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Neurogenesis in humans

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Abstract

Since 1944 increasing evidence has been emerging that the adult human brain harbours progenitor cells with the potential to produce neuroblasts. However, it was not until 1998 that this fact was confirmed in the adult human brain. With the purpose of human neurogenesis being hotly debated, many research groups have focussed on the effect of neurodegenerative diseases in the brain to determine the strength of the endogenous regenerative response. Although most of the human studies have focussed on the hippocampus, there is a groundswell of evidence that there is greater plasticity in the subventricular zone and in the ventriculo-olfactory neurogenic system. In this review, we present the evidence for increased or decreased plasticity and neurogenesis in different diseases and with different drug treatments in the adult human brain. Whilst there is a paucity of studies on human neurogenesis, there are sufficient to draw some conclusions about the potential of plasticity in the human brain.

Introduction

The concepts of neurogenesis and cell proliferation in the brain are generally believed to have been conceived in the 1960s, revisited in the 1980s and confirmed in humans in the 1990s. However, the human neural stem cell story was first eloquently described in the 1940s, thereby predating the rodent neural stem cell story by more than 20 years. In 1944, Globus and Kuhlenbeck, a neurosurgeon and neuropathologist, respectively, described the origin of human subependymomas (tumours arising from the remnant of the developmentally active lateral ganglionic eminence) as arising from cells they termed 'bipotential mother cells'. According to Globus and Kuhlenbeck, the bipotential mother cells start out small and round, and end up differentiating into neuroblasts that have either unipolar or bipolar morphology. These studies were all performed using brain tissue from patients who had died with ependymal, subependymal and choroid plexus tumours. Histological staining of *post mortem* brain tissue enabled identification of neoplastic tumours that contained primitive cells, akin to what they described as bipotential mother cells and spongioblasts. Bipotential mother cells and spongioblasts were described as the products of cell division that occurred in the primitive medullary epithelium. The offspring of bipotential mother cells were considered to be neuroblasts, which become neurons or spongioblasts; the spongioblasts, in turn, were thought to form ependymal, choroid plexus, astrocytes and oligodendroglial cells. According to Globus and Kuhlenback, spongioblasts could be formed as a direct daughter cell of the medullary epithelium or as a daughter cell of the bipotential mother cells (Globus & Kuhlenbeck, 1944). Today we call these bipotential mother cells 'neural stem cells'; they have been the focus of substantial study from the viewpoint of localising them in the adult brain and manipulating their physiology to promote the replacement of dying neurons in degenerative brain conditions, such as Parkinson's disease and stroke.

In this paper, we will review the methods by which stem cells have been detected in the human brain and explore the evidence for cell proliferation in different regions of the adult human brain. This review will especially focus on diseases in which stem cell proliferation is known to be affected and will also critique existing literature on the rostral migratory stream (RMS), the major pathway by which neural progenitor cells migrate to replace olfactory bulb neurons.

Methods for detecting cell proliferation and neurogenesis in the human brain

Detection of neurogenesis and cell proliferation in the adult rodent brain has become commonplace in stem cell biology today. The most common technique used to label progenitor cells in the brain is the thymidine analogue bromodeoxyuridine (BrdU) assay. BrdU is a compound formerly used to radiosensitise cancer cells because its incorporation weakens the DNA strand and less radiation therapy is then required to permanently destroy the DNA strand. In practice, BrdU has failed to make a major impact on cancer treatment, but remains the gold standard for labelling progenitor cells in the brain, as it causes no discernable toxicity at low doses and labels the cell during the S-phase of the cell cycle (Dolbeare, 1995, 1996). The main advantage of using BrdU for detecting cell proliferation is that it can be used in conjunction with a wide range of other antibodies raised against neuronal and non-neuronal cell types in the brain. This technique has also been applied to humans, and allowed the first demonstration of human neurogenesis in the hippocampus. In order to examine the spread of oropharyngeal carcinoma, 250 mg BrdU was

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administered in a single injection to a cohort of patients. Subsequent studies performed by Eriksson *et al.*, (1998) revealed the presence of BrdU in the same cells that came to express neuronal markers. There are two weaknesses of the BrdU method in determining neurogenesis in the human brain: first, the detection of BrdU labelling in fluorescent studies is hampered by the autofluorescent staining of lipofuschin that is especially high in the brains of older people; and second, there are ethical issues regarding routine administration of BrdU to humans (Cooper-Kuhn & Kuhn, 2002). Furthermore, each time the cells divide, the amount of BrdU in the nucleus is halved until eventually the BrdU becomes undetectable, thus rapidly dividing cells are more likely to have low levels of BrdU leading to an underestimation of the real number of dividing cells (Karpowicz *et al.*, 2005). Thus, there is still a major role for the use of endogenous cell cycle proteins in detecting progenitor cells in the human brain. Human brain studies to date have primarily utilised proliferating cell nuclear antigen (PCNA), Ki67, phospho-histone H3 (Ph3) and minichromosome marker-2 (MCM2). The advantage of these markers over BrdU labelling is that they are endogenously-produced cell cycle markers made by dividing cells. The major disadvantages arise from the fact that the cell cycle is very tightly controlled, so the cell cycle proteins are only expressed for a very short period of time and are then rapidly degraded, and so the future fate of the cell can not be determined.

The most recent method developed to demonstrate neurogenesis and the age of neurons in the brain capitalises on the variation over time in the atmosphere's radioactive ^{14}C content. In the 1950s and 1960s when above-ground nuclear testing was being carried out, atmospheric levels of ^{14}C rose dramatically. However, after the nuclear test ban treaty in 1963 the levels of ^{14}C steadily and rapidly decreased due to absorption of the ^{14}C into the biosphere. Because carbon is a major constituent of the body, higher levels of ^{14}C could also be detected in the tissues of those born during periods of high atmospheric ^{14}C (Spalding *et al.*, 2005). The ^{14}C is stable in the DNA of tissues not undergoing cell division, and thus by detecting the levels of ^{14}C in neurons *post mortem* it is possible to determine the age of the neurons. This technique has been employed predominantly by a group from Stockholm led by Prof. Frisen. Frisen *et al.* have been interested in the age of the neurons in the human cortex, and the ^{14}C method is one suitable way to examine large populations of neurons (Spalding *et al.*, 2005; Bhardwaj *et al.*, 2006). Fluorescence-activated cell sorting is first used to isolate neurons from non-neuronal cell types, by NeuN labelling in neurons. Once sorted, the neuron's DNA is removed and ^{14}C levels in the DNA are measured using a radio-mass spectrometer (Spalding *et al.*, 2005).

The adult hippocampus in health and disease

In 1998, Eriksson *et al.* published the first pivotal study on hippocampal neural progenitor cells and neurogenesis in the human brain. This elegant study not only provided proof of principle that new neurons were indeed formed in the adult human brain, but also showed that the new neurons could be detected a long time after BrdU was administered (Eriksson *et al.*, 1998). However, the study did not reveal the total number of newly born neurons in the hippocampus in the adult brain, nor how this number changed with age. The next important study on the hippocampus came from Jin *et al.* in 2004, where brain tissue homogenates prepared from normal and Alzheimer's disease-affected brains were used in Western blotting and immunohistochemistry studies to determine the level of neurogenic markers in the hippocampi. The major markers they used for Western blotting were doublecortin, the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), TUC-4 and mature neuronal markers. This study reported increases in the amount of pro-neurogenic proteins in Alzheimer's disease tissue; however, the Western blotting technique was perhaps not sufficiently quantitative to allow them to draw solid conclusions and the Alzheimer's cases were run on different blots to the controls making it difficult to compare between normal and diseased samples. In this study, they also detected the presence of doublecortin-positive cells in the CA1 region of the hippocampus, and they determined by way of immunohistochemistry that the new cells in the hippocampus express the cell division marker, TUC-4, and the neuronal marker, Hu (Jin *et al.*, 2004). The work of Jin *et al.* paved the way for other studies investigating neurogenesis in Alzheimer's disease. In 2006, Boekhoorn *et al.* studied neurogenesis in the hippocampus in a younger presenile group of Alzheimer's diseased cases. In their study they performed extensive counting of doublecortin, glial fibrillary acidic protein (GFAP) and Ki67-labelled cells in the CA1–3 regions (Boekhoorn *et al.*, 2006). Their results showed a generalised increase in hippocampal proliferation, but this was primarily a gliotic and vascular response rather than a neurogenic response. What was striking in both of these studies was that the most extensive proliferation was apparent in the CA1 region, but the overall number of proliferating cells was very low. A subsequent paper by Jin *et al.* in 2006 revealed an increase in the number of proliferating cells in the cortex affected by stroke. Interestingly, they saw less than one Ki67-positive nucleus per mm^2 in the normal cortex and no cells that were double-labelled with doublecortin, whereas in the infarct core they saw more than 63 Ki67-positive cells and 10 Ki67 cells that co-expressed doublecortin (Jin *et al.*, 2006). This study provided evidence of a rapid neurogenic response after significant injury to the cortex, and indicated that significant cell death is a powerful signal to induce proliferation. However, the origin of the new-born cells was not identified in this study.

Another recent study by Lucassen *et al.* looked at hippocampi dissected from aged *post mortem* human brains in cases who were either neurologically normal or who had depression for which they had taken antidepressants. The results demonstrated no stimulatory effect of antidepressants despite the large volume of data to the contrary in rodents (Lucassen *et al.*, 2010). The key message to note from this study is the very small number of proliferating cells present in the hippocampus and the likely minimal effect that the small number of cells could exert on repairing the host circuitry, at least in this cohort of cases over 50 years old.

Subventricular zone (SVZ) proliferation and neurogenesis

During development, progenitor cells migrate from the SVZ, differentiating *en route* and at their final destination, where they become incorporated in the functional circuitry of the olfactory bulb and other regions of the forebrain. In the adult brain, rodent and human studies reveal that neurogenesis continues in the SVZ throughout adult life (Altman, 1969; Luskin & Boone, 1994; Lois *et al.*, 1996; Eriksson *et al.*, 1998; Benraiss *et al.*, 2001). One of the key reasons for the highly proliferative nature of the SVZ is that the neighbouring lateral ventricle is a ready source of nutrients and growth factors as well as having guidance cues for neuroblast migration such as 'slit' proteins that are organised into gradients according to the activity of cilia on ependymal cells, as demonstrated in rodent studies (Schanzer *et al.*, 2004; Sawamoto *et al.*, 2006). We now know that the SVZ in the adult brain continues to produce stem cells and new neurons throughout life. Under normal circumstances the function of the SVZ is to produce neuroblasts for the RMS (Lois *et al.*, 1996; Doetsch *et al.*, 1997;

Bedard & Parent, 2004; Hack *et al.*, 2005; Curtis *et al.*, 2007). After experimental injury in animals, for example striatal quinolinic acid lesion or middle cerebral artery occlusion, the SVZ not only supplies the RMS with neuroblasts but SVZ progenitor cells also migrate toward the site of injury and cell death (Arvidsson *et al.*, 2002; Tattersfield *et al.*, 2004). The SVZ harbours two types of glial cells; one of the glial cell types gives rise to rapidly proliferating type C cells termed transit amplifying cells that ultimately give rise to the migratory neuroblasts (type A cells) that migrate toward sites of injury. What is clear from a number of studies in the human brain is that there are more proliferating cells in the SVZ compared with the hippocampus (Curtis *et al.*, 2007; Lucassen *et al.*, 2010). Interestingly, the studies of Eriksson *et al.*, (1998) detected relatively few SVZ proliferating cells, and this is likely to be attributed to the fact that many cells labelled with BrdU would have migrated out of the SVZ by the time the brain was studied, whereas in the hippocampus the proliferating cells would have only migrated a small distance from the subgranular zone (SGZ) to the granule cell zone.

In the SVZ, both throughout life and in disease, plasticity is maintained in response to the progressive and massive cell death in nearby regions. The work of our laboratory has focussed on the SVZ in Huntington's disease (HD), which is significantly thicker, and has an altered cellular composition where the numbers of progenitor cells is increased and the mature cell types present are altered (Curtis *et al.*, 2003, 2005b). Overall there is a 2.8-fold increase in SVZ thickness (mostly in layer 3) compared with the normal SVZ, which results from a large increase in the number of proliferating cells in the HD SVZ in HD grades 1–3. The greatest number of proliferating cells is seen in the central and ventral regions of the SVZ in HD brains, whilst the lowest number of proliferating cells is seen in rostral locations where no significant differences in the SVZ cell proliferation between the HD and normal brains was demonstrated (Curtis *et al.*, 2005b). In HD, there is an approximate doubling of type A and C cells in the SVZ, but this increase is dwarfed by the increase in the number of type B (glial) cells comprising layer 3, which resulted in a shift in the ratio of type A : B : C from 1 : 3 : 1 in the normal brain to 1 : 7.5 : 1 in HD grade 3; in the rodent brain the ratio is 3 : 2 : 1 (Curtis *et al.*, 2005a). Predictably, the increase in the number of type B/glial progenitor cells corresponds to GFAP-positive gliosis in the HD SVZ. Also, the number of newly produced neurons is increased in the HD SVZ in proportion to the increase in PCNA-positive dividing cells. Thus, although there is a 2.6-fold increase in the number of new neurons, the proportion of new neurons to astrocytes remains unaltered.

However, not all neurodegenerative diseases are associated with increased proliferation in the SVZ; Parkinson's disease is one such disease. Dopaminergic denervation of the striatum from the substantia nigra leads to hypo-excitation in the motor cortex and thus a paucity of movement in Parkinson's disease. The SVZ is also innervated by the same pathway of dopaminergic fibres that supply the striatum, and normal dopaminergic innervation and signalling in the SVZ profoundly increases the proliferative capacity of this layer. Höglinger *et al.*, (2004) showed in a rodent model that dopamine can diffuse as far as 15 μm from the synapse, but because the SVZ is well separated from the striatum by myelinated fibres, the dopaminergic effect on the SVZ is almost certainly from its own innervation. Furthermore, in mice the dopaminergic innervation, as shown by tyrosine hydroxylase staining, overlaps the entire rostrocaudal extent of the SVZ (Baker *et al.*, 2004). In particular, the D2 receptor seems to play a vital role in upregulating the proliferation of progenitor cells in the SVZ. One study by Baker *et al.*, (2004) used the rodent excitotoxic 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease that leads to the degeneration of the medial forebrain bundle (nigrostriatal pathway) to determine the effect of dopamine innervation on proliferation in the SVZ. The results demonstrated an almost halving of the number of BrdU-positive cells in the SVZ. Furthermore, they demonstrated that the amount of proliferation in the SVZ after a 6-OHDA lesion was proportional to the amount of remaining dopaminergic innervation in the striatum. When the dopamine precursor, levodopa, was administered there was a restoration of normal proliferation levels in the ipsilateral side to the lesion; however, on the contralateral side the levodopa had no apparent affect (Höglinger *et al.*, 2004). Agonists of both D2 and D3 receptors in mice have a powerful effect at upregulating proliferation in the SVZ (Diaz *et al.*, 2000; Ohtani *et al.*, 2003). D1 receptors have not been demonstrated in the SVZ. Based on cell counts of PCNA-positive cells in clinically diagnosed Parkinson's-affected cases, there is a decrease in cell proliferation in the SVZ by approximately 30%, and in the dentate gyrus there was also a significant 60 and 70% reduction in the number of β-III tubulin- and nestin-positive cells, respectively (Höglinger *et al.*, 2004). However, another recent report has demonstrated in a large cohort of Parkinson's disease brains that proliferation in the SVZ, as demonstrated with Musashi1 staining, is increased in patients treated with levodopa (the precursor to dopamine; O'Sullivan *et al.*, 2010). The study by Höglinger *et al.* used only four Parkinson's disease brains, and it is not clear whether those patients had been treated with levodopa. Because levodopa is the precursor to dopamine and dopamine has a significant proliferative effect on the cells in the SVZ, knowing whether the patient received levodopa is important for interpreting studies on cell proliferation in the SVZ. Further studies will be essential for confirming the up- or downregulation of proliferation in Parkinson's disease.

Proliferation, migration and neurogenesis in the RMS

In animal models, neurogenic cells from the SVZ form a constitutively migrating pathway of neuroblasts, the RMS, destined for the olfactory bulb (Altman, 1969; Doetsch *et al.*, 1997). The collective term for the SVZ together with the RMS is the 'ventriculo-olfactory neurogenic system' (VONS; Curtis *et al.*, 2007). The RMS in the adult brain is an extension of precursor cells arranged around a vestigial lumen that connects the lateral ventricle to the olfactory ventricle. The migrating neuroblasts in rodents extend from the anterior region of the SVZ, course ventrally and laterally between the striatum and the anterior forcep of the corpus callosum, forming the vertical arm, and then as cells continue to extend rostrally they become concentrated within the olfactory tract, forming the horizontal arm before reaching the olfactory bulb (Peretto *et al.*, 1997, 1999, 2005; Ponti *et al.*, 2006; Bonfanti & Peretto, 2007; Bonfanti *et al.*, 2008; Bonfanti & Theodosis, 2009). Once the neuroblasts arrive at the olfactory bulb, they disperse into the various layers of the olfactory bulb and eventually mature into interneurons (Lois & Alvarez-Buylla, 1994; Peretto *et al.*, 2005). In neurologically diseased brains, cells exit from the RMS and migrate to distant areas of neuronal death in an attempt to replace dying neurons, therefore making the RMS an important region of study for the future development of endogenous neuronal replacement strategies.

The RMS was recently demonstrated in the adult human forebrain (Curtis *et al.*, 2007). Histological staining, immunological staining and magnetic resonance imaging revealed that the human RMS also follows a vestigial ventricular extension (Curtis *et al.*, 2007; Kam *et al.*, 2008). The human RMS comprises a large number of proliferating cells that form a continuous stream from the SVZ towards the olfactory bulb. The RMS first extends ventro-caudally from the lateral ventricle immediately adjacent to the caudate nucleus

to form a long descending limb before extending ventro-rostrally to form a shorter rostral limb that enters the olfactory tract towards the olfactory bulb. This 'funnel-shaped' stream of cells shows anatomical characteristics that are consistent with observations in the primate brain (Pencea *et al.*, 2001). Due to the extensive frontal lobe development in humans, the olfactory tract in humans is located relatively more caudally, therefore the RMS in the adult human brain takes a path that is rotated by about 75° when compared with the rat RMS (Kam *et al.*, 2008). In the neurologically normal adult human forebrain, there was a calculated average of 108 300 ± 13 310 proliferating cells in the RMS alone (Curtis *et al.*, 2007). This suggests that a far greater number of neurogenic cells populate the entire VONS in the human brain compared with the rodent (Curtis *et al.*, 2007; Kam *et al.*, 2008).

There have been suggestions that glial tubes could support the proliferation and migration of the neuroblasts. In 1997, Peretto *et al.* showed in the rodent that GFAP-positive glial cells formed tubes, and within these glial tubes there were chains of neuroblasts. The neuroblasts appeared to associate with GFAP-positive glia throughout the SVZ and RMS (Peretto *et al.*, 1997). Therefore it is not surprising that in the human, the proliferating cells in the RMS are also surrounded by a GFAP-positive network of glial cells. However, instead of multiple branching glial tubes and multiple chains of neuroblasts, there is simply one single 'meshwork' of glial cells (Kam *et al.*, 2008). This observation is in keeping with the features reported in the adult primate brain and the rabbit RMS (Pencea *et al.*, 2001; Fasolo *et al.*, 2002), which show that cells in the RMS in the respective species are surrounded by a thick 'astrocytic net'.

The neurogenic properties of the human RMS have been investigated using immunohistochemical techniques. Some of the proliferating (PCNA-positive) cells found in the adult human RMS show co-expression with the migratory neuroblast marker PSA-NCAM (Curtis *et al.*, 2007; Kam *et al.*, 2008). These PSA-NCAM-positive cells possess a migratory morphology with elongated cell bodies, unipolar or bipolar processes, with the occasional appearance of cone-like end-feet. Furthermore, these cells are often arranged in parallel to the direction of the RMS (Kam *et al.*, 2008). This suggests that they are type A migratory neuroblasts that are not yet neuronally committed. Whilst clusters of cells with a migratory morphology can be seen occasionally in the human RMS, there do not appear to be distinct chains of migrating neuroblasts like that seen in the rat (Doetsch *et al.*, 1997).

As age increases in rodents, the number of neurogenic cells decreases (Kuhn *et al.*, 1996; Luo *et al.*, 2006). However, the rate of decline in different neurogenic regions has been debated. Kuhn *et al.*, (1996) showed that neurogenic cells in the SVZ do not decrease significantly with age, whilst those in the hippocampus decreased dramatically in 6- and 21-month-old rats. On the other hand, Luo *et al.* (2006) showed the contrary – where the proliferative cells in the SVZ displayed a dramatic decline in mice aged 2 and 10 months (Luo *et al.*, 2006).

In the human, the number of proliferative cells in the SVZ and the hippocampus, and the effect of aging on these populations have not been directly compared. However, in 1998, Eriksson *et al.* calculated the density of BrdU-positive cells in the SGZ of the aged adult human brain (age range 57–72) to be approximately 200–250 cells/mm^3 (Eriksson *et al.*, 1998). Given that the total area of the VONS is much larger than that of the SGZ alone, one can conclude that proliferative cells are much more abundant in the RMS and SVZ than in the hippocampus. In addition, the more accessible location of progenitor cells in the SVZ, RMS and the olfactory tract provides a pool of progenitor cells that may offer a greater potential than hippocampal progenitors for the development of neuron replacement therapies.

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Abbreviations

6-OHDA, 6-hydroxydopamine; BrdU, bromodeoxyuridine; GFAP, glial fibrillary acidic protein; HD, Huntington's disease; PCNA, proliferating cell nuclear antigen; PSA-NCAM, polysialylated form of the neural cell adhesion molecule; RMS, rostral migratory stream; SGZ, subgranular zone; SVZ, subventricular zone; VONS, ventriculo-olfactory neurogenic system.

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