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Regulation and function of neurogenesis in the adult vertebrate brain

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Abstract

Most adult tissues retain a reservoir of self-renewing, multipotent stem cells that can generate differentiated tissue components. Until recently, the brain was thought to be an exception to this rule and for many years the pervasive dogma of neurobiology relegated neurogenesis to the embryonic and earlier postnatal stages of development. The discovery of constant neuronal replacement in the adult brain has changed the way we think about neurological diseases and about the exploration of new strategies for brain repair. In this review we will explore the potential of adult neural stem cells and we will present some of our own work on this subject. We will also discuss the possibility that adult neurogenesis and neuronal replacement may also play a role in therapies aimed at restoring impaired brain function. A better understanding of the various aspects of spontaneous neuronal replacement may also be used to increase the success of procedures with cell therapies.

Key words
- Neural stem cells
- Adult neurogenesis
- 9-O-acetyl GD3
- Gangliosides

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Introduction

It has long been thought that the mature central nervous system lacks regenerative capacity. Most neurons in the adult central nervous system are terminally differentiated and are not replaced when they die. For this reason, therapeutic approaches are not efficient in the treatment of neurodegenerative diseases and neurological traumas.

In 1962, Joseph Altman (1) was the first to challenge the notion that new neurons were not produced in adulthood. In a series of papers using ^{3}H-thymidine labeling to identify proliferating cells, he suggested that in mammals neurogenesis continued postnatally in specific regions of the brain (2-4). However, the lack of cell-specific markers at that time made it difficult to conclude unequivocally that these cells were neurons. In 1977, Kaplan and Hinds (5) labeled cells with ^{3}H-thymidine in the granular layers of the dentate gyrus and olfactory bulb of adult rats and confirmed that they were neurons by electron microscopy. Despite Altman's and Kaplan's pioneering work, the dogma that no neurons are produced in the adult nervous system of mammals remained unchanged until the earlier 80's when new techniques permitted more direct proofs of the findings

reported by Altman and Kaplan.

The dogma started to change with the work of Nottebohm and co-workers (for a review, see Refs. 6 and 7). In a series of papers they were the first to show explicitly that new neurons were added to a key nucleus in the song-learning system of adult song birds' brains. They also showed that the new neurons were born in the same proliferative regions that gave origin to neurons during embryogenesis - the subependymal layer adjacent to the lateral ventricle. In addition, they showed that radial glial fibers guided the migration of new neurons from their birth site around the ventricular zone lining the lateral ventricle to the proximity of their final destination, the high vocal center, a structure correlated to the mammalian hippocampus.

Later studies showed that the same phenomenon also occurs in mammals, including rodents, monkeys and even humans (8-11). Eriksson and co-workers (11) used the brains of cancer patients that had received bromodeoxyuridine injections to prove the generation of new neurons in the human adult brain. Bromodeoxyuridine is a thymidine analogue incorporated in the S phase of the cell cycle that allows the identification of proliferating cells (12).

The proliferative capacity of the adult central nervous system, however, is very limited. This limitation seems to be related to low levels of signal factors and the presence of inhibitory signs. Several stimuli modulate the basal proliferation of neurons in the adult central nervous system. Adult neurogenesis increases, for example, after an injury or a neurological disease (13,14). Even so, this proliferation is not enough to reconstitute neuronal losses. Because of this, there is a fast growing number of researchers who have been showing ways to increase the classes and numbers of neurons that the brain can produce in adulthood.

Another approach used to amplify the number of new neurons generated includes growth factor infusion. The new neuroblasts proliferate and migrate to the infusion site (15,16). The prospect of developing strategies to stimulate neurogenesis in neurodegenerative diseases has spurred industrial activity in this field in the past few years.

Neurogenesis in the adult mammalian brain

In recent years, it has become evident that in the adult brain there are two regions of active proliferation that generate neurons continuously throughout life: the subependymal zone (or subventricular zone) of the lateral ventricle and the subgranular layer of the dentate gyrus in the hippocampus (17-20).

The subependymal zone of the adult lateral ventricle is seen as a residual proliferative matrix left over from the embryonic neural tube. In the adult, the new neurons generated in this region enter the rostral migratory stream, complete their last divisions, and continue to migrate into the olfactory bulb where they differentiate into new neurons (21,22). In the hippocampal formation, progenitor cells divide along the border between the hilus and the granule cell layer, and daughter cells differentiate into granule cell neurons (23).

Neural stem cells have been successfully isolated, cloned and expanded from these proliferative regions of the adult brain. Moreover, under *in vitro* conditions these stem cells are able to proliferate and form dividing clusters of cells called "neurospheres" (18) in the presence of growth factors, such as fibroblast growth factor 2 or epidermal growth factor. They can be clonally expanded and the differentiation of the progeny can be achieved by either growth factor withdrawal alone, or by subsequent stimulation of the cAMP pathway, or of retinoic acid receptors (18). These cells can give rise to the three major cell types of the nervous system: neurons, astrocytes, and oligodendrocytes. In

some cases, neural stem cells can be passaged *in vitro* for years (24).

To better understand the biology of neural stem cells and to fully understand their therapeutic value, we need to known more about the factors that influence neurogenesis in the adult brain. Several strategies have been used to modulate neurogenesis both in the hippocampus and subependymal region. For example, enriched environment paradigms whereby animals are housed under conditions similar to their natural surroundings, increase neurogenesis at all ages, including senescence (25). These animals also show improved motor skills and better performance in learning tasks. In addition, it has been suggested that physiological parameters, such as blood flow, glucose uptake, and neovascularization could be mediators of this effect (26).

It has also been shown that learning through hippocampus-dependent tasks improved the survival of newborn cells, whereas hippocampus-independent learning tasks had no impact on the generation of new neurons (27). The functional integration of granule cells generated during adulthood into the hippocampal circuitry has been postulated in studies using retrograde and anterograde labeling (28). Subsequent studies indicated that environmental stimulation affects the proliferation and differentiation of these cells *in vivo*. Specifically, exposure to an enriched environment increases dentate gyrus neurogenesis.

In our laboratory we have tested the effect of physical activity on the proliferation of cells in the subgranular region of the dentate gyrus. Initial results indicate that in hamsters voluntary exercise in a running wheel results in an increase in neurogenesis in the dentate gyrus (Jacini W, Santiago MF and Mendez-Otero R, unpublished data). Further studies are needed to understand whether this adult neurogenesis contributes to functional roles of the hippocampus such as synaptic plasticity, learning and memory.

Identity of the adult neuronal stem cells

Adult neurogenesis is now accepted as a common feature of vertebrate brains. It is important, however, to clarify that although new neurons are incorporated into certain adult brain regions, there are other regions where this does not seem to take place. In addition, in the regions in which new neurons are incorporated, only a small percentage and only a subset of neuronal types are involved. This raises the question of the identity of neural stem cells within adult germinal regions and the relation of these cells to those in the developing brain.

It has been assumed that the subventricular zone of the adult brain harbors at least three distinct cell phenotypes: A, B and C cells. The A cells are the migratory neuroblasts that proliferate and migrate from this region to the olfactory bulb through tubes of slowly proliferating astrocytes (B cells). The C cells are rapidly dividing precursors that give rise to the A cells. The B cells are claimed to be the adult neural stem cells that can originate both C and A cells *in vivo* (29). Lining the ventricle lumen are the ependymal cells (E cells). In contrast to the classical model that suggests that neurons and glia are derived from two separate branches of a lineage tree, it has been hypothesized that neural stem cells are contained within a continuum that forms the trunk of a lineage tree (30). From this trunk, the different committed progenitors or terminally differentiated cells emerge. Depending on the time of development, cells within this trunk have neuroepithelial, radial glial or astrocytic characteristics. These cells have different morphologies and can express different markers. However, they also share some common characteristics such as the expression of nestin (30).

Recently, Johansson and co-workers (31) have suggested that the neural stem cell may in fact reside within the ependymal lining of

the ventricles, or may even be the ciliated-ependymal cell itself (E cell). This cell population is generally homogeneous and is characterized by the presence of functional cilia.

Searching for adult neuronal stem cells

Reynolds and Weiss (18) were the first to show that by culturing tissue from the adult rodent forebrain, a population of cells having the hallmark properties of stem cells, i.e., multipotency and self-renewal capacity, was expanded. An obvious question following the discovery of stem cells in the adult brain was the localization and identity of these cells. This task has been difficult due to the lack of specific markers for these cells. In comparison with the best studies on the stem cell population - the hematopoietic system - there are very few markers for different cell types in the nervous system and even fewer for neuronal stem cells. However, further elucidation of stem cell identity and potential will be important for the development of stem cell-based therapies for nervous system diseases in which a pure population of neuronal stem cells may be obtained.

In our laboratory we have studied for many years the functional role of a specific ganglioside expressed by neurons and radial glia during development (32-34). In the adult, this ganglioside expression is downregulated in most of the brain but persists in very few regions including the subependymal layer around the lateral ventricles (35). Recently, we have focused on asking whether 9-O-acetyl GD3 can be used as a marker of neuronal stem cells. Using this marker, we are studying the cellular, molecular as well as environmental influences that regulate neurogenesis in the adult brain. We have found that 9-O-acetyl GD3 is expressed by a subpopulation of cells that also express markers associated with different neuronal stem cell phenotypes. For example, 9-O-acetyl GD3-positive cells do not bind to the peanut agglutinin antigen that was described as an excluding marker for neural stem cells (36). On the other hand, a subpopulation of 9-O-acetyl GD3-positive cells co-label with the early neuroepithelial intermediate filament Nestin and with the carbohydrate Lex (Gubert F, Zaverucha-do-Valle C, Santiago MF and Mendez-Otero R, unpublished data). The Lex antigen was recently associated with a non-ependymal adult neural stem cell population (37). In addition, using magnetic activated cell sorting, we have isolated a fraction of cells from the ependymal/subependymal region of the adult rat brain that express high amounts of 9-O-acetyl GD3. In the neurosphere formation assay we show that in the presence of fibroblast growth factor 2 and epidermal growth factor, the group containing 9-O-acetyl GD3-positive cells forms more neurospheres than the group negative for this marker. Furthermore, neurospheres generated by the 9-O-acetyl GD3-positive population differentiate into neurons and glia after deprivation of growth factors. Based on these results, we suggested that 9-O-acetyl GD3 can be used as a surface marker to isolate neural stem cells from the rat adult nervous system (Gubert F, Zaverucha-do-Valle C, Santiago MF and Mendez-Otero R, unpublished data). Figure 1 summarizes some of the findings of our group concerning the characterization of adult neural stem cells.

Regulation of neurogenesis in pathological conditions

Recent studies have shown that acute brain injury, including that caused by ischemia, seizures and trauma, can stimulate neurogenesis in the adult hippocampus. However, enhanced neurogenesis is also caused by seizure activity, mild global forebrain ischemia and stroke not associated with neuronal death in the hippocampus. Thus, cell death is not necessary for insult-induced neurogenesis. Stroke has also been reported

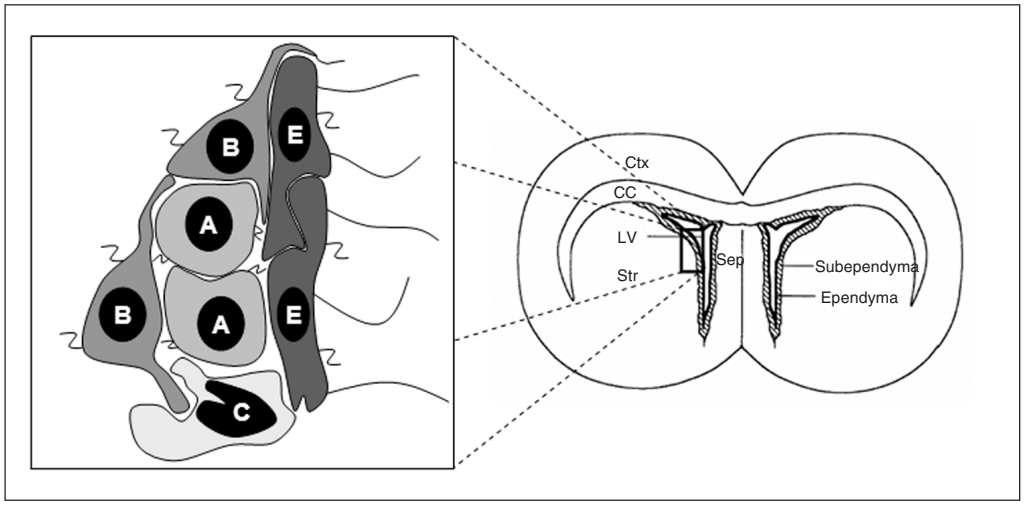

Figure 1. Schematic diagram of the organization of the adult subventricular zone. Migrating neuroblasts (type A cells) are surrounded by astrocytes (type B cells) that separate the migrating cells from the striatum. The type B cells eventually possess a single cilium that touches the ventricular lumen, are self-renewable and express glial fibrillary acid protein (GFAP). Ependymal cells (type E cells) line the ventricular surface and possess functional cilia. In close association with the neuroblasts are the C cells. The type C cells are putative intermediate precursors that give rise to the migrating neuroblasts and are self-renewable as well. Two different views in the literature point to type B and type E cells as good candidates for the true adult neural stem cell identity (29,31). Note that both of them express the ganglioside 9-O-acetyl GD3 (Z) in their membranes. Therefore, this ganglioside can be used as a surface marker to isolate an enriched population of adult neural stem cells. Since type A cells also express the ganglioside 9-O-acetyl GD3 this antigen could not be considered an exclusive marker of adult neural stem cells. A = type A cells; B = type B cells; C = type C cells; E = type E cells; Ctx = cerebral cortex; CC = corpus callosum; LV = lateral ventricle; Sep = septum; Str = striatum.

to increase neurogenesis in the subependymal region. In our laboratory, we have investigated the expression of 9-O-acetyl GD3 following global ischemia in the rat. Ischemia was induced with bilateral carotid occlusion and the subependymal region was examined 7 days after occlusion. Preliminary results have shown that in the ischemic brain there was a change in the expression pattern of 9-O-acetyl GD3 in the subependymal region. The results suggest that this ganglioside may play a role in the response of neural stem cells to injury (Zaverucha-do-Valle C, Gubert F, Santiago M and Mendez-Otero R, unpublished data). The functional consequences of insult-induced neurogenesis are still unclear. It is possible that increased neurogenesis might be a compensatory mechanism replacing lost neurons and counteracting functional impairments.

Final comments

Evidence now exists that small populations of neurons are formed continuously in specific regions of the adult brain (38). Although there is no guarantee that all this knowledge will yield any new clinical tools for brain and spinal cord repair, our hopes are that, in the future, research on the brain's own neurogenic potential will result in ways of reconstituting damaged or senescent circuits and functions in the brain (39). The discovery of neurogenesis and neuronal replacement in adult brains is likely to affect the ways in which we think about neurological diseases and neuronal repair.

References

1. Altman J (1962). Are new neurons formed in the brains of adult mammals? *Science*, 135: 1127-1128.
2. Altman J (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anatomical Records*, 145: 573-591.
3. Altman J (1969). DNA metabolism and cell proliferation. In: Lajtha A (Editor), *Structural Neurochemistry*. Vol. 2. *Handbook of Neurochemistry*. Plenum Press, New York, 137-182.
4. Altman J (1967). Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. In: Quarton GC, Melnechuk T & Smith FO (Editors), *The Neurosciences. First Study Program*. Rockefeller University Press, New York, 723-743.
5. Kaplan MS & Hinds JW (1977). Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science*, 197: 1092-1094.
6. Nottebohm F (2002). Neuronal replacement in adult brain. *Brain Research Bulletin*, 57: 737-749.
7. Alvarez-Buylla A, Buskirk DR & Nottebohm F (1987). Monoclonal antibody reveals radial glia in adult avian brain. *Journal of Comparative Neurology*, 264: 159-170.
8. Morshead CM, Reynolds BA, Craig CG et al. (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron*, 13: 1071-1082.
9. Luskin MB (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron*, 11: 173-189.
10. Gould E, Reeves AJ, Graziano MS et al. (1999). Neurogenesis in the neocortex of adult primates. *Science*, 286: 548-552.
11. Eriksson PS, Perfilieva E, Bjork-Eriksson T et al. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, 4: 1313-1317.
12. Gratzner HG (1982). Monoclonal antibody to 5-bromo and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science*, 218: 474-478.
13. Zhang RL, Zhang ZG, Zhang L et al. (2001). Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience*, 105: 33-41.
14. Parent JM, Yu TW, Leibowitz RT et al. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *Journal of Neuroscience*, 17: 3727-3738.
15. Emsley JG & Hagg T (2003). Endogenous and exogenous ciliary neurotrophic factor enhances forebrain neurogenesis in adult mice. *Experimental Neurology*, 183: 298-310.
16. Scharfman H, Goodman J, MacLeod A et al. (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology*, 192: 348-356.
17. Bayer SA (1983). ^{3}H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. *Experimental Brain Research*, 50: 329-340.
18. Reynolds BA & Weiss S (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, 255: 1707-1710.
19. Thomas LB, Gates MA & Steindler DA (1996). Young neurons from the adult subependymal zone proliferate and migrate along an astrocyte, extracellular matrix-rich pathway. *Glia*, 17: 1-14.
20. Taupin P & Gage FH (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. *Journal of Neuroscience Research*, 69: 745-749.
21. Menezes JR, Marins M, Alves JA et al. (2002). Cell migration in the postnatal subventricular zone. *Brazilian Journal of Medical and Biological Research*, 35: 1411-1421.
22. Menezes JR, Smith CM, Nelson KC et al. (1995). The division of neuronal progenitor cells during migration in the neonatal mammalian forebrain. *Molecular and Cellular Neurosciences*, 6: 496-508.
23. Seri B, Garcia-Verdugo JM, McEwen BS et al. (2001). Astrocytes give rise to new neurons in the adult mammalian hippocampus. *Journal of Neuroscience*, 21: 7153-7160.
24. Bazan E, Alonso FJ, Redondo C et al. (2004). *In vitro* and *in vivo* characterization of neural stem cells. *Histology and Histopathology*, 19: 1261-1275.
25. van Praag H, Kempermann G & Gage FH (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, 2: 266-270.
26. Isaacs KR, Anderson BJ, Alcantara AA et al. (1992). Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. *Journal of Cerebral Blood Flow and Metabolism*, 12: 110-119.
27. Gould E, Beylin A, Tanapat P et al. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nature Neuroscience*, 2: 266-270.
28. Markakis EA & Gage FH (1999). Adult generated neurons in the dentate gyrus of adult rats extend axonal projections to field CA3 and are surrounded by synaptic vesicles. *Journal of Comparative Neurology*, 406: 449-460.
29. Doetsch F, Caille I, Lim DA et al. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*, 97: 703-716.
30. Alvarez-Buylla A, Garcia-Verdugo JM & Tramontin A (2001). A unified hypothesis on the lineage of neural stem cells. *Nature Reviews*, 2: 287-292.
31. Johansson CB, Momma S, Clarke DL et al. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. *Cell*, 96: 25-34.
32. Mendez-Otero R & Santiago MF (2003). Functional role of a specific ganglioside in neuronal migration and neurite outgrowth. *Brazilian Journal of Medical and Biological Research*, 36: 1003-1013.
33. Santiago MF, Costa MR & Mendez-Otero R (2004). Immunoblockage of 9-O-acetyl GD3 ganglioside arrests the *in vivo* migration of cerebellar granule neurons. *Journal of Neuroscience*, 24: 474-478.
34. Miyakoshi LM, Mendez-Otero R & Hedin-Pereira C (2001) The 9-O-acetyl GD3 gangliosides are expressed by migrating chains of subventricular zone neurons *in vitro. Brazilian Journal of Medical and Biological Research*, 34: 669-673.
35. Mendez-Otero R & Cavalcante LA (1996). Expression of 9-O-acetylated gangliosides is correlated with tangential cell migration. *Neuroscience Letters*, 204: 97-100.
36. Rietze RL, Valcanis H, Brooker GF et al. (2001). Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature*, 412: 736-739.
37. Capela A & Temple S (2002). LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron*, 35: 865-875.

38. Zhao M, Momma S, Delfani K et al. (2003). Evidence for neurogenesis in the adult mammalian substantia nigra. *Proceedings of the National Academy of Sciences, USA*, 100: 7925-7930.

39. Lindvall O & Mckay R (2003). Brain repair by cell replacement and regeneration. *Proceedings of the National Academy of Sciences, USA*, 100: 7430-7431.