et al²⁹ reported a ribbon of SVZ astrocytes lining the lateral ventricles that proliferate *in vivo* and behave as clonal precursor cells of self-renewing, multipotent neurospheres *in vitro*. Although the precise location of the stem cells cannot be established, Sanai et al²⁹ identified SVZ astrocytes as NSCs in the adult human brain, lending further support to the glial origin of adult NSCs.

Neurogenesis and CNS Pathophysiology

Neurogenesis in the adult DG and OB is modulated by various environmental stimuli, and in pathophysiological conditions. Environmental enrichment was first reported to promote neurogenesis by Kempermann et al.⁴⁹ Using a combination of toys, wheels, tubes, and food supplements, Kempermann et al⁴⁹ reported that the survival of newly generated neuronal cells was increased in adult mice DG. Various form of exercises, like voluntary running, forced running and swimming, were reported to enhance hippocampal neurogenesis,⁵⁰⁻⁵² an activity that was found to be dependent on the circadian rhythm – running activity significantly increases neurogenesis only in animals with wheel access during the middle of the dark period, when mice are normally active.⁵³ Behaviours and social environments were also reported to modulate neurogenesis. Learning, novel environment, dietary restriction enhance neurogenesis in the adult hippocampus.⁵⁴⁻⁵⁶ Social isolation, alcohol consumption, stress and sleep deprivation decrease hippocampal neurogenesis.⁵⁷⁻⁶⁰ The rate of neurogenesis decreases with age in the DG and SVZ, in laboratory animals, as well as in wild-living rodents.^{9,61,62} In pathological conditions, like neurological diseases, strokes and traumatic brain injuries, neurogenesis is increased in the DG and SVZ,⁶³⁻⁶⁶ and new neuronal cells have been reported to be generated at the sites of injury or degeneration.^{67,68} It is estimated that 0.2% of the degenerated nerve cells are replaced in the striatum after middle cerebral artery occlusion, a model of focal ischaemia.⁶⁷ Cell tracking studies revealed that new neuronal cells at the sites of degeneration originate from the SVZ. They migrate to the site of degeneration, partially through the RMS.^{67,68}

Most of these studies have been performed in rodent, and quantified by BrdU immunohistology and stereological analysis. The modulation of neurogenesis and particularly its quantification have been subject of debates, partly due to the use of BrdU as a method of labelling. As BrdU

crosses the blood brain barrier (BBB), it is generally administered intraperitoneally. Activity, like exercise, certain drug treatments and various pathophysiological conditions, like epilepsy, affect cerebral flow, metabolism and permeability of the BBB.⁶⁹⁻⁷¹ Hence, changes in cerebral flow, metabolism and permeability of the BBB would affect the uptake of BrdU in the brain, and thereby the level BrdU incorporation in brain cells. Some investigators suggest that the variation of BrdU quantification observed in these conditions would reflect the change in BrdU uptake by the cells, rather than the modulation neurogenesis.⁷² This remains to be further evaluated.

The modulation of neurogenesis by various environmental stimuli, and in pathophysiological conditions suggests the involvement of newly generated neuronal cells in these processes. The function and contribution of adult neurogenesis to the CNS pathophysiology remain to be understood. Some reports also suggest that exposure to environmental enrichment, known to stimulate neurogenesis, could compensate for the decrease in neurogenesis observed in certain conditions. For example, physical exercise reverses the decrease of neurogenesis with alcohol consumption, and prevents the age decrease of neurogenesis in the DG.⁷³⁻⁷⁵ In contrast, alcohol exposure impairs the neurogenic response to an enriched environment in adult mice.⁷⁶ The physiological consequences and benefits of such compensatory mechanisms on neurogenesis remain to be understood. The determination of the function(s) of newly generated neuronal cells in the adult brain may lead to a better understanding to the contribution of adult neurogenesis to CNS pathophysiology.

Investigators have attempted to determine the function of newly generated neuronal cells in the adult brain. Evidence suggests that newly generated neuronal cells participate to processes like learning and memory, and depression. The behavioural effects of chronic antidepressants may be mediated by the stimulation of neurogenesis in the hippocampus.⁷⁷ In learning and memory, hippocampal neurogenesis is involved in the formation of trace memories that depends on the hippocampal formation, but not in all types of hippocampal-dependent learning processes.^{78,79} Further support of the involvement of adult hippocampal neurogenesis in learning and memory come from studies where adult rats were subjected to brain irradiation. Hippocampal brain irradiation blocked the formation of new neurons in the DG, and in the following weeks after irradiation, the animals performed poorer than controls in various hippocampus-dependent tasks that can be said to be vulnerable to the effects of neurogenesis suppression, like the short-term memory hippocampal-dependent test (place-recognition task) and non-matching-to-sample task.^{80,81} However, the involvement of adult neurogenesis

in learning and memory has been challenged by other studies. Increased hippocampal neurogenesis has been observed without improvement of learning and memory performances, in the Morris water maze test, in mice selectively bred for high levels of wheel running, and the contribution of adult neurogenesis to the formation of trace memory remains for months and beyond the time required for the retention of trace memories.^{82,83} Therefore, there are evidences of the involvement of adult neurogenesis in learning and memory, but its contribution in learning and memory remains to be elucidated.

In all, neurogenesis is modulated by a variety of environmental stimulus and pathophysiological conditions, and newly generated neuronal cells participate to processes like learning and memory and depression.^{84,85} The modulation of neurogenesis may therefore play a role in neuroadaptation and plasticity of the CNS.⁸⁶ Reports show that new neuronal cells are also generated at the sites of injury or degeneration, where they replace some of the lost nerve cells, suggesting that newly generated neuronal cells may also participate in a regenerative attempt by the CNS. Further studies will aim at better understanding the function and contribution of adult neurogenesis to CNS pathophysiology, and its mechanisms.^{87,88}

Cellular Therapy

The evidence that neurogenesis occurs in the adult brain and that NSCs reside in the adult CNS suggests that the adult CNS may be amenable to repair, and opens new opportunities for cellular therapy. Cell therapeutic intervention may involve the stimulation of endogenous or the transplantation of adult-derived neural progenitor and stem cells.

Neural progenitor and stem cells have been isolated and characterised *in vitro* from various areas, neurogenic and non-neurogenic – including the spinal cord – of the adult brain, suggesting that neural progenitor and stem cells reside throughout the adult CNS. Hence, hypothetically, regeneration could be promoted by locally stimulating neural progenitor and stem cells at sites of degeneration. Other evidences show that new neuronal cells are generated at sites of degeneration in the diseased brain and after CNS injuries, where they replace some of the degenerated nerve cells. The SVZ origin of these newly generated neuronal cells suggests that conditions enhancing SVZ neurogenesis could promote regeneration and functional recovery after CNS injuries.⁸⁹

Neural progenitor and stem cells can be isolated from the adult brain providing a valuable source of tissue for cellular therapy. Adult-derived NSCs elicit several advantages over other cell types for cellular therapy, among them, the ability to perform autologous transplantation, in which

neural progenitor and stem cells would be isolated from an undamaged area of the CNS, grown in culture, and grafted back to restore brain function. On the one hand, the ability to perform autologous transplantation represents a considerable advantage for adult-derived neural progenitor and stem cells over other cell types for cell transplantation strategy, as it would obviate the need to find a matching donor, or the use of immune-suppressive drug, like cyclosporine. On the other hand, harvesting neural progenitor and stem cells from patients would involve invasive surgery and the destruction of healthy brain tissue, limiting its clinical application. Neural progenitor and stem cells have also been isolated from human *post-mortem* tissues, providing an alternative source of tissues for cellular therapy.^{90,91} The generation of a bank of post-mortem tissue would allow to find matching donors, without the ethical and political concerns associated with embryonic and fetal tissues.

Conclusion

With the confirmation that neurogenesis occurs in the adult brain and NSCs reside in the adult CNS, determining the identification, origin, and functions of newly generated neuronal cells has been the subject of intense research, debates and controversies. Studies showed a broad range of stimuli and pathophysiological conditions modulate neurogenesis. Newly generated neuronal cell would be involved in processes like learning and memory, and depression. However, the contribution of newly generated neuronal cells to CNS functioning remains to be fully understood. Further, recent data show the particularity of neurogenesis in the human brain. Future studies will aim at unravelling the contribution of newly generated neuronal cells to CNS functioning and pathophysiology, redefining our current knowledge of the CNS. Future study will also aim at studying human adult neurogenesis and NSCs.

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