Neurogenesis in the Adult Mammalian Brain – Implications for Self Repair and Treatment of Acute Brain Trauma

Joella Joseph

Abstract: The adult mammalian brain, previously thought to be incapable of repair after acute injury, is now known to contain discreet populations of neural cell progenitors. These stem cells have been shown to proliferate and establish neural connections in response to acute brain injury. This may represent a capacity for self repair that can be used to restore loss of cognitive abilities and movement associated with ischaemia, stroke and other acute trauma. This essay examines both the potential and limitations of stimulating the proliferation of endogenous neural stem cells.

Introduction

Few organs in mammals possess the intricacy that characterises the brain. Comprised of millions of interconnected neurons, the brain and spinal cord coordinate involuntary activities such as breathing, heart beat and digestion, as well as our capacity for conscious activities such as thought, reasoning and abstraction (Haggard and Clark, 2003). This vast network of synaptic connections allows us to perceive and process information, and to adjust our responses based on a combination of past experience and present knowledge (Haggard *et al.,* 2002).

During the late 19th century, when neuroscience emerged as a defined field, it was assumed that the brain lacked any capacity for regeneration or development in maturity. The mammalian central nervous system became structurally stable soon after birth, and remained in this state unless otherwise disrupted by injury, disease or death (Chichung Lie *et al.,* 2004). The fundamental assumption of this stability was that no new neurons were added to the brain in adulthood (Gould and Cross, 2002).Unlike tissues such as the skin and the liver, which can replace dead cells by proliferation of nearby cells or activation of resident stem cells (Bjorklund and Lindvall, 2000), most of the neurons of the adult central nervous system are terminally differentiated and are not replaced when they die (Mendez-Otero *et al.,* 2005). The result is a brain with functionally stable circuitry, but also with vulnerability to injury and disease (Bjorklund and Lindvall, 2000).

While the brain is indeed sensitive to these deleterious conditions, research has shown that the brain is not as inflexible as previously thought. In his pioneering studies, Altman (1962, 1963, 1966, as cited by Gould and Cross, 2002) demonstrated the proliferation of neural stem cells in the hippocampus and cerebral cortex of adult rats and cats by monitoring the uptake of ^{3}H-thymidine by cells actively undergoing DNA synthesis in preparation for mitosis. The resulting radioactive signal was used as a marker for proliferating cells. Although further studies substantiated these findings, it was not until the development of techniques such as cell-type specific marker identification that these cells were positively identified as neuronal precursors (Cameron *et al.,* 1993). The generation of new neurons, or neurogenesis, appears to be localised to two regions in the adult brain – the subventricular zone (SVZ) of the lateral ventricle, and the subgranular zone of the dentate gyrus in the hippocampus (Bjorklund and Lindvall, 2000)

For the most part, the functions of these cells in the adult brain remain speculative; however, their presence may represent a dormant capacity of the adult brain for repair and recovery after injury (Bjorklund and Lindvall, 2000). Acute brain injury caused by interrupted blood flow (ischemia) or seizures is accompanied by proliferation of neural cell precursors in the SVZ and dentate gyrus (Zhang *et al.,* 2001; Parent *et al.,* 1997). Resultant neuronal death may also trigger increased neuron addition in regions where neural stem cell proliferation normally does not take place, such as in the neocortex (Parent *et al.,* 1997). It is therefore possible that neural stem cells can replace damaged neurons in order to restore lost function.

This potential for self repair provides a novel basis for a therapeutic approach to brain injury. If these endogenous cells can somehow be induced to multiply in the region of damaged neurons, this may provide a non-invasive treatment for persons suffering reduced mental capacities or loss of function as a result of stroke, seizures or traumatic brain injury. The immediate, synchronous damage to multiple neurons brought about by acute injury may induce neuroregenerative signals, as opposed to the slower, more widespread cell death associated with neurodegenerative disease (Mendez-Otera *et al.,* 2005). Although neurogenesis occurs in response to brain injury, it is still unclear whether or not they actually replace damaged neuronal circuits (Vergara *et al.*, 2005). The sequence of steps leading to the integration of functional neurons in the adult brain is equivalent to that in the embryo, but with an important difference – new adult neurons undergo development in a mature environment and must integrate into pre-existing circuits (Chichung-Lie *et al.,* 2004).

Are adult stem cells responsive to the cues that guided their development in the embryo, or are they regulated by a different set of cues? Can these signals be used to generate functional, fully integrated neurons in the adult brain? What are the factors which influence these processes? These questions can be answered by understanding the processes that guide neurogenesis in both the embryo and the adult brain. This essay will compare the events in neurogenesis, focussing on the differentiation, migration and

integration of neural stem cells. It will also examine the studies surrounding this phenomenon in both animal and human models, and assess the therapeutic potential of harnessing endogenous neural stem cells for brain repair.

Neurogenesis in the Mammalian Embryo and the Adult Brain – A Comparison

Neurogenesis is the process by which functionally integrated neurons are generated from progenitor cells (Ming and Song, 2005). It is also from these cells that the support cells of the nervous system – astrocytes and oligodendrocytes – are produced (Chichung Lie *et al.,* 2004). Integration of neurons is a highly regulated process, beginning with proliferation of neural stem cells and proliferation of a rapidly amplifying progenitor cell. This is followed by differentiation into an immature neuron, migration to the final location, growth of axon and dendrites and the formation of synapses with other neurons. Ultimately, these steps lead to the maturation of a fully functional neuron (Chichung Lie *et al.,* 2004). The processes of differentiation, migration/targeting and integration are of particular interest, as they may be the most heavily influenced by the fully developed neural network surrounding them in the mature brain. For this reason, these three steps in neurogenesis will be considered.

Differentiation

As an unspecialised cell matures, it takes on distinctive characteristics to reach its mature (specialised) form and function (Kintner, 2002). In the human embryo, neurons are produced by almost all regions of the neuroepithelium, a group of cells that constitute the primordium of the central nervous system (Stagaard and Mollgard, 1989). Gene expression involved in differentiation is regulated by basic helix-loop-helix (bHLH) transcriptions factors. bHLHs prompt neuronal differentiation by causing neuroepithelial cells to exit the cell cycle and delaminate out of the epithelium, and by activating genes specific for neuronal differentiation (Kintner, 2002). In the adult brain, astrocytes promote proliferation and neuronal fate specification in the SVZ and dentate gyrus by releasing neurogenic signals (Seni *et al.,* 2001). Although these signals are largely unknown, they are thought to antagonise the action of bone morphogenic protein (BMP), which promotes glial cell as opposed to neuron differentiation (Lim *et al.,* 2000). Thus differentiation in the adult brain depends on extracellular signal from pre-existing cells.

Neuronal Migration and Nerve Targeting

Migration of neurons over relatively long distances is an essential activity in the development of the vertebrate brain. Neurons actively migrate from their site of generation in the center of the developing brain to their permanent residence in the cortex and other structures (al-Ghoul and Miller, 1993). In embryos, this movement is supported by a scaffold of radial glial cells and guided by secreted molecules and contact with the extracellular matrix (Ming and Song, 2005; Huber *et al.,* 2003). These secreted signalling molecules, which include netrins, ephrins and semaphorins, bind to receptors on the surface of the migrating cells and initiate cellular events that dictate the neuron's response to guidance cues (Huber *et al.,* 2003).

Newborn neurons migrate from the SVZ to the olfactory bulb in rats and non-human primates by chain migration, a process in which neuroblasts move closely together in tube-like structure formed by glial cells. Factors such as netrins and ephrin-B2 guide this movement (Ming and Song, 2005). In the dentate gyrus, newly generated neurons migrate a short distance to the inner granule cell layer where they become granule neurons. The dendrites of these neurons rapidly reach their target in the pyramidal cell layer in the brain, and continue to grow and increase in complexity over months. The molecular mechanisms guiding nerve growth and axon/dendrite guidance in granule neurons are unknown, but it has been found that semaphorins retain their expression in the adult brain, indicating a possible role in neuronal guidance (Huber *et al.,* 2003).

Synapse Formation and Neuronal Integration

There are marked differences in maturation of neurons in the adult brain and in the developing embryo. In the olfactory system, neuroblasts migrating from the SVZ express Gamma-aminobutyric acid (GABA) receptors first, followed by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. These receptors act at inhibitory and excitatory synapses respectively. *N*-methyl-D-aspartic acid (NMDA) receptors, which act at excitatory synapses, are expressed last (Ming and Song, 2005). In the embryo's developing brain, however, NMDA receptors are detected before AMPA receptors (Durand *et al.,* 1996). In addition, granule cells acquire the ability to fire action potentials after synaptic inputs are made – another significant divergence from neuronal integration in the embryo (Carleton *et al.,* 2003). The sequence of events that occur during neuronal maturation and synapse formation in the dentate gyrus are unclear, as are the overall cellular and molecular mechanisms that regulate the integration of neurons into the existing circuits of the brain (Ming and Song, 2005).

Is the Brain capable of Self Repair?

Despite the differences in the physical and possibly chemical environments in which neurons become functional in the adult and developing brains, there still exists the possibility that the neurons of the brain can be regenerated once damaged. Adult neural stem cells retain the ability to respond to proliferation and differentiation cues *in vitro* (Medez-Otero *et al.,* 2005). Neural stem cells that have been isolated from the proliferative regions of the brain continue to grow in the presence of growth factors such as fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF), producing clusters of cells that can further differentiate into astrocytes and oligodendrocytes (Reynolds and Weiss, 1992, as cited by Mendez-Otero *et al.,* 2005). However, these experiments were carried out *in vitro,* in the absence of the complex chemical environment of the developed brain. It is possible that these pathways may still exist, but it is unclear whether or not these growth factors are endogenously produced in the brain in response to injury, thus contributing to neurogenesis (Mendez-Otero *et al.,* 2005).

Introduction of exogenous factors has been shown to induce neuron formation in the brain. Intraventricular infusion of EGF in mouse brains resulted in large increases in proliferation of neural stem cells, and more importantly, their migration away from the lateral ventricle to the adjacent brain parenchyma (Craig *et al.,* 1996). When this infusion was attempted in rats, however, the differentiative effect of the growth factor was lost. Clearly, there are many details to the mechanism of neurogenesis that must be elucidated before it can be effectively applied in treatment of acute brain injury.

It has been established that neuronal death in the proliferative regions of the brain can trigger increased neuron addition (Parent *et al.,* 1997). This may also occur in regions not necessarily associated with neurogenesis, such as in the cortex. Magavi *et al.* (2000) found that synchronous degeneration of neurons in the adult mouse neocortex induced the differentiation of cortical neurons. Seizures involving the temporal lobe can produce an

increase in the granule neurons from the dentate gyrus in the hippocampus of rats (Parent *et al.,* 1997). When ischaemia is induced in the cortex and subcortex adjacent to the SVZ, the numbers of proliferating cells in the SVZ and olfactory bulb were found to increase, while those of the dentate gyrus were unchanged (Zhang *et al.,* 2001).This may be indicative of not only a proliferative response to injury, but of a region-specific response as well.

Neural progenitor cell differentiation relies on extracellular signals from surrounding neurons and support cells (Seni *et al.,* 2001). In the region of injury, neuronal cell death can cause surrounding cells to upregulate the expression of specific developmental signalling molecules that guide neuronal differentiation (Magavi *et al.,* 2000). Although the studies performed involved the migration and differentiation of transplanted immature neuronal progenitor cells, it is thought that similar mechanisms may underlie the migration and differentiation of endogenous stem cell populations. Indeed, the expression of neurotrophin-4/ 5 (NT-4/ 5) and brain-derived neurotrophic factor (BDNF) is increased while neurons of the cortex are undergoing targeted neuronal degradation. BDNF is expressed by surrounding interneurons rather than the degenerating neurons themselves, indicating intercellular signalling between the tissues coordinating neuronal regeneration (Wang *et al.,* 1998).

The proof of self repair is the return of normal function to the damaged neurons (Kempermann *et al.,* 2004). Craig *et al.,* (1996) have demonstrated that neural regeneration in rats stimulated by infusion of EGF resulted in restoration of brain functions associated with the hippocampus, such as spatial learning and memory, after induced ischemic damage. This restoration is shown by the improved navigation of water mazes, a task which tests the ability of rats to recall physical factors in their environment. Newly differentiated neurons receive afferent synapses and reform the appropriate connections in the damaged neocortex of adult mice (Magavi *et al.,* 2000). While such findings are promising, there must be more supporting evidence that restored neuronal connections consistently result in the restoration of the functions they control.

Mobilisation of Endogenous Neural Stem Cells – Possibilities and Limitations

The ability to utilise endogenous neural stem cell populations in the brain to replace damaged neural tissue may be beneficial to the recipient. Not only is a source of stem cells readily available for use, but using cells from the recipient's brain will reduce the likelihood of tissue rejection. It also presents a comparatively non-invasive means of treatment when compared with transplantation of donor neural stem cells.

Numerous studies have shown that neuronal progenitors found in the mammalian brain are capable of responding to the chemicals that initiate differentiation and integration into the existing circuitry (Bjorklund and Lindvall, 2000). However, in the cases of non-laboratory induced brain injury, this repair mechanism is limited to the germinal regions of the brain (Mendez-Otero *et al.*, 2005). Infusion of the necessary growth factors will be essential in generating the numbers of neural progenitors required. The survival of these newly generated neurons is another important consideration. Even when neurogenesis has been reported in areas other than the SVZ and the dentate gyrus, the resulting neurons do not survive long enough to become functional (Hallbergson *et al.*, 2003). As is the case in development, there must be specific temporal and spatial expression of appropriate signalling molecules. These molecules not only generate progenitor cells, but also direct their migration, differentiation and survival (Hallbergson, *et al.,* 2003). It is possible that the levels of signalling molecules present in the adult brain are insufficient to produce the required response. The natural neuroregenerative capacity of the brain can therefore be amplified by the infusion of the growth factors previously mentioned (Medez-Otero *et al.,* 2005).

The return of lost function is the key criterion for determining whether or not adult neurogenesis is successful (Kempermann *et al.,* 2004). So far, newly generated neurons have only proven functional in terms of improvement of hippocampal memory related tasks. Further research would be necessary to determine if this is fact applicable in humans. In this case, neurogenesis would have applications in the restoration of memory and possibly movement after stroke or acute trauma. It is necessary to note that the actual function of neurons is extremely complex, so although the appropriate connections have been made, unless the role of the neurons was previously established, determining the restoration of function will be difficult.

The survival of new neurons has been found to be enhanced by the activity of GABA (Miller *et al.*, 2006). Although GABA inhibits the firing of mature neurons, it actually has the opposite effect on newly developed neurons – it stimulates the electrical activity that is crucial to their integration into neural circuits and hence, their survival. This finding gives yet another point of consideration in the potential augmentation of neurogenesis. The effect of low levels of signalling molecules may also be increased by the presence of chemicals that inhibit neurogenesis. Although the majority of these factors are unknown, they undoubtedly play a significant role in preventing neurogenesis in the non-proliferative regions of the brain. If these interactions can be overcome, it may be possible to dampen the effects of these factors while enhancing those of pro-neurogenetic molecules and pathways.

Caution must be taken however, in the manipulation of any of the brain's processes. It is unclear what effects such changes would have on the other developmental pathways; unintentional disruption of function is an unacceptable trade for attempted restoration. Based on the studies reviewed, the potential for the brain to undergo self repair is great – however, this process must be enhanced by the infusion of exogenous growth factors to promote the differentiation, migration and integration of the newly developed neurons. There exist within the adult brain conditions for regeneration of damaged neurons; careful study of the complex interactions that produce this phenomenon will enable the development of relatively safe methods of treatment for acute brain trauma.

References

al-Ghoul WM, Miller MW. (1993). Orderly Migration of Neurons to the Principal Sensory Nucleus of the Trigeminal Nerve of the Rat. *J. Comp. Neurol.* 330(4):464-75

Bjorklund A, Lindvall O. (2000). Self repair in the brain. *Nature.* 405: 892-894.

Cameron HA, Woolley CS, McEwen BS, Gould, E. (1993). Differentiation of Newly Born Neurons and Glia in the Dentate Gyrus of the Adult Rat. *Neuroscience.* 56:337-344

Carleton A, Petreanu LT, Lansford R., Alvarez-Buylla A, Lledo PM. (2003). Becoming a New Neuron in the Adult Olfactory Bulb. *Nat. Neurosci.* 6:507-18

Chichung-Lie D, Song H., Colamarino SA, Ming G, Gage FH (2004). Neurogenesis in the Adult Brain: New Strategies for Central Nervous System Diseases. *Annu. Rev. Pharmacol. Toxicol.* 44:399-421

Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D. (1996). *In vivo* Growth Factor Expansion of Endogenous Subependymal Neural Precursor Cell Populations in the Adult Mouse Brain. *The Journal of Neuroscience.* 16(8): 2649-2658

Durand GM, Kovalchuk Y, Konerth A. (1996). Long-term Potentiation and Functional Synapse Induction in Developing Hippocampus. *Nature.* 381:71-75

Gould E, Cross, CG. (2002). Neurogenesis in Adult Mammals: Some Progress and Problems. *The Journal of Neuroscience.* 22(3):619-623

Haggard P, Clark S, Kalogeras J. (2002). Voluntary Action and Conscious Awareness. Nature Neuroscience. 5:382-385

Haggard P and Clark S. (2003). Intentional Action: Conscious Experience and Neural Prediction. Consciousness and Cognition. 12(4):695-707

Hallbergson AF, Gnatenco C, Peterson D. (2003). Neurogenesis and Brain Injury: Managing a Renewable Resource for Repair. *The Journal of Clinical Investigation.* 112(8): 1128-1133

Huber AB, Kolodkin AL, Ginty DD, Cloutier J-F. (2003). Signalling at the Growth Cone: Ligand-Receptor Complexes and the Control of Axon Growth and Guidance. *Annu. Rev. Neurosci.* 26:509-563

Kempermann G, Wiskott L, Gage FH. (2004). Functional Significance of Adult Neurogenesis. *Current Opinion in Neurobiology.* 14:186-191

Kintner C. (2002). Neurogenesis in Embryos and in Adult Stem Cells. *The Journal of Neuroscience.* 22(3):639-643

Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A. (2000). Noggin Antagonizes BMP Signaling to Create a Niche for Adult Neurogenesis. *Neuron*. 28:713-26

Magavi S.S, Leavitt B.R., Macklis J.D. (2000). Induction of Neurogenesis in the Neocortex of Adult Mice. *Nature*. 405: 952-955

Mendez-Otero R, Zaverucha-do-Valle C, Gubert F, de Freitas GR, Santiago MF. (2005). Regulation and Function of Neurogenesis in the Adult Vertebrate Brain. Brazillian *Journal of Medical and Biological Research*. 38: 1553-1559

Ming GL, Song H. (2005). Adult Neurogenesis in the Mammalian Central Nervous System. *Annu. Rev. Neurosci*. 28:23-50

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. (1997). Dentate Granule Cell Neurogenesis is Increased by Seizures and Contributes to Aberrant Network Reorganisation in the Adult Rat Hippocampus. *The Journal of Neuroscience.* 17(10): 3727-3738

Stagaard M, Mollgard K. (1989). The Developing Neuroepithelium in human Embryonic and Fetal Brain Studied with Vimentin-Immunocytochemistry. *Anat. Embryol.* 180(1):17-18

Vergara MN, Arsenijevic Y, Del-Rio-Tsonis K. (2005). CNS Regeneration: A Morphogen's Tale. *J. Neurobiol*. 64:491-507

Wang Y, Sheen VL, Macklis JD. (1998). Cortical Interneurons Upregulate Neurotrophins *In Vivo* Response to Targeted Apoptotic Degeneration of Neighbouring Pyramidal Neurons. *Experimental Neurology.* 154:389-402

Zhang RL, Zhang ZG, Zhang L, Chopp M. (2001). Proliferation and Differentiation of Progenitor Cells in the Cortex and Subventricular Zone in the Adult Rat after Focal Ischemia. *Neuroscience.* 105(1):33-41