The Role of SGZ Neurogenesis in Hippocampal Dependent Learning and Memory

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Abstract

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Advances in technology have allowed for the discovery of adult neurogenesis inthe human brain. Adult neurogenesis is found only in the subventricular zone (SVZ) and subgranular zone (SGZ) in the dentate gyrus region of the hippocampal formation. There are two types of progenitors found in the SGZ and their growth is thought to be influenced by their immediate environment called the neurogenic niche. Research suggests that new neurons generated in the brain are integrated into existing brain circuitry. This paper will focus on neurogenesis in the SGZ and provide evidence supporting its influence on the hippocampal functions of learning and memory.

It appears that some forms of hippocampal dependent learning influence levels of neurogenesis and that the number of adult born neurons influences learning and memory. SGZ neurogenesis is thought to be regulated by factors such as genetics, environment, and age. These factors regulate some forms of hippocampal dependent tasks in a correlative manner. Research on the function of SGZ neurogenesis in hippocampal dependent learning and memory comes to varying conclusions that are caused by differences in procedures. Research indicates that the relationship between adult neurogenesis in the SGZ and hippocampal dependent learning and memory may be dependent on: the age of the neurons, the age of the subjects and the type of learning that is occurring. More work needs to be done before a comprehensive understanding of the functionality of adult neurogenesis can be made.

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INTRODUCTION

The plasticity of the human brain is a phenomenon that is poorly understood but highly studied. Originally it was thought that new neurons did not develop in the postnatal brain. However, with advances in technology it was slowly discovered that neurogenesis does occur after birth. Adult neurogenesis is consistently found only in the subventricular zone (SVZ) and subgranular zone (SGZ) in the dentate gyrus region of the hippocampal formation. The growth of progenitor cells in both regions is thought to be influenced by their immediate environment known as the neurogenic niche. The neurogenic niche surrounding the SGZ supports the development of two types of progenitor cells. Research suggests that new neurons generated in the brain are integrated into existing brain circuitry. This paper will focus on neurogenesis in the SGZ and its possible influence on the hippocampal functions of learning and memory.

SGZ neurogenesis is thought to be regulated by internal and external sources. In addition, it appears that some forms of hippocampal dependent learning influence levels of neurogenesis and that the number of adult born neurons present, in return, influence learning and memory. Research on the function of SGZ neurogenesis comes to varying conclusions indicating that more work needs to be done before a comprehensive view on the functionality of adult neurogenesis can be made.

The discovery of adult neurogenesis in the hippocampal formation has changed scientists' view of the way that the brain adapts to physiological and environmental changes. Future studies in neurogenesis will continue to help scientists to understand how the brain functions. Information on neurogenesis in the SGZ and its function in

memory formation and learning may help treat related diseases such as Alzheimer's disease.

HISTORY

Existence of Adult Neurogenesis

Central Dogma

For almost a full century, a central dogma of developmental biology declared that the birth and addition of new neurons to the brain concluded shortly after birth (Gross, 2000). This hypothesis was based on evidence from Santiago Ramon y Cajal, who in 1913 maintained that neurons were generated only during the prenatal phase of development (Ming & Song, 2005). This view of brain development did not exist without contrary evidence.

Also at the turn of the 20th century, Edward Hamilton and Edward Allen provided opposing evidence to Cajal's theory which showed that dividing cells did exist in the central nervous system of postnatal mammals (Ming & Song, 2005). Despite their limited but convincing data that postnatal neurogenesis was a plausible phenomenon; the scientific world rejected the idea for many years and instead supported the idea that neurons were generated only during the prenatal phase of development.

Advancements in Technology

Acceptance of the adult neurogenesis hypothesis was hindered by the technological limitations of the time. In the early 20th century techniques for visualization and analysis of cell division and cellular phenotyping were not yet developed. Therefore there were no procedures available to provide definitive proof that cells of the postnatal mammalian brain were newly dividing neurons. The discovery of

new technologies that provided mechanisms for labeling and visualizing brain cells led scientists to eventually accept the concept of adult neurogenesis in the human brain.

Tritiated Thymidine

The first technological breakthrough came in the late 1950's when Sidman and colleagues developed a technique to label dividing cells. Triated thymidine ($[H^3]$ – thymidine) is a radioactive thymidine analog which incorporates into replicating DNA during the S phase of the cell cycle and is detectable by autoradiography (Sidman *et al.,* 1959). Sidman and colleagues injected pregnant mice with $[H^3]$ -thymidine and prepared autoradiograms of brain sections throughout embryonic development. Impressively, the data showed that the cells of the primitive ependymal layer in the wall of the cerebral vesicle behave synchronously and that the sites of DNA synthesis and mitosis are different (Sidman *et al*., 1959). Through this experiment Sidman and colleagues not only presented their discovery of $[H^3]$ – thymidine but they were able to validate its use as a means to identify dividing cells.

The development of new methods capable of detecting dividing cells enabled scientists to investigate postnatal cell generation in the brain. Development of $[H^3]$ -thymidine autoradiography was pivotal to the discovery of neurogenesis in postnatal mammals. Approximately ten years after Sidman and colleagues discovery, Smart was able to utilize the $[H^3]$-thymidine technique to identify the generation of new neurons in postnatal mice. He found that in three day old animals, cells labeled by $[H^3]$ -thymidine gave rise to neuroblasts and spongioblasts respectively, which migrated from the subependymal layer and became neurons and neuroglia cells (Smart *et al.,* 1961).

Shortly after Smart's experiments, Jennifer Altman published a series of papers which used [H^3] -thymidine to detect new neurons in various brain regions of juvenile rats. By conducting the study with juvenile rats Altman was able to make a smooth transition from pre and perinatal neurogenesis to postnatal neurogenesis. She injected [H^3] -thymidine into six and thirteen day old rats and monitored cellular proliferation and survival over a range of one hour to sixty days. Altman's data provided definitive evidence that cells were being generated in the postnatal mammalian brain and that these new cells migrated from germinal sites through defined migratory channels (Altman, 1966). The series of papers concluded that new neurons grew in the dentate gyrus of the hippocampus (Altman & Das, 1965), neocortex (Altman, 1966), and olfactory bulb (Altman, 1969). However, Altman's work on adult neurogenesis was ignored by the scientific community for almost a decade, serving as a classic case of a discovery made before society was ready to accept it.

Electron Microscopy

The issue of adult neurogenesis was revisited in the late 1970's at a time when scientists had the ability to visualize the morphology of the cells using electron microscopy. Electron microscopes use electrons to illuminate a specimen and create an enlarged image. They have much greater resolving power than light microscopes and can obtain higher magnifications. A study by Kaplan and Hinds in 1977 provided evidence that neurons born in adulthood survive for at least thirty days. Autoradiography of three month old animals allowed to survive thirty days after [H^3] -thymidine injection revealed labeled cells in the granular layers of hippocampal dentate gyrus and olfactory bulb.

These labeled cells were confirmed to be neurons by electron microscopy. This was some of the first direct evidence that newborn neurons in the hippocampus survived for at least up to thirty days, and this was considered a relatively long period of time (Kaplan & Hinds, 1977). In a follow up study, Kaplan and Bell (1983) injected nine-month-old rats with [H^3] -thymidine and allowed them to survive for twenty days. In light-microscopic autoradiographs, labeled cells were found in the granule cell layer of the hippocampus. Importantly, analysis of the labeled cells with electron micrographs clearly demonstrated their neuronal nature with synapses along their cell bodies and dendrite (Kaplan & Bell, 1983). Similarly, Stanfield and Trice (1988) were able to show that within the adult hippocampal formation newly generated dentate granule cells were capable of extending axonal projections for considerable distances (Stanfield & Trice, 1988). This suggested that these cells were capable of being integrated into existing circuitry. Electron microscopy served as a tool to confirm that the newly generated cells found in the adult brain were indeed neurons and to show that these cells became a part of the brain structure.

Avian Neurogenesis:

The initial research finding the existence of neurogenesis in the brains of adult birds suggested to some members of the scientific community that neurogenesis could occur in brains of adult humans as well. In 1985, Fernando Nottebohm began an analysis of the neural basis of song learning in birds and discovered that neurogenesis occurs in two specific regions of the avian brain (Nottebohm, 1985). However, despite evidence of neurogenesis in the telencephalon of the adult bird brain, known to be

homologous to primate cerebral cortex and primate hippocampus, these studies were thought to be irrelevant to the human brain by the vast majority of the scientific community. Instead, the evidence for avian neurogenesis was considered a specialization related to the necessity for flying creatures to have light cerebrums and the seasonal singing cycles of birds (Gross, 2000).

Today studies of adult neurogenesis in songbirds provide convincing evidence not only for the existence of adult neurogenesis but for an actual functional role of these new cells. The development of new cells in the brain of adult birds has been found to be a critical component to their seasonal song learning. Neurons are constantly added to the telencephalon region of the brain in the high vocal center. New neurons replace older ones that have died, and peaks in neuronal replacement are seasonal affecting some types of neuronal cells but not others. The highest amount of replacement occurs when the animals are doing the greatest amount of learning (Nottebohm, 2004). This has lead scientists to believe that adult neurogenesis plays a role in birds' ability to learn new songs. The fact that there seems to be a function for adult neurogenesis in birds has lead scientists to hypothesize that there may be a function for human neurogenesis as well.

Bromodeoxyuridine

The discovery that bromodeoxyuridine, another thymidine analogue, could be used to detect DNA synthesis once again revolutionized the field of neurogenesis. Bromodeoxyuridine (BrdU), like [H^3] –thymidine, labels DNA during the S phase of the cell cycle (Figure 1). However BrdU, unlike [H^3] -thymidine, allows adult born cells to be double labeled with neuronal markers. These cells can be easily and rapidly detected

by an immunofluorescent staining method and can also be quantified by flow cytometry (Gratzner, 1982).

In 1998, Eriksson and colleagues took advantage of this technology. Using postmortem human brain tissue from patients given systemic BrdU injections for medical evaluations of cancerous tumors, they demonstrated that new neurons are generated from dividing progenitor cells in the dentate gyrus of adult humans and that the human hippocampus retains its ability to generate neurons throughout life (Eriksson et al., 1998). This finding provided substantial evidence suggesting that neurogenesis could be found in the adult human brain.

Immunohistochemistry:

In the 1980's another important advance in the field of adult neurogenesis was discovered. Scientists began the use of cell type specific markers for the immunohistochemical identification of the newly generated cells. Among the markers for mature neurons are the following: neuron specific enolase (NSE), microtubule-associated protein 2 (MAP-2), class III beta-tubulin (Tuj1) and the nuclear antigen neuronal nuclei (NeuN) (Gross, 2000) (Figure 1). However, in certain conditions some of these markers can stain cells that are not neurons and others do not stain all neuronal types. Therefore, it is necessary to look for the expression of several of these antigens in a population of adult generated cells to be sure that the new neurons are identified correctly.

Reynolds and Weiss used immunohistochemistry to show that cells isolated from the striatum of the adult mouse brain could be induced to proliferate in vitro by epidermal growth factor. The proliferating cells initially expressed nestin, an intermediate filament

found in neuroepithelial stem cells, and subsequently developed the morphology and antigenic properties of neurons and astrocytes. The newly generated cells with neuronal morphology were immunoreactive for two neurotransmitters of the adult striatum in vivo (gamma-aminobutyric acid (GABA) and substance P). Thus, they concluded that the cells of the adult mouse striatum have the capacity to divide and differentiate into neurons and astrocytes (Reynolds & Weiss, 1992). In 1999, Kukekov and colleagues were the first to isolate proliferative stem/progenitor cells of the SVZ and the SGZ from surgical biopsy specimens of adult human brains. Using light and electron microscopy, immunocytochemistry for several neuronal, glial, and developmental markers, and the reverse transcriptase polymerase chain reaction to demonstrate different gene transcripts found in neurospheres, it was shown that the adult human brain has a complex population of stem/progenitor cells that can generate neuronal and glial progeny under particular *in vitro* growth conditions (Kukekov *et al.*, 1999). The ability to look for immunohistochemical neuronal markers on cells greatly helped to ensure that scientists were indeed seeing adult born neuronal cells as opposed to other types of cells in the brain.

Retroviral Based Lineage Tracing:

Retroviral based lineage tracing is another technology that has helped to solve the mysteries of neurogenesis (Figure 1). Oncoretroviruses, such as Muloney murine leukemia virus (M-MuLV), enter the nucleus when the nuclear envelope breaks down during the prophase-prometaphase transition at the onset of mitosis. Therefore, oncoretroviruses cannot infect cells that are not dividing cells. They are useful for

accessing and inserting a transgene into dividing cells such as new neurons in the brain. In comparison, lentiviruses infect both mitotic and quiescent cells (Gage *et al.*, 2008). As a result, these two viruses are used for different scientific applications.

One study constructed a defective recombinant retrovirus in which Escherichia coli beta-galactosidase (lac Z) was inserted into the genome of M-MulV. Expression of lacZ was detected with a histochemical stain that could be applied to both cultured cells and embryonic tissue. Infection of cultured cells showed that lacZ had no detectable deleterious effects on cell viability or growth, that the enzyme was stably expressed in the progeny of infected cells for many generations in the absence of selective pressure, and that virus-mediated lacZ expression occurred in a variety of cell types. The researchers then injected the virus into mid-gestation mouse embryos and found that clones of lacZ positive cells were detected in skin, skull, meninges, brain, visceral yolk sac, and amnion. In each tissue, evidence was obtained that several cell types have a pluripotential ancestor and that cell fate is progressively restricted as development proceeds in each type of tissue (Sanes *et al.*, 1986). Similar retroviral techniques employing other types of traceable markers have been used to study lineage relationships in the developing vertebrate nervous system, both in vivo and in culture (Price *et al.*, 1987). Retroviral systems have broad applicability as a means of gene transfer and expression in the nervous system and are very useful for visualizing and tracing cells during neurogenesis. They have been extremely useful as a means to trace the development of newly born neurons in the adult brain.

New Neurons Integrate Into Established Hippocampal Circuitry

Once it was finally established that neurogenesis did occur in the hippocampus of the adult brain, the question still remained whether these cells became integrated into existing circuitry. As mentioned, studies that suggested a functional role of adult avian neurogenesis provided some of the strongest evidence to suggest that adult neurogenesis was not just a vestigial process but had important functional relevance.

Song and colleagues presented a mini-symposium at the 2005 Society for Neuroscience Meeting which supported the idea that newly generated neurons become synapticaly integrated into the existing circuitry of the adult brain. They claimed that current data indicated adult neurogenesis not only increases the number of neurons but also provides a continuous source of new neurons that are qualitatively distinct functional units. They suggested that the adult hippocampal neurogenic pool constitutes a heterogeneous neuronal population that originates at three distinct developmental stages: late embryonic, early post natal and adult brain. Song and colleagues focused on the physiological properties of these three stages of newborn cells as evidence for their integration into existing circuitry (Song *et al.*, 2005).

In 2003, Carleton and colleagues performed a study that used a high dose of BrdU in addition to [H^3] -thymidine to investigate the number of newborn neurons in the SVZ brain region of young adult rats. They found that there were 9,400 dividing cells proliferating with a cell cycle time of twenty-five hours. This indicated that the brain was generating 9,000 new cells each day, or more than 250,000 per month in the SVZ alone. Within five to twelve days of BrdU injection a substantial amount of immature granule neurons, which made up fifty percent of all the BrdU labeled cells in the dentate gyrus,

were identified to also contain neuron specific antibodies. The scientists claim that this large number of adult generated granule cells supports the idea that these new neurons play an important role in hippocampal function (Carleton et al. 2003).

However, there is controversy surrounding this finding. The opposition points out that if the brain is producing 250,000 new cells per month in just one region, the SVZ, without even taking into account the new neurons generated in the SGZ there must be some explanation for what is happening to this large number of cells before they reach maturity. It is unclear how many of these cells are dying at an early age and how many are surviving and differentiating. The issue of how many new cells are produced and survive during adult neurogenesis is unresolved.

Van Praag and colleagues also looked at the functionality of the new neurons generated in the dentate gyrus of the adult mammalian hippocampus. They used a retroviral vector expressing green fluorescent protein that only labeled dividing cells and that could be visualized in live hippocampal slices. They found that newly generated cells in the adult mouse hippocampus have neuronal morphology. More importantly, these cells could display passive membrane properties, action potentials and functional synaptic inputs similar to those found in mature dentate granule cells. They concluded that the newly generated cells mature into functional neurons in the adult mammalian brain (Van Praag *et al.*, 2002).

Scientist Pasko Rakic is a current opponent of the idea that adult neurogenesis has a functional role. Rakic believes that the desire for curing neurological disorders has fostered a willingness to accept unsound evidence for the importance adult neurogenesis under normal and experimental conditions. He claims that some studies looking at adult

neurogenetic function do not satisfy even basic criterion for the identification of newly born adult neurons and that very few studies meet rigorous criteria. Rakic believes that the pitfalls to research in this area of science lie in the methods that are used. Therefore, he states that it is important to review the methods used for identification of new neurons in the adult brain. To assure scientifically sound advances in the field of neurogenesis, Rakic calls for the formation of standard and uniform criteria to be used by both authors and journal reviewers when identifying new cells and the neuronal phenotype (Rakic, 2002). While it does appear that there is a need for some standardization in adult neurogenic research, studies strongly indicate that adult neurogenesis does exist and may indeed have a functional role.

Over the last century, the progress in technology has led to a new understanding of adult neurogenesis (Table 1). The scientific world has come a long way from the central dogma that stated neurogenesis did not occur in postnatal mammals. While some scientists, like Rakic, hold on to the belief that adult neurogenesis does not have a functional role, the idea that these neurons influence the brain has been accepted by most. Today the central question in the field of adult neurogenesis is not whether or not it exists but instead whether there is a functional significance for this biological phenomenon in mammals.

CURRENT STATUS

Neurogenesis in the Hippocampal Formation

Neurogenic Niche

Neural stem cells (NSCs) have been found in different regions of the adult nervous system, however adult neurogenesis is consistently found only in the SVZ and SGZ in the dentate gyrus region of the hippocampal formation. This paper will focus solely on neurogenesis in the SGZ of the dentate gyrus of the hippocampus. The microenvironment of the SGZ has components that allow the differentiation and integration of new neurons (Zhao *et al*., 2008). This microenvironment is termed the hippocampal neurogenic niche.

In the neurogenic niche of the SGZ, adult hippocampal progenitors lie close to a layer of granule cells that also include: mature and immature neurons, astrocytes, oligodendrocytes, and other types of neuronal cells (Zhao *et al.*, 2008). Astrocytes are known to regulate synapse formation and synaptic transmission. It has been discovered that adult astrocytes from the hippocampus are capable of regulating adult neurogenesis by instructing stem cells to develop into neuronal cells (Song *et al.*, 2002). SVZ progenitors have a distinct neurogenic niche as well; they lie next to the ependymal cell layer of the lateral ventricles. This paper will focus on the current information regarding SGZ progenitors.

The neurogenic niche is highly vascularized and there is a large amount of research that focuses on determining exactly how stem cells and endothelial cells interact. Dividing neural progenitor cells in the SGZ have been found in dense clusters in close

proximity to vasculature structures. Research suggests that neurogenesis is closely associated with active vascular recruitment and remodeling. New neuronal cells and new endothelial cells seem to respond to similar signals. This angiogenic environment provides an area where mesenchyme derived cells and circulating factors meet and can influence plasticity in the adult central nervous system (Palmer *et al.*, 2000). For example, vascular endothelial growth factor (VEGF) is a growth factor that signals to endothelial cells involved in angiogenesis. Interestingly, VEGF has been found to regulate SGZ neurogenesis (Cao *et al.*, 2004). The presence of VEGF is required for increased neurogenesis in adult mice exposed to an enriched environment or given the opportunity of voluntary exercise; both situations which are known to enhance adult neurogenesis.

There is still much to learn about the critical components of the SGZ neurogenic niche. It is important to remember that factors outside of the immediate area will also play a role in the development of new neurons in the adult brain. These outlying cells can influence neural progenitors indirectly by moving neurotransmitters into the niche or directly by making synaptic contacts with cells in the niche (Zhao *et al.*, 2008). Therefore, there are many factors that could affect the neurogenetic niche in a way that influences the growth and differentiation fate of neural stem cells.

Types Neural Progenitors in the SGZ

There are two types of neural progenitors located in the SGZ of the dentate gyrus (Figure 2). These progenitors can be differentiated according to their specific morphologies and molecular markers. Type 1 hippocampal progenitors, also called

radial astrocytes, have a radial process that spans the granule cell layer (GLC) and sends out branches in the inner molecular layer (MOL). Type 2 hippocampal progenitors are morphologically distinct because of their short processes and are typically thought of as transit amplifying cells or intermediate progenitors. Nestin is an example of a typical molecular marker for SGZ precursor cells. There are two distinct subpopulations, type I and type II, of nestin-positive cells in the SGZ (Fukuda *et al.,* 2003). Glial fibrillary acidic protein (GFAP) is an astrocyte marker that is expressed in cells all over the brain. It is considered a hippocampal stem cell marker when used in conjunction with cell placement (in the SGZ), and morphology (radial process traversing the GCL). GFAP serves as molecular marker of progenitor cells for Type I adult neurogenesis while Type 2 cells lack GFAP expression (Garcia *et al.,* 2004). Sry-related HMG box transcription factor, Sox-2, is a general stem cell molecular marker which can be applied to the hippocampus to identify SGZ neural stem cells. Sox-2 is a transcription factor that is essential to maintain self-renewal of undifferentiated embryonic stem cells. It has been found to be present on two morphologically distinct populations that differ in proliferation levels in the SGZ. $Sox2^{+}$ cells in the SGZ give rise to both neurons and astrocytes indicating that these cells are multipotent. A subpopulation of $Sox2^{+}$ cells give rise to cells that retain $Sox2^{+}$, indicating that they are regenerative. Therefore, $Sox2^{+}$ neuronal stem cells in the hippocampal region help maintain the constant size of the neuronal stem cell pool in addition to producing newly born neurons during adult neurogenesis (Suh *et al.,* 2007). It is very likely that there are other markers for the two types of SGZ neural progenitors that have not yet been discovered.

Migration of Newborn Cells

Studying the cell cycle progression and the molecular markers of neurogenic progenitors helps to gain an understanding of the mechanisms of neurogenetic regulation. Scientists have found that factors such as neurotransmitters, growth factors and extrinsic signals, and intracellular mechanisms all play critical roles in the regulation of the growth and migration of new cells.

Newborn neurons that exist as a result of adult neurogenesis are integrated into the existing neuronal circuitry. In the SGZ, the survival and integration of newborn neurons is determined during a critical time window of 1 to 3 weeks (Figure 2). During this time the new neurons are immature and display unique physiological properties (Zhao *et al.*, 2008). This distinct regulation of the different stages of developing neurons has led to the hypothesis that different stages of adult neurogenesis may contribute to different hippocampal dependent functions.

Functions of the Hippocampus in Learning and Memory

The hippocampus is a part of the forebrain located in the medial temporal lobe. It belongs to the limbic system and plays major roles in short term memory and spatial navigation. The hippocampus' role in memory has been most lucidly portrayed by patient H.M. H.M. suffered from epileptic seizures and as a therapeutic result had his medial temporal lobes removed bilaterally. The results were unexpected in that H.M. began to suffer from severe amnesia. He was unable to consciously remember events that occurred both after his surgery and for several years immediately before (Scoville & Milner, 1957). In the years since this case, numerous other patients with similar levels

of hippocampal lesions have commonly suffered from some degree of amnesia. There is now universal agreement that the hippocampus plays an important role in memory; however, the precise nature of this role remains widely debated. Another important function of the hippocampus is its role in spatial coding. In 1971, O'Keefe and his student Dostrovsky were the first to discover that neurons in the rat hippocampus appeared to show activity that encoded the rat's location within its environment (O'Keefe & Dratrovsky, 1971). Today along with the memory theory, there is universal agreement that hippocampal function somehow plays an important role in spatial coding, but once again the details are widely debated.

Hippocampal lesions commonly lead to amnesic effects in patients but they can also result in behavioral disinhibition and reduced anxiety. Research using rodents as subjects suggests that these diverse behavioral effects are associated with different hippocampal sub regions. Selective lesion studies show that the hippocampus is functionally subdivided along the septotemporal axis into dorsal and ventral regions and that each of these regions is associated with a distinct set of behaviors. The dorsal hippocampus has a role in certain forms of learning and memory, notably spatial learning. Spatial learning requires that the animal learns how to find their way around their world. The ventral hippocampus has a role in brain processes associated with anxiety related behaviors. The ventral hippocampus also has a role in emotional processing (Bannerman *et al.*, 2004). It is thought that the human brain is divided into similar sub regions with similar specificity of function.

SGZ adult neurogenesis is known to occur in the dentate gyrus (DG) of the hippocampus which is not equally involved in all types of hippocampus dependent tasks

(Morris, 2006). The DG has been implicated to contribute to the formation of new memories. The birth of these new neurons generates a substantial population of young neurons. Adult generated neurons play a significant role in synaptic plasticity in the DG. Since the DG is the major source of the afferent inputs into the hippocampus, the production and the plasticity of new neurons may have an important role in some hippocampal functions (Synder *et al.,* 2001). The fact that SGZ neurogenesis is occurring in the dentate gyrus of the hippocampal formation has led scientists to hypothesize that SGZ neurogenesis and hippocampal functions interact..

Two Way Relationship Between Neurogenesis and Hippocampal Dependent Learning

Some scientists believe that there is an interrelationship between neurogenesis and hippocampal dependent cognition, however the exact causal mechanisms are not yet known. Research suggests that the rate of neurogenesis determines hippocampal dependent learning performance and memory retention (Gage *et al.*, 2008). In addition, hippocampal dependent learning tasks appear to enhance neurogenesis by enhancing the survival of adult born neurons at a particular stage in their development (Gage *et al.,* 2008). What is not known is how the rate of neurogenesis affects which types of hippocampal dependent learning and memory, nor is it known how and which hippocampal dependent learning tasks enhance the survival of different stages of new neuronal growth. Science is at a stage where it is constantly collecting evidence on the function of SGZ neurogenesis in hopes to put together a broad correlative map.

Evidence of Correlations Between SGZ Neurogenesis and Hippocampal Dependent Cognition

Correlative evidence suggests that the conditions that enhance neurogenesis also improve hippocampal dependent learning. Similarly the conditions that decrease neurogenesis also impair hippocampal dependent learning. Conditions that will be discussed in this paper include: genetics, environment, and age. There is also research to suggest quantitative correlations between neurogenesis and hippocampal dependent learning performances.

Genetic Conditions

The genetic background of an animal has been shown to affect both hippocampal neurogenesis and hippocampal dependent cognitive performance. Cell proliferation, differentiation, and survival during adult hippocampal neurogenesis are influenced by the genetic background of mice. Kempermann and Gage investigated whether the baseline level of adult neurogenesis is predictive of performance on the water maze task, which is a spatial learning task that is also a test of hippocampal function. They used a strain of recombinant inbred mice which were good learners and had high baseline levels of neurogenesis, and a strain of recombinant inbred mice which were poor learners and had low levels of neurogenesis. All strains showed a significant correlation between the number of new neurons generated in the dentate gyrus and the slope of the learning curves for the water maze task. There was no correlation between neurogenesis and tasks that did not require hippocampal functioning (Kempermann & Gage, 2002).

Therefore, genes that support a high level of neurogenesis appear to aid mice in specific aspects of hippocampal function, particularly the acquisition of new information.

Environmental Conditions

In addition to genetic determinants, environmental factors play a role in hippocampal neurogenesis and correspond with changes in hippocampal dependent cognitive performance. Environmental enrichment and voluntary exercise have been shown to increase adult hippocampal neurogenesis and improve spatial learning ability. Evidence suggests environment enrichment and voluntary exercise act through different pathways to affect different phases of the neurogenesis process in distinct ways. It has been suggested that environmental enrichment increases the likelihood of the survival of new cells and voluntary exercise increases the level of proliferation of progenitor cells (Olsen *at al.,* 2006). Therefore, environmental factors and voluntary exercise act as distinct interventions, and in combination with genetic background, affect hippocampal plasticity and associated behaviors.

Exposure of animals to an enriched environment leads to improvement of performance in several hippocampal depedent learning tasks in addition to enhancing neurogenesis specifically in the hippocampus. One study found that environmental enrichment led to improved long term recognition memory and increased hippocampal neurogenesis. Exposing the animals to antimitotic agent methylazoxymethanol acetate (an antimitotic drug) during environmental enrichment, completely prevented both the increase in neurogenesis and enrichment induced long term memory improvement (Bruel-Jungerman *et al.,* 2005). These results establish that newborn cells in the dentate

gyrus contribute to the behavioral effects of environmental enrichment, and provide further support for the hypothesis that adult generated neurons participate in modulating memory function.

Previous exposure to an enriched environment has been found to increase the total number of new neurons and the number of new neurons responding to reexposure to the same environment. The increase in the density of activated new neurons occurred specifically in response to exposure to the same environment but not to a different experience. These experience specific modifications are affected exclusively by previous exposure around the second week after neuronal birth but not later than three weeks. Thus, the animal's experience within a critical period during an immature stage of new neurons determines the survival and population response of the new neurons and may affect later neural representation of the experience in the dentate gyrus (Tashiro *et al.*, 2007). This experience specific functional modification through different stages of adult neurogenesis could be a mechanism by which new neurons exert a long term influence on the functions of the hippocampal dependent learning and memory.

Another study performed by Kee and colleagues used immunohistochemical approaches to visualize the recruitment of new neurons into dentate gyrus circuits during the formation of water maze memory in mice. They found that new neurons in the SGZ make a unique contribution to memory processing in the dentate gyrus. As new granule cells in the SGZ mature, they are increasingly likely to be incorporated into the circuits supporting spatial memory in the dentate gyrus. However, their study found a different critical period during which new neurons participate in cognitive function. Kee found that by the time the cells are four or more weeks of age, they are more likely than

existing granule cells to be recruited into circuits supporting spatial memory (Kee *et al.*, 2007).

This conflicting data could be the result of a number of factors that are not common between the two experiments such as the subject species, the number of subjects, and the type of learning being tested. The likely explanation is that different stages of neuronal growth participate in different phases of hippocampal dependent cognition. There is a substantial amount of research to support the claim that environmental conditions play a role in adult neurogenic growth which, in turn, can mediate hippocampal related tasks.

Age Conditions

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Aging is an important co-variable for many regulatory mechanisms affecting adult neurogenesis. The hippocampus is one of the brain regions that is prominently affected by neurodegeneration and functional decline as a normal process of aging (Klempin & Kempermann, 2007). The process of neurogenesis declines with age and as a result the decline in neurogenesis that occurs throughout the lifespan of an animal can be related to a decreasing proliferation of granule cell precursors (Kuhn *et al.*, 1996). One explanation for this phenomenon is that the decline in neurogenesis is partially caused by the senescence of the neural progenitors with increasing age *(*Molofsky *et al.*, 2006). Voluntary exercise can fix some of the deleterious morphological and behavioral consequences of aging with respect to adult SGZ neurogenesis (Van Praag *et al.*, 2005).

Stress has been shown to inhibit cell proliferation and neurogenesis in the hippocampus. This effect is constant across mammalian species, life stages, and most

types of stressors (Mirescu & Gould, 2006). Stress also seems to play a role in neurogenesis and subsequent performance on hippocampal related spatial working tasks. Prenatal stress in rats has been found to induce a lifespan reduction of neurogenesis in the dentate gyrus and to cause impairment on spatial tasks. Prenatal stress has also been found to blocks increases of learning induced neurogenesis. This data supports the hypothesis that there is an early neurodevelopmental origin for behavioral vulnerabilities in aging (Lemaire *et al.,* 2000). Both age and stress play a role in manipulating the rate of neurogenic growth during different developmental stages and these changes appear to subsequently affect hippocampal dependent learning and memory function.

Quantitative Correlations

Age related data shows a quantitative correlation between learning abilities and neurogenesis. Drapeau and colleagues found that a quantitative relationship exists between learning and the number of newly generated adult neurons (Drapeau *et al.*, 2003). When tested with a hippocampal dependent learning task animals with preserved spatial memory, aged-unimpaired rats, exhibited a higher level of cell proliferation and a higher number of new neurons in comparison with rats with spatial memory impairments, aged-impaired rats. Therefore, the extent of memory dysfunction in aged rats seemed to be quantitatively related to the level of hippocampal neurogenesis. These data reinforce the assumption that neurogenesis is involved in memory processes and suggests that neurogenesis is also involved in aged related cognitive alterations.

It is known that the hippocampus is responsible for select types of learning and memory formation. What is not yet known is whether or not adult neurogenesis is

involved in hippocampal dependent learning and memory. Research provides evidence of correlations between SGZ neurogenesis and hippocampal dependent cognition. The same factors that contribute to changes in neurogenesis seem to cause changes in hippocampal dependent learning and memory as well. However, these correlations do not prove that adult neurogenesis is the actual cause of any of the functional changes seen. It is also possible that another aspect of the hippocampus is responsible for the modulations seen learning and memory performance. Modifications in synaptic and structural plasticity at the level of dendrites and spines, and changes in vascularization and metabolic adaptation occurring within and outside the hippocampal formation could change learning and memory behaviors independently of neurogenesis (Gage *et al.*, 2008). Therefore, it is difficult to determine whether hippocampal neurogenesis is the major causal factor for the changes seen in hippocampal function. Current research focuses on the complicated relationship between adult neurogenesis in the SGZ and hippocampal dependent functioning.

Evidence for Neurogenic Involvement in Learning and Memory

The Effect of Learning on SGZ Neurogenesis:

Hippocampal function is thought to play a role in the regulation of neurogenesis in the SGZ. This suggests that hippocampal dependent learning could induce the activation of adult newborn neurons and enhance their survival and incorporation into functional circuits. New cells produced during adult neurogenesis are thought to integrate into the functional circuitry and influence both normal and pathological

behaviors (Zhao *et al.*, 2008). Research suggests that various factors affect adult neurogenetic growth including: the type of hippocampal dependent tasks, the act of learning, the stage of neuronal growth, and the phase of learning.

Hippocampal Dependent Tasks Affect Neurogenesis:

SGZ neurogenesis has been found to be enhanced exclusively by learning tasks that depend on the hippocampus. The number of surviving seven day old neurons in the rat SGZ has been found to be increased by hippocampus dependent tasks such as trace eye blink conditioning and the Morris water maze (Zhao *et al.*, 2008). However, it is not increased by hippocampus independent tasks, such as delay eye blink conditioning and active shock avoidance (Zhao *et al.*, 2008). In addition, the survival of newborn neurons is increased by a long delay conditioning task which is also hippocampal dependent. Conversely, pretraining in delayed conditioning, which makes the subsequent trace conditioning independent of the hippocampus, causes trace conditioning to be ineffective at promoting the survival of newborn neurons (Zhao *et al.*, 2006). This suggests that hippocampal dependent tasks specifically affect neurogenesis.

Act of Learning Affects Neurogenesis:

Other research confirms that it is the act of learning and not the act of training that causes the survival of new cells in the adult brain. In fact, the time course in which learning occurs affects adult neurogenesis. The spacing effect is a condition in which information that is presented spaced over time is better remembered than the same amount of information presented together. Sisti and colleagues explored the spacing

effect with respect to its effect on learning and neurogenesis in the adult dentate gyrus of the hippocampal formation. Since cells are generated over time and learning enhances their survival, they hypothesized that training with spaced trials would rescue more new neurons from death than the same number of trials administered close together in time. Their results indicate that learning, and not mere exposure to training, enhances the survival of cells that are generated one week before training. They also indicate that learning over an extended period of time induces a more persistent memory, which then relates to the number of cells that remain in the hippocampus (Sisti *et al.*, 2007). This research contributes to the hypothesis that hippocampal dependent learning affects neurogenesis.

Hippocampal Dependent Learning Affects Different Stages of Neuronal Growth

Hippocampal dependent learning causes different effects on neural precursors at different developmental stages. Spatial learning has been found to modify neurogenesis by inducing a cascade of events that is similar to the selective stabilization process which occurs during development. Learning promotes survival of relatively mature neurons, apoptosis of more immature cells, and proliferation of neural precursors. These three interrelated events, in return, mediate hippocampal dependent learning. Blocking neuronal apoptosis impairs memory formation and inhibits hippocampal dependent learning induced cell survival and cell proliferation. During learning, like during development, neuronal networks are created by a regulated selection and suppression of different populations of newly born neurons (Dupret *et al.*, 2007). Similarly, another study confirmed an influence of spatial learning on the survival of newly born cells

depending on the neurons birth date. Learning increased the survival of cells generated before learning and decreased survival of cells produced during the early phase of learning (Drapeau *et al.,* 2007). These results highlight the importance of hippocampal dependent learning induced changes on different stages of adult born cell survival.

Phases of Learning Affects Neurogenesis

Learning occurs in different phases and each distinct phase has varying modulatory effects on neurogenesis. Water maze learning, a hippocampal dependent spatial task, is divided into two phases; an early phase during which performance improves rapidly, and a late phase during which learning increases until a certain level of performance is reached. The late phase of learning has a different effect on neurogenesis depending on the birth date of the new neurons. The number of newly born cells increases with the late phase and many survive for at least four weeks and differentiate into neurons. In contrast, late phase learning decreases the number of newly born cells produced during the early phase. This decline in neurogenesis is positively correlated with performance in the water maze. Therefore, rats with a higher number of new cells born in the early phase were less able to acquire and use spatial information than those rats with lower numbers of new cells born in the early phase (Dobrossy *et al.*, 2003). Therefore, different phases of learning have different effects on hippocampal neurogenesis, which in turn, influences hippocampal dependent tasks.

Research suggests that the regulation of SGZ neurogenesis by hippocampus dependent learning is complicated and can be affected by factors such as the age of newborn neurons, the stage of learning, and the type of learning (Zhao *et al.*, 2008).

Interestingly, these learning induced changes in neurogenesis have been proposed to be involved in subsequent hippocampal dependent memory and spatial learning.

Evidence for Effect of SGZ Neurogenesis on Learning and Memory

The functional impact of neurogenesis on animal physiology and behavior is unclear. To investigate the function of adult neurogenesis in hippocampal dependent learning and memory, several experimental methods have been developed to decrease or ablate SGZ neurogenesis in adult animals. These methods include low dose irradiation, (Snyder *et al.* 2005), systemic treatment with antimitotic drugs, such as methylazoxymethanol acetate (MAM) (Shors *et al.,* 2001), the natural process of aging, and mice which have been genetically engineered to eliminate neural progenitors (Saxe *et al.*, 2006). These tools are useful to demonstrate correlations between SGZ neurogenesis and hippocampal dependent cognition. Current research on the function of SGZ neurogenesis reaches conflicting conclusions suggesting that either: 1) all hippocampal dependent tasks are influenced by neurogenesis, 2) some hippocampal dependent tasks are influenced by neurogenesis or 3) no hippocampal dependent tasks are influenced by neurogenesis. Explanations for the discrepancies in data will be discussed.

Neurogenesis Affects Hippocampal Dependent Tasks

Shors and colleagues were among the first to study the function of neurogenesis in learning. They found that a significant reduction in the number of newly generated neurons in the adult rat impairs hippocampal dependent trace conditioning. A similar

reduction does not affect learning when the same stimuli are not separated in time, a task that is hippocampal independent. The reduction in neurogenesis does not induce death of mature hippocampal neurons or permanently alter their neurophysiological properties. Recovery of cell production allows for the ability to acquire trace memories (Shors *et al.*, 2001). This suggests that SGZ neurogenesis is important for hippocampal dependent learning. Another report ablated the formation of new neurons in the dentate gyrus of adult rats and tested the animals in two tests of short term memory, (that differ with respect to their dependence on hippocampal function), at different time points after the procedure. Eight and twenty-one days after irradiation, the animals with blocked neurogenesis performed more poorly than controls in a hippocampus dependent place recognition task. The animals were never impaired in a hippocampus independent object recognition task (Madsen *et al.*, 2003). This research confirms the theory that the presence of newly generated adult neurons is necessary for the normal function of the hippocampus.

Neurogenesis Affects Some Hippocampal Dependent Tasks

The majority of research indicates that adult born neurons in the SGZ make a distinct contribution to some but not all hippocampal dependent functions. One study subjected two month old mice to localized X irradiation of the hippocampus/cortex and tested their behavior on hippocampal related tasks three months later. The study found significant cognitive impairments after a modest dose of radiation and demonstrated that the Barnes maze, (more so than the Morris water maze), is particularly sensitive for the detection of radiation induced cognitive deficits in young adult mice. This study

demonstrated that cognitive impairments in spatial learning on some, but not all, hippocampal dependents tasks were associated with a significant loss of proliferating SGZ cells (Raber *et al.*, 2004). Similarly, another study found that three months after irradiation changes in neurogenesis were associated with spatial memory retention deficits. However, this study found that the Morris water maze, (more so than the Barnes maze), is particularly sensitive for the detection of radiation induced cognitive deficits in young adult mice. Behavioral training and testing increased the numbers of immature neurons, most prominently in irradiated animals. The researchers propose that the irradiation of young animals induces a long term impairment of SGZ neurogenesis that is associated with some hippocampal dependent memory deficits but not all (Rola *et al.*, 2004). Both studies came to the same conclusion but had opposing data on which hippocampal dependent task was affected by the depletion in adult neurogenesis.

Early work by Shors and colleagues demonstrated that certain forms of associative learning can enhance the survival of new neurons and that a reduction in neurogenesis coincides with impaired learning of one hippocampal dependent task (trace eye blink conditioning). In follow up research, Shors and colleagues decided to study the function of these new neurons in more depth. Using MAM on proliferating cells, they tested whether reduction of neurogenesis affected learning and performance associated with several different hippocampal dependent tasks: spatial navigation learning in a Morris water maze, and fear response to context and an explicit cue after training with a trace fear paradigm. They also examined exploratory behavior in an elevated plus maze. Rats were injected with MAM for fourteen days, concurrent with BrdU, to label new neurons on days ten, twelve, and fourteen. After treatment, groups of

rats were tested in the various tasks. They found that a significant reduction in new neurons in the adult hippocampus was associated with impaired performance in some hippocampal dependent tasks, but not with others. Interestingly, treatment with MAM reduced the amount of fear acquired after exposure to a trace fear conditioning paradigm but did not affect contextual fear conditioning or spatial navigation learning in the Morris water maze. Nor did MAM treatment affect exploration in the elevated plus maze (Shors *et al.*, 2002). These results suggest that neurogenesis may be associated with the formation of some but not all types of hippocampal dependent memories.

Similarly, Saxe and colleagues used two independent methods, Focal X irradiation of the hippocampus and genetic ablation of GFAP positive neural progenitor cells, to ablate hippocampal neurogenesis and found that each procedure caused a limited behavioral deficit and a loss of synaptic plasticity within the dentate gyrus. Both procedures impaired contextual fear conditioning but not cued conditioning. Hippocampal dependent spatial learning tasks such as the Morris water maze and Y maze were unaffected. Their findings suggest that adult born neurons make a distinct contribution to some but not all hippocampal functions. In a parallel set of experiments, it was shown that new hippocampal neurons can be preferentially recruited over mature granule cells *in vitro*, suggesting a mechanism for how this small cell population can influence behavior (Saxe *et al.*, 2006).

In their own study, Winocur and colleagues found hippocampal irradiated rats were impaired on the hippocampal dependent Non Matching To Sample (NMTS) task when the intervals between sample and test trials were relatively long. These rats also performed poorly when attempting to associate shock induced fear with contextual cues

in the hippocampal dependent fear conditioning task. Irradiated rats were not impaired in learning the basic NMTS rule or in performing that task when the intervals between the sample and test trials were short. Nor were there group differences in conditioning the fear response to the control stimulus in the fear conditioning task. The results suggest that new hippocampal cells generated in adulthood participate in a broad range of hippocampal functions but not all (Winocur *et al.*, 2006).

The research of Snyder and colleagues suggest a new functional role for adult born neurons. They found that new neurons aged four to twenty-eight days old at the time of training are required for long term memory formation in a spatial version of the water maze. Interestingly, irradiation just before or after water maze training had no effect on learning or long term memory. Relationships between learning and new neuron survival, as well as proliferation, were investigated but were found not to be significant. These results actually suggest a new role for adult neurogenesis in the formation and consolidation of long term, hippocampus dependent, spatial memories but not for learning (Synder *et al.*, 2005).

The majority of evidence suggests that the type of hippocampal dependent tasks determines whether or not it will be affected by neurogenesis. However, individual studies reach contradictory conclusions as to which types of hippocampal dependent learning tasks are influenced and which are not. These discrepancies will be discussed below.

Neurogenesis Does Not Affect Any Hippocampal Dependent Tasks

In contrast, there is a body of research that suggests SGZ neurogenesis is not involved in modulating hippocampal dependent tasks at all. One study found that a reduction in neurogenesis is not associated with a general decrement in learning or a decrement in hippocampus dependent learning specifically. This study examined hippocampal dependent and independent working memory using different radial maze tasks. Surprisingly, ablating neurogenesis actually caused an improvement of hippocampal dependent working memory when repetitive information was presented in a single day. These findings suggest that adult born cells in the dentate gyrus have different, and sometimes opposite, roles in distinct types of hippocampal dependent memory (Saxe *et al.*, 2007).

Similarly, another study ablated adult hippocampal neurogenesis and analyzed the effect of enrichment on spatial learning and anxiety-like behavior. They found that environmental enrichment alters behavior in mice regardless of their hippocampal neurogenic capability. This suggests that the environment is the influencing factor and that adult newborn cells do not mediate the effect of environmental enrichment and are not involved in influencing behavior (Meshi *et al.*, 2006).

The exact function of adult neurogenesis in hippocampal dependent functioning is a very controversial topic. Currently it is difficult to reach definitive conclusions due to the seemingly inconsistent research results.

Explanation for Discrepancies

Functional studies suggest potential roles for adult SGZ neurogenesis in hippocampal dependent learning and memory. While scientific conclusions of adult neurogenic research may be seemingly contradictory this may not be the case. If you look at the data alone it is clear that any effect that learning has on neurogenesis depends on the age of the newborn neurons, the age of the animal, and the type of learning that is occurring. It is probable that these differences are the result of distinct electrophysiological properties of the cells at different time points in development (Zhao *et al*, 2000). In order to have a better understanding of the mechanisms by which learning affects adult neurogenesis, scientists will have to break these factors apart and test each one independently.

The methodologies used in research on adult neurogenesis indicate that the contrasting conclusions reached may be caused by differences in the protocols. Factors such as: the number of subjects, subject species, age of subjects, gender of subjects, means of neurogenic ablation, learning tasks used, type of learning being tested, duration of treatments before the testing procedures, age of adult born neurons, time to testing and various other aspects that vary from experiment to experiment will likely lead to different experimental conclusions. It seems as if Rakic, who calls for a unification of procedures among both scientists and reviewers, is correct in identifying the fact that you cannot compare the results of studies that are so different in their controls. In order to gain some understanding of the functional significance of adult neurogenesis it is necessary to start with a standard system of methods throughout each experiment so that the results can be effectively compared.

In addition, all studies that use knockdown methods of the hippocampus are affecting the whole hippocampus which will cause side effects in the animals. These side effects could affect behavior and influence the results of the study. While it is true that ablation studies are extremely useful for indentifying the functional role of newly born adult neurons it is possible that, because the brain is a highly plastic region, other pathways takeover as a result of the neurological loss. Therefore scientists may not be getting an accurate picture of the full function of new neuronal cells in adults.

Similarly, new neurons in the SGZ make up only a small part of the anatomical structure of the hippocampus. Most ablation studies knockout out all neurogenesis in the hippocampus. Therefore, it is difficult to identify impaired behavior caused by a lack of SGZ neurogenesis alone. In addition, it is evident that different stages of neuronal growth affect hippocampal functioning differently, therefore ablating neurogenesis completely does not help decipher the functions of the developing and mature neurons.

The majority of research on the functional role of adult neurogenesis indicates that subject performance on different hippocampal dependent tasks is affected differently by the stage of the developing neuronal cells and by the age of the subjects. Therefore it is necessary to control for the type of learning task, the stage of the newly born neuronal cells, and the age of the subjects, in order to determine a causal relationship between adult neurogenesis and hippocampal dependent learning and memory.

To definitively demonstrate the influence of SGZ neurogenesis on hippocampal function, extremely selective knockdown approaches and very specific behavioral tests need to be developed for future research (Zhao *et al.*, 2008). There also needs to be a

standardization of the protocols so that the results of future studies can be effectively and appropriately compared.

CONCLUSION

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Future Directions

Further research is needed in order to make concrete conclusions about the function of SVG neurogenesis for modulating hippocampal dependent tasks. Computational models of hippocampal functioning are often used to help unveil the role of adult hippocampal neurogenesis. Current computation models suggest several potential functions of hippocampal neurogenesis and provide theoretical guidance for future experimental work (Becker, 2005, Wiscott *et al*., 2006). Interactions between these theoretical models and scientific research should help to advance scientific knowledge on adult hippocampal neurogenesis.

Future experiments should continue to study both correlative and causative relationships in order to get a unified picture of how neurogenesis participates in learning and memory. Behavioral tasks should also be accessed since neurogenesis is likely to have different roles in different behavioral circumstances (Gage *et al.*, 2008).

Scientists also need to continue to study the significance of the different ages of new cells in order to determine the specific learning functions at different developmental stages. The development of new transgenic models that ablate adult born neurons with emphasis on targeting different developmental stages of neurons will be the key to success in this area. It is also important to determine which of the plastic properties of new neurons are relevant to learning. Finally, if scientists develop a way to induce and inactivate specific neurons individually they will be able to manipulate their activity and observe their function. Given how far technology has carried the field of adult

neurogenesis research, there is good reason to believe that it will lead us the rest of the way and help us determine the exact function of adult neurogenesis in the human brain.

Final Thoughts

The discovery and acceptance of adult neurogenesis in the mammalian brain has been a long and controversial process. It took new and increasingly sensitive technologies to uncover and prove that neurogenesis was occurring in the adult mammalian brain. Scientists now have information on the various types of neural progenitors and the factors that affect their growth. Today it is believed that increases in adult neurogenesis are paralleled by improvements on hippocampal dependent tasks. At the same time, learning seems to promote the rate of production and number of surviving newly generated neurons in the adult hippocampus. Conversely, there is some research that suggests inhibition of adult neurogenesis in the hippocampus plays no significant role in hippocampal dependent learning. These discrepancies can be explained by differences in protocols as it is not scientifically valid to compare two studies that lack similar controls. What is certain is that the age of the new neurons plays an important role in the type of learning and memory that is affected. These differences need to be explored further. As in the past, it is going to take increasingly sensitive technologies to discriminate the different functions of the different stages of adult neuronal growth. However, once information is known scientists will have unveiled the significance of adult neurogenesis and will be able to determine its role in neurological lesions and diseases. History shows that the advent of new technologies is limitless and, as a result,

so is our ability to overcome the current obstacles that face research on adult neurogenesis.

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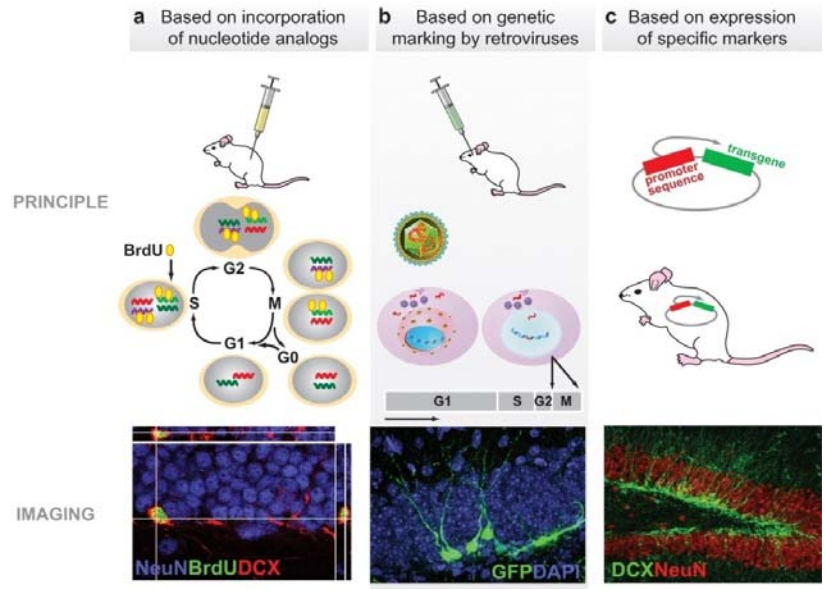
Zhao, C., Deng, W., and Gage, F. 22 February 2008. Mechanisms and Functional Implications of Adult Neurogenesis. *Cell.* 132 (4): 645-660

Figure 1
Diagram of Three Techniques Used to Study Adult Neurogenesis

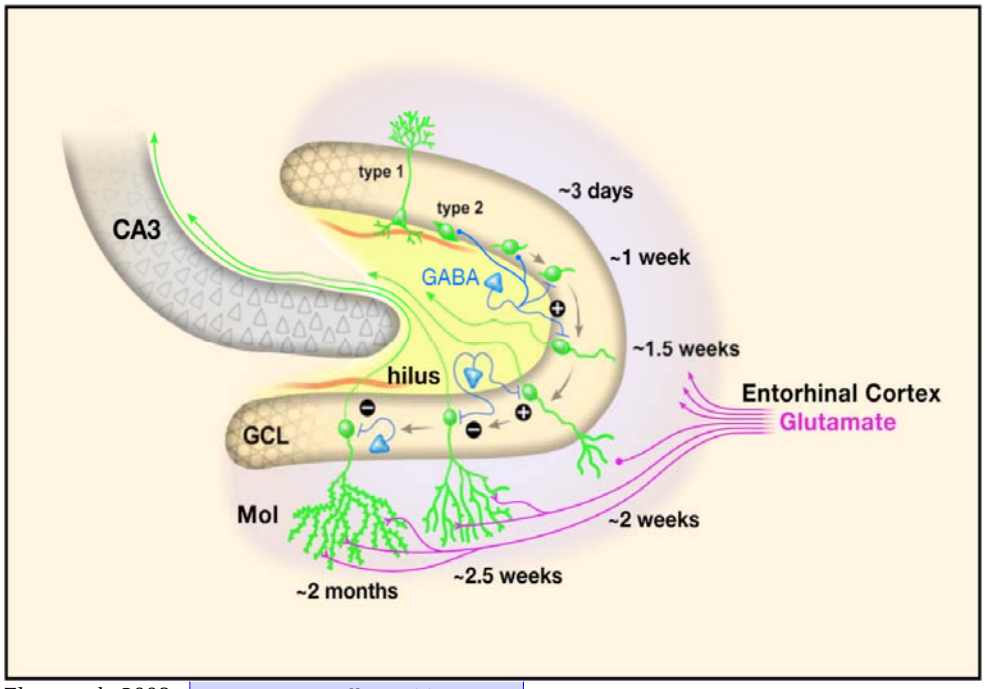


Birth dating	good	good	poor
Tracing	permanent	permanent	transient / permanent
Cell Population	whole population	limited cells	whole population
Visualization	fixation / processing	direct	processing / direct
Morphology	nuclear	whole cell	cellular / whole cell
Concerns	DNA repair	invasive	specificity

Ming, G and Song, H. 2005
Annu. Rev. Neurosci. 28: 223–50

Figure 1: Diagram of three different techniques used for analysis of adult neurogenesis in vivo. (A) Analysis using a nucleotide analog. Bromodeoxyuridine (BrdU), like [H^3] –thymidine, labels DNA during the S phase of the cell cycle. The picture shows co localization of BrdU (green) and cell-type-specific markers (NeuN (blue) and DCX (red)). (B) Analysis using retroviral genetic marking. Retroviruses will integrate their DNA into that of its host. Retroviral systems have broad applicability as a means of gene transfer and expression in the nervous system and are very useful for visualizing and tracing cells during neurogenesis. The picture shows the expression of green fluorescent protein (GFP) in newborn cells after injection with a retrovirus containing the GFP gene. (C) Analysis using cell-type specific markers for the immunohistochemical identification of the newly generated cells. This analysis was performed by making a transgenic mouse in which the marker is placed behind a promoter specific for the cells in the dentate gyrus of the hippocampal formation. The picture shows the expression of markers for immature neurons (DCX (green)) and mature neurons (NeuN (red)) in the adult mouse dentate gyrus of the hippocampus. Each of these technologies allowed for advancements in the field of adult neurogenic research. (Ming & Song, 2005)

Figure 2
Two Types of SGZ Progenitors in the Dentate Gyrus of the Hippocampal Formation



Zhao *et al.*, 2008. Neurogenesis. *Cell.* 132 (4): 645-660

Comment [CSL3]: Cite this figure just the way you cite the previous one.

Figure 2: There are two types of neural progenitors located in the SGZ of the dentate gyrus; Type 1 and Type 2. These progenitors can be differentiated according to their specific morphologies and molecular markers. Type 1 hippocampal progenitors, also called radial astrocytes, have a radial process that spans the granule cell layer (GLC) and sends out branches in the inner molecular layer (MOL). Type 2 hippocampal progenitors are morphologically distinct because of their short processes and are typically thought of as transit amplifying cells or intermediate progenitors. In the neurogenic niche of the SGZ adult hippocampal progenitors lie close to a layer of granule cells that includes mature and immature neurons, astrocytes, oligodendrocytes, and other types of neuronal cells (not shown). They have also been found in dense clusters in close proximity to vasculature structures (red). Research suggests that neurogenesis is closely associated with active vascular recruitment and remodeling.

Newborn neurons that exist as a result of adult neurogenesis are functionally integrated into the existing neuronal circuitry. In the SGZ, the survival and integration of newborn neurons is determined during a critical time window of 1 to 3 weeks. During this time the new neurons are immature and display unique physiological properties. Factors such as neurotransmitters (GABA- blue), growth factors and extrinsic signals, and intracellular mechanisms all play critical roles in the regulation of the growth of new cells.

Table 1: Technological Advancements that Helped Bring About the Discovery of Adult Neurogenesis

Technological Advancement	**Year First Used in Adult Neurogenic Research**	**Explanation of Technological Advancement**	**How Advancement Helped Adult Neurogenic Research**
Tritiated Thymidine [H^3] –thymidine	1959	Radioactive thymidine analog which incorporates into replicating DNA during the S phase of the cell cycle and is detectable by autoradiography	Allowed scientists the ability to label dividing cells in the brain
Electron Microscopy	1970's	Uses electrons to illuminate specimen and create enlarged image. Have greater resolving power than light microscopes and can obtain much higher magnification	Allowed scientists to view labeled cells and confirm their identity as neurons
Avian Neurogenesis	1985	Discovery that neurogenesis occurs in two specific regions of the avian brain. Implicated that adult neurogenesis is involved in song acquisition and learning. Showed that the amount of neurogenesis was directly related to song complexity and season	Provides convincing evidence not only for the existence of human adult neurogenesis but for an actual functional role of these new cells
Bromodeoxyuridine (BrdU)	1982	Bromodeoxyuridine (BrdU), like [H^3] –thymidine, is thymidine analog which incorporates into replicating DNA during the S phase of the cell cycle	BrdU, unlike [H^3] - thymidine, allowed adult born cells to be double labeled with neuronal markers. These cells could be easily and rapidly detected by immunofluorescent staining method and could also be quantified by flow cytometry
Immunohistochemical Cell-type Specific Markers	1980's	Molecular markers that can be used to identify different stages of neuronal cells	Allowed scientists the ability to use several antigens in a population of adult-generated cells to correctly identify different stages of adult born neuronal cells
Retroviral Based Lineage Tracing	1980's	Retroviruses enter the nucleus of infected cells and insert their DNA into that of hosts	Allowed scientists a means of gene transfer and expression in the nervous system. Very useful for visualizing and tracing cells during neurogenesis

Comment [CSL4]: Remove all the stuff after this. When you are all done, remember to accept all changes so you don't send me a file that still has revision marks and comments.

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Start text here. Everything should be double spaced. You can put figures or tables in between text. If so, make sure there is enough space between the text and the figure. However, if there are a lot of big figures and table, you may want to put them all at the end, after "references". In that case, each figure/table should have its own page. All figures must have a title and legends. Do not use fine print for figure legends.

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