

Research Update:

Neurogenesis in Adult Brain and Neuropsychiatric Disorders

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Abstract

Until recently neurogenesis in mammals was considered to occur only during the embryonic and early post-natal periods and to have no significant role in the adult nervous system. However, it is now accepted that neurogenesis occurs in two brain regions in adult mammals, namely, the hippocampus and olfactory bulb. In both regions new neurons arise from a resident population of neural progenitor cells that are maintained throughout adult life.

Hippocampal neurogenesis is required for some types of hippocampal-dependent learning. Many factors enhance hippocampal neurogenesis including hormones, growth factors, drugs, neurotransmitters, and physical exercise as well as learning a hippocampal-dependent task. Other factors suppress hippocampal neurogenesis; these include aging, stress, glucocorticoids and stimuli that activate the pituitary/adrenal axis. Recently much attention has focused on the relevance of hippocampal neurogenesis to the pathophysiology and treatment of mood disorders. Indeed all major pharmacological and non-pharmacological treatments for depression enhance hippocampal neurogenesis and suppressing hippocampal neurogenesis in mice blocks behavioral responses in some antidepressant-sensitive tests. Altered hippocampal neurogenesis may also play a pathophysiological role in neurodegenerative disorders such as Alzheimer's disease. How much neurogenesis occurs normally in other brain regions is unclear. Neural progenitors are found throughout the neuraxis including both neurogenic and non-neurogenic regions. When cultured *in vitro* or isolated and transplanted back into neurogenic brain regions, these cells can differentiate into neurons, although in their *in situ* location they seem to behave as lineage-restricted glial progenitors. The environmental cues that limit the potential of progenitor cells in non-neurogenic brain regions are unknown. However, an emerging view is that astrocytes, a subset of which also functions as neural progenitor cells, are critical in regulating the local environment.

After transplantation into adult brain, neural stem cells are capable of surviving and differentiating into both neurons and glial cells, offering hope that stem cell therapy may be utilized to treat a variety of neurological and perhaps psychiatric disorders. The widespread existence of endogenous neural progenitors even in non-neurogenic brain regions also offers hope that the potential of these cells may be harnessed to repair cellular injuries caused by injuries such as stroke, trauma or neurodegenerative diseases. While obstacles remain to both approaches, stem-cell-based therapies remain an area of intense research interest.

Key Words: Alzheimer's disease, bromodeoxyuridine, depression, hippocampus, major affective disorders, neural progenitors, neural stem cells, neurodegenerative diseases, neurogenesis, stem cell therapy.

Historical Perspective

IN MAMMALS, NEUROGENESIS, i.e., the production of new neurons, has until recently been considered to occur only during the embryonic and early post-natal periods and to have no significant role in the adult brain. Indeed, Santiago Ramon Y Cajal, who first described neurons using the technique of Golgi staining, concluded that neurons were only generated during prenatal development, and this "no new neuron" doctrine has prevailed until recently. Various reasons accounted for the persistence of this view, including the lack of mitotic figures in mature neurons, the fact that neuronal numbers in adult brain appear to be stable or declining

in number with age as well as a generally more "hard wired" view of the adult brain.

Following the introduction of [³H]-thymidine labeling, scattered reports, in particular the studies of Altman (1), described labeling of neurons in both cortical and subcortical regions of adult rodents. However, the number of cells labeled was low, and using the morphological criteria available at the time, doubt remained as to whether the labeled cells were actually neurons. In the 1980s the question of neurogenesis in adult brain was extensively reexamined using [³H]-thymidine labeling in non-human primates with the conclusion that neurogenesis was confined to embryonic and early post-natal life and that there is no neurogenesis in adult brain (2).

During the last 10 years this "no neuron doctrine" has had to be revised to acknowledge that at least "limited" neurogenesis occurs normally in selected regions of adult mammalian brain and that neurogenesis may be stimulated in these as well as other regions in response to injury. This modified view has come about in part because of technical

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improvements in methods available for labeling dividing cells as well as the availability of a broad array of cell-type-specific markers that allowed cells to be identified on other than morphological grounds. This concept has also been bolstered by the finding that a resident population of neural progenitor cells exists in adult brain, obviating the need for mature neurons to become mitotic.

Neurogenic Brain Regions

It is now accepted that neurogenesis occurs in two brain regions in adult mammals, namely, the hippocampus and olfactory bulb (3). Neurogenesis in both regions has been demonstrated in the brains of rodents, non-human primates and humans. In the hippocampus, new granule cell neurons arise from neural progenitor cells located within the hippocampus itself in a region known as the subgranular zone (see below). By contrast the neural progenitors that give rise to new neurons in the olfactory bulb reside in the region around the anterior lateral ventricle termed the subventricular zone. Within the subventricular zone neural progenitors proliferate throughout life before differentiating and migrating through a tract known as the rostral migratory stream, ultimately giving rise to new granule cell neurons and a special type of interneuron in the olfactory bulb. Neurogenesis in the olfactory bulb in rodents has been extensively studied experimentally. Since it has less direct clinical relevance it will not be discussed here further. However, extensive reviews on this subject are available elsewhere (4, 5).

In contrast to hippocampus and olfactory bulb, other brain regions including the cerebellum, brainstem, basal ganglia, and spinal cord appear to be non-neurogenic. While neurogenesis may be stimulated in some of these regions by injury, it remains unclear how much if any constitutive neurogenesis occurs in these regions normally. The issue of neurogenesis in neocortex remains controversial, as discussed below. In the peripheral nervous system (PNS), only the olfactory neuroepithelium of the nasal mucosa has been demonstrated to be neurogenic in adults (6).

How Neurogenesis Is Studied in Adult Brain

Mature neurons are post-mitotic cells. By contrast, neural progenitor cells periodically divide. Therefore, the behavior of neural progenitors can be tracked by labeling them with nucleotide analogs that become incorporated into the DNA of dividing cells. Once incorporated the nucleotide

analog remains within the progeny during subsequent rounds of cell division. Historically [^3H]-thymidine was the first analog widely used. However, the [^3H]-thymidine signal penetrates thick tissue sections poorly and is difficult technically to combine with immunohistochemical labeling. It has now been largely replaced by bromodeoxyuridine (BrdU). BrdU is a thymidine analog that once incorporated into dividing cells can be labeled by immunohistochemical staining, which can be combined with cell-type-specific markers, allowing more precise identification of neuronal and non-neuronal cells. Examples of BrdU-labeled cells in the hippocampus of an adult mouse brain are shown in Fig. 1. Labeling with cell-type-specific markers combined with BrdU immunohistochemistry is shown in Fig. 2.

Using BrdU labeling, it is possible to measure three parameters of neural progenitor behavior. Typically several doses of BrdU are given over a 48-hour period. If the animal is sacrificed at the end of this labeling period and the number of BrdU labeled cells in the hippocampal subgranular zone are counted, an index of neural progenitor proliferation rate can be established. If the same dosing schedule is followed, but the animal is allowed to survive for several weeks, the number of BrdU labeled cells remaining in the dentate gyrus can be counted, giving an index of the fractional survival of the initially labeled progenitor population. Furthermore, the phenotype of surviving BrdU-labeled cells can be determined by dual immunocytochemical staining, allowing the fraction cells that became neurons or glial cells to be determined. Finally, by multiplying the number of surviving cells by the fraction of those cells that acquired neuronal markers the number of the new neurons produced from the initially labeled BrdU population can be calculated.

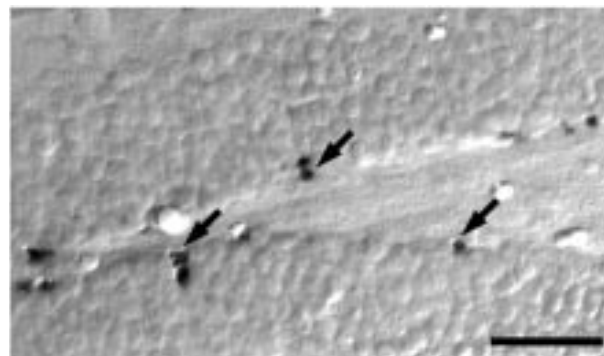


Fig. 1. An example of immunoperoxidase staining for bromodeoxyuridine (BrdU) in the hippocampus of an adult mouse is shown. Arrows indicate examples of BrdU-labeled cells in the subgranular zone.

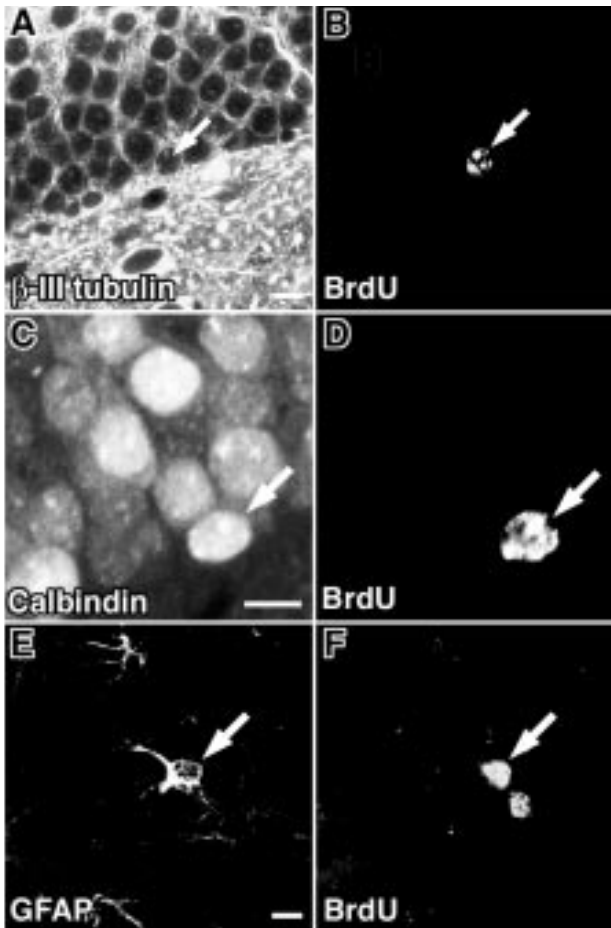


Fig. 2. Confocal laser-scanning microscopy images of bromodeoxyuridine (BrdU)-labeled cells 4 weeks after the last BrdU injection. Cells are shown co-labeled with BrdU and the neuronal markers β -III tubulin (A–B) or calbindin (C–D) or with the astrocyte marker GFAP (E–F). Arrows in each set of panels point to co-labeled cells. Scale bars: 10 μ m.

While extensively utilized to study progenitor cell fate, the general strategy has limitations. First, BrdU labeling will not permanently mark highly proliferative cells since the signal will be diluted with each round of cell division until ultimately it is no longer detectible. In addition, BrdU's limited bioavailability after injection may be no more than two hours in the adult brain (7), meaning that repeated injections must be given to quantitatively label adult progenitor cells, which have a cell cycle of ≈ 25 hrs (7). Doses must be further adjusted to capture the population of slow-cycling progenitor cells (8).

Indeed the true efficiency of BrdU labeling is unknown and recent studies have suggested that the most commonly used doses of BrdU fail to label all dividing cells (7). The issue of labeling sensitivity is of particular concern in regions where basal levels of neurogenesis may be low at

any given time but significant over a lifetime. Concerns have also been raised about BrdU's possible toxic effects on progenitor cells and even its specificity for dividing cells since cells undergoing DNA repair may also be labeled. Indeed, mature neurons may reenter the cell cycle prior to undergoing apoptosis. Concerns have also been raised about the specificity of some of the widely utilized cell-type-specific markers. Despite the fact that BrdU labeling has become the industry standard for studies of neurogenesis in the adult nervous system limitations remain which, in particular, limit BrdU's usefulness for following progenitor cell behavior over long time periods in adult brain.

The Fate of BrdU-Labeled Cells in Adult Mouse Hippocampus

Using a labeling protocol as described above, at the end of the injection period several thousand BrdU-labeled cells can be detected in the hippocampal subgranular zone of a young adult mouse. At four weeks after injection this number is reduced to about one-third of the original. Most of the two-thirds of cells that are lost are presumed to undergo programmed cell death through apoptosis although some loss through label dilution is difficult to exclude. Of the BrdU-labeled cells that remain approximately half express neuron-specific markers, another 10–15% glial-specific markers and the $\approx 35\%$ remaining have no identifiable phenotype. From such studies, the rate of new neuron generation in young adult mice or rats has been estimated to be from 1.5% (9) to 6% (7) of the total granule cell population per month, amounting to the hippocampus of a three-month-old mouse producing about one new neuron per day per 2,000 existing granule cells (9). Since at this age an adult mouse hippocampus contains $\approx 250,000$ granule neurons, the hippocampal granule cell layer could theoretically turn over in about four months. Whether this indeed happens and whether all granule cells are equally likely to be replaced is unknown.

Origins of Neural Progenitors in Adult Brain

Neural progenitors in regions such as the hippocampal subgranular and subventricular zone are generally thought to represent a resident population of cells that is established at birth and maintained throughout life by a combination of symmetric and asymmetric cell divisions that both renew the progenitor population and generate daughter cells that adopt neuronal or glial fates. The designation of a cell as a "stem cell" implies

both the capacity for self-renewal as well as multipotentiality, in the brain implying the ability to give rise to progeny with both neuronal and glial fates. Within the population of BrdU-labeled cells in the hippocampal subgranular zone, there is probably a population of true stem cells. However, since there are no markers that will differentiate whether an individual cell is a stem cell or has adopted a more restricted fate, BrdU-labeled cells in the subgranular zone are frequently referred by the more generic term "neural progenitor."

A peripheral origin would seem to constitute the only major alternative to the "resident since birth view." Notably, neural progenitor cells in both the hippocampal subgranular zone and the subventricular zone exist in close proximity to blood vessels in what has come to be referred to as a vascular niche (10). Why neural progenitor cells reside in a vascular niche is unclear, although reasonable speculations include that blood vessels may condition the niche with factors that help neural progenitors maintain their proliferative and undifferentiated nature; and recent *in vitro* studies have supported a role for soluble factors secreted by endothelial cells in maintaining neural stem cells in an undifferentiated state (11). However, a vascular niche might also imply that neural progenitor cells are derived from outside the central nervous system (CNS). Indeed, it is now well accepted that a circulating population of stem cells exists in adult blood and that at least under some *in vitro* conditions non-CNS stem cells may transdifferentiate into neurons (12). Whether transdifferentiation occurs *in vivo* is controversial and no studies to date have provided evidence that circulating stem cells repopulate the progenitor niches in the hippocampus and subventricular zone.

Functional Significance of Neurogenesis in the Adult Hippocampus

If new neurons are generated in adult brain, what might their function be? An immediate question is, do the new granule cell neurons in the hippocampus make functional connections? Hippocampal granule neurons are part of a synaptic circuit that receives input from the entorhinal cortex and sends projections to the pyramidal neurons in the CA3–CA1 region of the hippocampus. If newly generated granule neurons are to participate functionally in these circuits, they must send axonal projections into the mossy fibers that carry granule cell output to the hippocampal pyramidal cells. Indeed, retrograde tracer studies using injections into the terminal fields of the mossy fibers have established that newly born BrdU-labeled

neurons in the granule cell layer do send axonal projections to the pyramidal cell layers (13).

Since the hippocampus is best known for its role in learning and memory, one immediate question is whether neurogenesis might facilitate hippocampal-dependent learning. A general correlation does exist within inbred strains of mice between higher rates of neurogenesis in adult hippocampus and learning potential, as judged by the Morris water maze (14). To test this hypothesis experimentally Shors et al. (15) abolished hippocampal neurogenesis by treating adult rats with the DNA-methylating agent methylazoxymethanol acetate (MAM). They then asked whether disrupting hippocampal neurogenesis would impair performance in a test in which an eye blink elicited by an electrical stimulus was conditioned to occur in response to a white noise. In one version of the test, known as "delayed conditioning," the white noise and the periorbital stimulation (or unconditioned stimulus) overlap temporally and learning does not require an intact hippocampus. In the second version, known as "trace conditioning," a gap is present between the unconditioned and conditioned stimulus, and an intact hippocampus is required for learning to occur. Interestingly, treatment with MAM impaired the hippocampal-dependent but not the hippocampal-independent learning, arguing that hippocampal neurogenesis is involved in formation of trace memories.

Agents such as MAM are highly toxic and could be exhibiting effects on non-dividing cells, a confounding variable that is difficult to ever completely exclude. However, arguing against a general toxic effect of MAM, there was no change in hippocampal volume, cell numbers or long-term potentiation in the MAM-treated animals. In addition, if hippocampal neurogenesis was allowed to recover, learning in the hippocampal-dependent task recovered as well. Most recent studies continue to support the notion that hippocampal neurogenesis plays a role in at least some forms of hippocampal learning and memory.

Factors that Affect Hippocampal Neurogenesis in Adult Brain

Many factors affect hippocampal neurogenesis, including hormones, growth factors, drugs and neurotransmitters (3). Not only may hippocampal neurogenesis be required for some types of learning, but learning a task can also stimulate hippocampal neurogenesis (16). Environmental manipulations can also enhance hippocampal neurogenesis. For example, environmental enrichment which consists of switching animals from housing

in standard laboratory cages to an environment with increased opportunities for social interaction, exploration and physical activity produces a range of morphological and behavioral changes in rodents including stimulating hippocampal neurogenesis (17). Physical exercise enhances hippocampal neurogenesis quite possibly by stimulating the systemic production of insulin-like growth factor 1 (18). Estrogen stimulates hippocampal neurogenesis and the rate of hippocampal neurogenesis is increased during phases of the estrus cycle when estrogen levels are high (19). Besides increasing longevity, dietary restriction also enhances hippocampal neurogenesis (20).

Other factors suppress hippocampal neurogenesis. In general, glucocorticoids or any stimulus that activates the pituitary/adrenal axis suppresses hippocampal neurogenesis. Activation of the pituitary/adrenal axis may at least partly account for the consistent effects of a variety of stress paradigms on suppressing hippocampal neurogenesis (21). Stress and pituitary/adrenal activation may also account for the suppression of hippocampal neurogenesis seen with social isolation (22) and lower social hierarchy in rodents in which hippocampal neurogenesis is decreased in subordinate males (23). Hippocampal neurogenesis also decreases with aging (24) and may be impaired in pathological states that provoke some types of inflammation (25).

Neurogenesis as a Response to Injury

In general, neurogenesis is stimulated by injuries to the hippocampus. Insults including ischemia (26), seizures (27) and direct excitotoxic lesioning of the hippocampal granule cell layer (28) all increase neural progenitor proliferation. Lesioning the hippocampus indirectly by removing its afferent input from the entorhinal cortex also stimulates neurogenesis (29).

Injury may also stimulate neurogenesis outside the hippocampus. Occlusion of the middle cerebral artery in rats, for example, in addition to enhancing hippocampal neurogenesis, induces neural progenitors in the subventricular zone to migrate into the cerebral cortex and striatum (30). What remains unclear is the degree to which such activation results in actual repair and the reestablishment of functional connections. While the answer to this question is not fully known, targeted injuries to select neocortical layers in mice has been shown to stimulate the appearance of BrdU-labeled cells within the neocortex (31), reflecting cell labeling induced within the neocortex itself as well as migration of BrdU-labeled cells from the subventric-

ular zone (31). Some of these labeled cells differentiate into neurons and, most interestingly, some fraction of them send projections to the thalamus and reestablish corticothalamic projections. While these studies do not establish full functionality, they do offer hope that following brain injuries more plasticity exists in adult brain than might have been expected and that the capacity exists to reestablish functional connections between brain regions.

Hippocampal Neurogenesis and Depression

Hippocampal neurogenesis might at first be thought to have little relevance to major affective disorders. Yet recently, much attention has been focused on hippocampal neurogenesis in relation to the pathophysiology and treatment of mood disorders. Interest was initially stimulated by the observation that all major pharmacological and non-pharmacological treatments for depression enhance hippocampal neurogenesis (32). Indeed, clinically effective agents including selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors, tricyclics, lithium, and monoamine oxidase (MAO) inhibitors enhance hippocampal neurogenesis. Similar effects are also seen with newer agents predicted to have antidepressant efficacy, including rolipram (a type IV phosphodiesterase inhibitor), substance P (NK1 receptor) antagonists, group II metabotropic glutamate receptor antagonists and AMPA (α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid) receptor potentiators. The effects are specific in that non-antidepressant agents, such as haloperidol, do not consistently enhance hippocampal neurogenesis. Interestingly, the time course of the effects that agents such as fluoxetine have on neurogenesis are delayed in a manner consistent with the known delay in the clinical efficacy of the drugs. Non-pharmacological manipulations such as electroconvulsive shock therapy (ECT) also increase neural progenitor proliferation and neurogenesis. Indeed, of all antidepressant treatments tested to date, only one putative therapy, transcranial magnetic stimulation, may not affect hippocampal neurogenesis (32).

In major depression, it has been hypothesized that neuronal systems may be unable to respond to stressful stimuli with appropriate adaptive plasticity (33). Neurogenesis may be seen as one form of adaptive plasticity. Various acute and chronic physical or psychosocial stress paradigms all reduce neural progenitor proliferation and neurogenesis in the hippocampus of adult animals (34, 35). Stress-induced reductions in neural progenitor

proliferation in the hippocampus can also be reversed with antidepressant treatments including tricyclics, SSRIs and NK1 receptor antagonists (34, 36, 37). These effects have been seen in models of psychosocial stress (36) and learned helplessness (34). Thus, one current working hypothesis is that reduced neural progenitor proliferation leads to decreased hippocampal neurogenesis and that reversing or blocking this decrease may be one way that antidepressant treatments exert therapeutic effects.

Admittedly, it was well known before the effects of antidepressants on neurogenesis were discovered that stress adversely affects the hippocampus in other ways, including producing atrophy and reduced dendritic arborization of CA3 pyramidal neurons (38), and that some of these effects can be reversed by antidepressants (38). Indeed, the degree to which effects on hippocampal neurogenesis underlie the therapeutic benefits of antidepressant treatments remains to be determined and several recent commentaries have summarized arguments, both pro and con, on this issue (39–41).

However, recent studies by Santarelli et al. (42) in mice seem to strongly support the notion that hippocampal neurogenesis plays some role in the actions of antidepressant compounds. In these studies, hippocampal neurogenesis was blocked with fractionated radiation directed to the hippocampus. Mice were then tested in an antidepressant-sensitive behavioral test called the novelty-suppressed feeding test, in which following fasting, the time that it takes for a mouse to eat in a novel environment is measured. The test is considered a measure of anxiety since anxiolytic drugs reduce feeding latency. It is also considered a measure of antidepressant effect since commonly used antidepressant compounds have a similar effect. Reducing neural progenitor proliferation blocked the responses of mice to both fluoxetine and imipramine in the novelty-suppressed feeding test, arguing that hippocampal neurogenesis is necessary for the actions of antidepressants in this test.

As with cytotoxic agents, interpretation of such studies is confounded by whether radiation may be affecting factors other than hippocampal neurogenesis since in these studies other regions including the hypothalamus and amygdala, which might also be involved in mediating antidepressant effects, were included in the irradiated area. While such effects are difficult to completely exclude, no changes were found in brain histology, numbers of granule cell neurons or hippocampal long-term potentiation. A variety of neuroendocrine responses were also normal and a separate behavioral test, the cued fear conditioning test, which is regarded as a test of amygdala function, were in-

tact. Collectively, these studies strongly argue that at least some of the effects of antidepressants are mediated through their influence on hippocampal neurogenesis.

How antidepressants exert their actions is not completely understood. At the cellular level, antidepressants seem to principally increase the proliferation rate of neural progenitors without effecting fractional survival rate or the rate at which surviving cells differentiate into neurons (32). Thus antidepressants probably enhance neurogenesis by increasing the size of the progenitor pool. Although effects have been principally studied *in vivo*, fluoxetine (43) and lithium (44) have been noted to increase cell proliferation *in vitro* as well.

At the molecular level it is not known how antidepressants influence neural progenitor proliferation. As noted above, stress adversely affects hippocampal function at least in part through hyperactivity of the pituitary/adrenal axis and hypersecretion of glucocorticoids (45). A connection between activity of the glucocorticoid system and excitatory amino acids has been suggested by the finding that cortisol-induced decreases in neural progenitor proliferation can be prevented by administration of the *N*-methyl-D-aspartate (NMDA) antagonist MK-801 (45).

Antidepressants affect additional pathways that may be relevant to their effects on hippocampal neurogenesis, the best studied of these being the interactions of antidepressants with the brain-derived neurotrophic factor (BDNF) signaling pathway (46). BDNF is of interest since both BDNF mRNA and protein increase in response to antidepressant treatments and BDNF is one of a number of growth factors that enhance hippocampal neurogenesis. BDNF administered exogenously to brain either intraventricularly or intrahippocampal mimics the effects of antidepressants in a number of behavioral models of depression. Stress also downregulates BDNF levels and antidepressants reverse this effect.

Thus, by impacting both the glucocorticoid and BDNF pathways, antidepressants intersect with two major pathways that also regulate hippocampal neurogenesis. That these are the only pathways, however, seems unlikely: indeed, recent microarray and proteomic studies of antidepressant-treated animals have identified several other potential targets for antidepressant actions that might also impact hippocampal neurogenesis (47, 48)

Hippocampal Neurogenesis and Alzheimer's Disease

Alzheimer's disease (AD) is a degenerative disorder of the brain characterized clinically by

progressive loss of memory and other cognitive skills. Its pathological hallmarks include neuronal and synaptic loss, amyloid plaques, and neurofibrillary tangles. While most cases occur sporadically, the disease is inherited in some families in an autosomal dominant fashion. These familial AD (FAD) cases share the identical clinical and pathological features as sporadic AD except for their generally earlier age of onset (49). Mutations in the amyloid precursor protein as well as the presenilin genes have been found to cause early onset FAD (49).

Several studies have found that neurogenesis is impaired in the hippocampus of transgenic mice expressing presenilin 1 FAD mutants (50–52). Decreased progenitor proliferation has also been noted in mice expressing FAD mutations in the amyloid precursor protein (53). The molecular basis for this impairment is unknown, although Ab peptide, which is overproduced in FAD mutant brain and is the principle component of senile plaques, may be directly toxic to neural progenitor cells (54). At the cellular level, the presenilin-1 FAD mutants seem to primarily reduce the fractional survival of BrdU-labeled neural progenitors without decreasing total granule cell numbers, suggesting that the FAD mutant's primary effect is to alter the kinetics of new neuron production, in essence making the hippocampus in the FAD mutant generate more neural progenitors in order to maintain the same number of granule cell neurons (50).

Whether a similar impairment occurs in human FAD cases or in the more common sporadic disease is not known. One study has reported that the number of neurons expressing immature neuronal markers such as doublecortin, polysaturated nerve cell adhesion molecule (PSA-NCAM) and TUC-4 (TOAD [Turned On After Division]/Ulip/CRMP) are increased in the hippocampal granule cell layer of sporadic human cases (55). The authors of this study interpreted their findings as indicating that neurogenesis was increased in the hippocampus in Alzheimer's disease. However, given the lack of any method for tracking the fate of these immature neurons and, in particular, whether they ever progress to become mature neurons, these results could also be interpreted as showing that in AD hippocampal neurogenesis is blocked at the stage of immature neuron generation.

Of direct clinical relevance is whether impaired neurogenesis might contribute to cognitive dysfunction in AD. Pointing to a possibly relevant role, the hippocampus is pathologically affected early in AD with pyramidal cell loss and disruption of perforating path connections from the entorhinal cortex. Granule cell neurons exhibit cytoskeletal

changes that are similar to those found in other neurons in AD, possibly impairing neurogenesis. It is also interesting that the rate of hippocampal neurogenesis decreases with aging coincident with age-related memory decline (24) and that age-related memory impairments may be improved in rodents following transplantation of immortalized neural stem cells into brain (56). Moreover, factors that positively affect hippocampal neurogenesis, such as estrogen (57), and cognitively stimulating activities (58) may reduce the risk of AD. Yet of the major brain regions ultimately affected by AD pathology, only the hippocampus is currently regarded as neurogenic, and even in the hippocampus, the brunt of the pathology is in the pyramidal neurons, which are currently regarded as non-neurogenic. Thus, whether hippocampal neurogenesis can be considered a legitimate therapeutic target in AD is at present unclear.

Neurogenesis in Non-neurogenic Brain Regions

Discussion of disorders such as AD leads into the broader question of how much neurogenesis continues in regions of the adult brain beyond the hippocampus and subventricular zone/olfactory bulb. Neocortical regions have, for example, long been regarded as non-neurogenic. However, this view was challenged by Gould and colleagues in 1999 (59). Using a combination of BrdU labeling along with neuronal specific markers and retrograde tracers they reported that new neurons are continuously generated during adulthood in the neocortex of non-human primates. However, a latter study by Kornack and Rakic (60) in non-human primates found only glial cell labeling in neocortex and no evidence for neurogenesis. The differing conclusions of these two studies can be reduced to contrasting interpretations of data collected in stacks from a confocal microscope, highlighting how difficult a seemingly simple question can be to resolve. More recent studies have tended to side with Kornack and Rakic on this issue (61).

Separating the Neurogenic from the Non-neurogenic

What may be seen as the relatively limited neurogenic potential of most brain regions in the adult has led researchers such as Steven Goldman to comment that "the brain is generally not friendly to new neurons" (62). Indeed *in vivo*, most "stem"/progenitor cells in the adult CNS seem to behave as lineage-restricted glial progenitors. One might therefore ask if neurogenic progenitors are confined to neurogenic regions. The answer to this

question is clearly no. Progenitor cells that can give rise to neurons *in vitro* can be expected to be isolated from the adult hippocampus and subventricular zone, but they can also be isolated from essentially all non-neurogenic regions including the subcortical white matter, striatum and spinal cord.

Indeed, Goldman's group (63) has successfully fluorescence-activated cell sorting (FACS)-sorted adult neural progenitor cells from the temporal lobe white matter of human surgical specimens using the antibody A2B5. These cells formed neurospheres in culture that could differentiate and be induced to express neuronal-specific markers. In addition, they behaved as multipotent stem cells *in vivo* when transplanted into embryonic rat brain, giving rise to both neuronal and glial lineages. While such observations do not prove that multipotent stem cells exist in human temporal lobe white matter *in vivo*, they do indicate that progenitors isolated from white matter have not forgotten how to behave as stem cells.

What then limits the potential of these cells *in vivo*? An evolving view is that it is not an innate property of the progenitors themselves, but rather the environment, that controls their potential. Clues to these environmental controls have come from studies in which neural progenitors isolated from non-neurogenic regions such as the spinal cord were transplanted back into either spinal cord or hippocampus (64). Cells that were transplanted back into their natural home, the non-neurogenic spinal cord differentiated into only glial cells. However, cells that were transplanted into the hilus of the hippocampus integrated into the subgranular zone and began to express neuronal markers, suggesting that in the hippocampus local environmental factors had instructed the transplanted cells to adopt a neuronal fate.

How environmental cues control the potential of resident progenitor cells is unknown. However, emerging evidence points to a central role of astrocytes in determining neurogenic vs. non-neurogenic brain regions. Astrocytes have long been viewed as playing a support role in the CNS, being involved in metabolic support of neurons as well as injury repair. Astrocytes have more recently been recognized to also play a role as neural progenitor cells themselves in both the developing and mature CNS (62, 65). In addition, the major neural stem cell in the subventricular zone in adult brain has been identified as an astrocyte-like cell and a subset of astrocytes in the hippocampal subgranular zone also have stem-cell-like properties (62).

Besides serving as stem cells, astrocytes have also been reported to promote neurogenesis. For example, Song et al. (66) cultured neural progeni-

tors from the adult rat hippocampus with either primary cultures of hippocampal neurons or astrocytes. Interestingly, they found that neural progenitors cultured with neurons assumed an oligodendroglial fate while progenitors cultured with astrocytes were induced to differentiate into neurons. Moreover, the effect depended on the source of the astrocytes. While astrocyte cultures established from hippocampus promoted neurogenesis, astrocytes from spinal cord promoted only glial differentiation. These studies clearly argue that heterogeneity exists in the ability of astrocytes from different CNS regions to promote neurogenesis. What factors determine the regional specification of astrocytes and whether, for example, astrocytes in different brain regions secrete different complements of growth factors is unknown.

Therapeutic Implications

Following transplantation into the adult brain, stem cells are capable of surviving and differentiating into both neurons and glial cells. Stem cell therapy is thus being considered for a variety of neurological and even psychiatric disorders. Replacement strategies typically contemplate injecting neural or embryonic stem cells that may have been partially differentiated *in vitro* into the mature CNS, with the hope that these cells will restore lost function. Some disorders seem more amenable to this approach than others. Parkinson's disease, for example, is considered to be a good target since the cell type that needs to be replaced and its location are well known. Other disorders such as Alzheimer's disease pose greater challenges since the cell types lost are more diverse and the disease ultimately spreads diffusely.

Given the widespread existence of endogenous neural progenitors even in non-neurogenic brain regions, an attractive alternative to transplantation would be to harness the potential of endogenous progenitors to repair cellular injuries. As discussed above, whether this strategy can ever be exploited may well depend on understanding the environmental cues that determine the neurogenic from non-neurogenic.

Yet whether utilizing transplanted cells or endogenous stem cells, other obstacles remain. For example, even if stem cells can be recruited or induced in the correct location *in situ*, a mechanism must be in place to induce differentiation into the correct neurochemical or other phenotype. In addition, neurons in the adult brain exist not just as neurotransmitter pumps, but must be integrated into neuronal circuits, in many cases extending axons over long distances such as the long cortico-

cortical connections that are lost in AD. Additionally, even if axons succeed in growing into appropriate target regions, how readily will new fibers reestablish appropriate connections in the setting of an ongoing neurodegenerative process?

Yet while many obstacles remain, the capacity of engrafted stem cells to function in the adult nervous system and the existence of a resident potential stem cell population in the adult brain continue to raise hope that repair and rejuvenation through stem cell approaches might be possible. As such, neurogenesis in the adult brain can be expected to remain an area of intense research interest for many years to come.

Acknowledgments

This work was supported by grants from the National Institute on Aging (AG020139, AG023599), the National Institute of Mental Health (MH070603) and a Merit Award from the Department of Veterans Affairs. RDG is a recipient of a Young Investigator Award from the National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD).

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