

*Perspectives in Pharmacology*

## Regulation of Adult Neurogenesis by Psychotropic Drugs and Stress

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### ABSTRACT

Proliferation and maturation of neurons has been demonstrated to occur at a significant rate in discrete regions of adult brain, including the hippocampus and subventricular zone. Moreover, adult neurogenesis is an extremely dynamic process that is regulated in both a positive and negative manner by neuronal activity and environmental factors. It has been suggested to play a role in several important neuronal functions, including learning, memory, and response to novelty. In addition, exposure to psychotropic drugs or stress

regulates the rate of neurogenesis in adult brain, suggesting a possible role for neurogenesis in the pathophysiology and treatment of neurobiological illnesses such as depression, post-traumatic stress disorder, and drug abuse. As the mechanisms that control adult neurogenesis continue to be identified, the exciting prospect of developing pharmacological agents that specifically regulate the proliferation and maturation of neurons in the adult brain could be fulfilled.

Throughout the modern neurobiological era, it has been widely accepted that no new neurons are added to the *adult* brain. However, recent studies clearly demonstrate that neurogenesis can occur in the brain after development and even into old age. Adult neurogenesis has been documented in many different types of animals, including birds, rodents, nonhuman primates, and humans (see Gould et al., 1999a; Gage, 2000; Gross, 2000). Relatively high rates of neurogenesis are restricted to two major regions in adult brain: the olfactory bulb and the hippocampus. In hippocampus, a brain region implicated in learning, memory, and mood disorders, it is estimated that there are approximately 250,000 new neurons per month in the adult rodent brain or about 6% of the total number of granule cells. In nonhuman primates and humans, the numbers of new neurons are much smaller, although not insignificant. Neurogenesis has also been demonstrated to occur in other parts of the brain, such as cerebral cortex (Gould et al., 1999a), at a much lower rate, although it can also be induced by apoptotic degeneration (Magavi et al., 2000).

Recent studies also demonstrate that the rate of neurogenesis can be regulated by environmental, endocrine, and phar-

macological stimuli. This indicates that neurogenesis is a form of neural plasticity that contributes to the ability of the brain to process, adapt, and respond to stimuli. The focus of this perspective is to briefly describe adult neurogenesis, its regulation by environmental factors and psychotropic drugs, and how it may be a target for future drug development. The regulation of neurogenesis provides further support that *structural*, as well as neurochemical, adaptations, mediate the actions of psychotropic drugs and responses to stress and other environmental factors. However, additional studies are needed to directly test the functional relevance of neurogenesis in adult brain of humans, as well as laboratory animals.

### Adult Neurogenesis Defined

Neurogenesis in adult brain is characterized by DNA synthesis that occurs during the S phase of mitosis of dividing progenitor cells. Incorporation of labeled nucleotide precursors into the DNA of dividing cells is used as a marker of neurogenesis. The precursors that are commonly used are [<sup>3</sup>H]thymidine and bromo-deoxy-uridine (BrdU) (Gould et al., 1999b; Gage, 2000; Gross, 2000). Labeling with [<sup>3</sup>H]thymi-

**ABBREVIATIONS:** BrdU, bromodeoxyuridine; MAM, methylazoxymethanol acetate; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; HPA, hypothalamic-pituitary-adrenal; 5-HT, serotonin; NMDA, *N*-methyl-D-aspartate; CREB, cAMP response element-binding protein.

dine requires several weeks or months of film exposure to detect a signal, while immunohistochemical detection of BrdU-labeled cells is very sensitive and requires only a few days. For this reason, BrdU is the preferred technique for labeling newborn cells. One concern with this approach is that these nucleotide precursors are also incorporated into nicked or damaged DNA undergoing repair. For this reason, it is important to demonstrate in other ways that labeled cells are in fact newborn (see below).

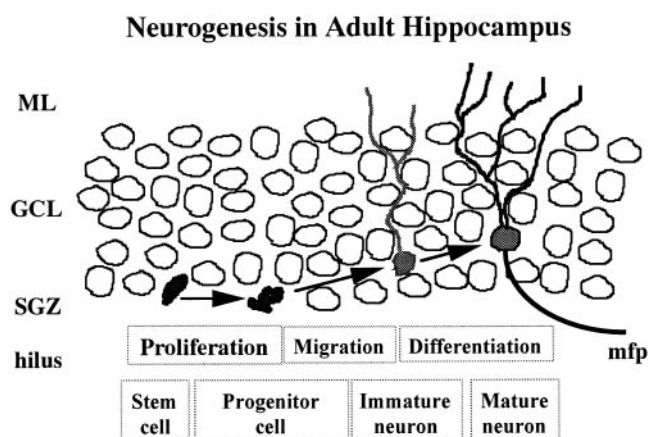
Several phases of neurogenesis can be studied, including *proliferation* and *survival* of newborn cells, and there are different protocols for each (Fig. 1). Proliferation is a measure of the number of newborn cells and is determined at a short time (2 h) after BrdU administration. At this early time point, the progenitor cells undergo only a single round of cell division, and the number of BrdU-labeled cells is not influenced by cell survival. Unbiased stereology is used to count the total number of labeled cells in hippocampus. Newborn cells visualized just after proliferation are found in the subgranular zone, are irregular in shape, and have very few or no processes (Fig. 2). In addition, it is possible to visualize BrdU-labeled cells in the process of mitosis and to observe mitotic spindles. This demonstrates that BrdU is incorporated into dividing cells and not just into damaged DNA that is undergoing repair.

Cell survival is determined at a relatively long time point (4 weeks) after BrdU administration (Fig. 1). At this time point, roughly 50% of the newborn cells survive and the remaining cells undergo a process of degeneration. Colocalization of BrdU with cellular markers of neurons (e.g., NeuN) or glia (e.g., glial fibrillary acidic protein or S100 $\beta$ ) can be used to determine the phenotype of these relatively mature cells (Fig. 2) (Gage, 2000; Gross, 2000). Approximately 75 to 80% of the surviving cells become neurons, 10 to 15% glia, and the remaining do not express markers of either neurons or glia (i.e., phenotype is undetermined). The cells expressing neuronal markers have migrated into the granule cell layer and display processes characteristic of mature cells. These neurons extend axons along the mossy fiber pathway to the CA3 pyramidal cell layer (Fig. 1) and exhibit long-term potentiation (van Praag et al., 1999). These findings demonstrate that neurons added to adult hippocampus have characteristics of mature granule cells and that they can integrate into the existing hippocampal circuitry.

## Regulation of Neurogenesis by Environmental Factors

Studies of the neurobiological mechanisms underlying the sex, endocrine, and seasonal variation of bird song have demonstrated an important role for neurogenesis (see Barnea and Nottebohm, 1996). This work has been extended to rodents and demonstrates that exposure to environmental factors, including enriched environment, exercise, and learning and memory, influence the rate of neurogenesis and the survival of new neurons (see Fig. 3).

Gage and colleagues have demonstrated that mice placed in an enriched environment where there are more social interactions, inanimate objects for play, and a wheel for voluntary exercise have an increased rate of neurogenesis relative to mice that are kept in standard cages (Kempermann et al., 1997). The factors underlying the positive ac-

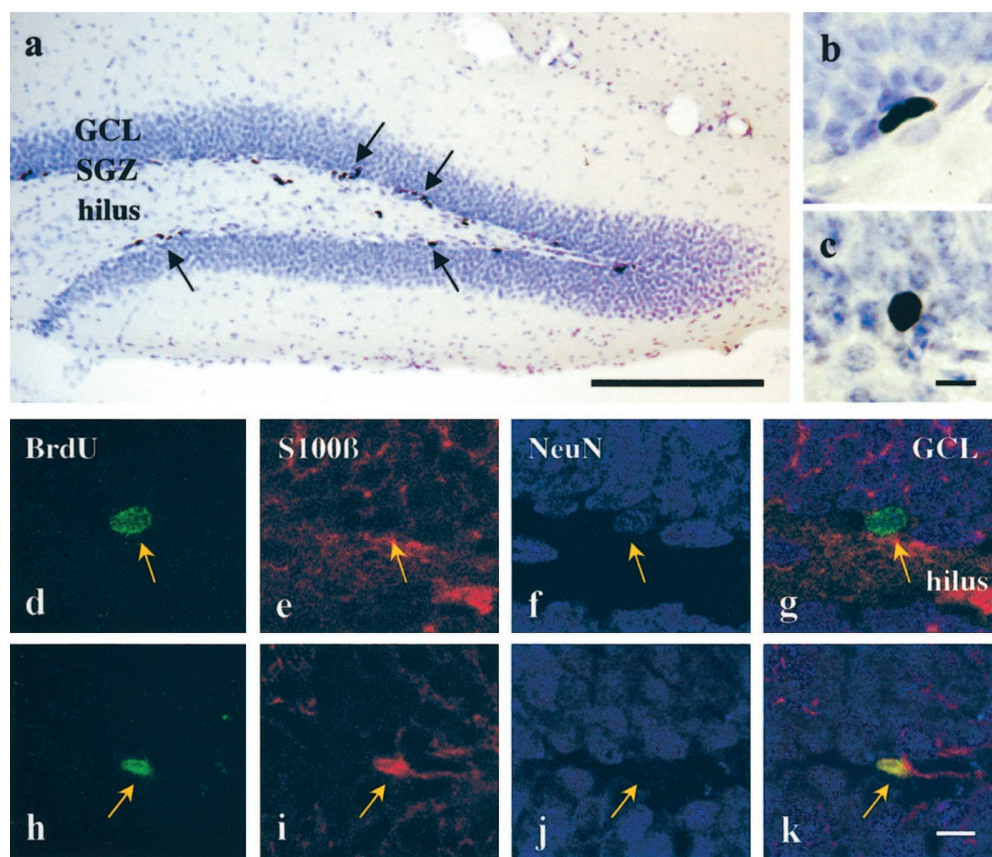


**Fig. 1.** Neurogenesis in adult hippocampus. Putative neural stem cells and/or progenitor cells are located in the subgranular zone (SGZ) of the hippocampus, located at the border between the granule cell layer (GCL) and the hilus. Proliferating cells are irregular in shape and have very few or no processes. Approximately 80% of the newborn cells take on characteristics of neurons, and the remaining cells are glial or have a phenotype that is undetermined. Cells destined to become neurons migrate into the granule cell layer, mature, and take on characteristics of adult granule cells. This includes extension of dendrites into the molecular layer (ML) and axons into the CA3 pyramidal cell layer via the mossy fiber pathway (mfp).

tions of an enriched environment include a combination of social interactions, learning and memory, and behavioral activity (see van Praag et al., 2000). Attempts to specifically examine the influence of learning and memory have been mixed. One study has reported that hippocampal-dependent learning increases the survival of newborn granule cells, while another study found no effect (see Gould et al., 1999b). The difference between these two studies may be attributable to the timing of the BrdU administration. In the study reporting a training-induced increase in survival, the BrdU was administered before training. In the study reporting no effect, BrdU was administered during or after training. This difference illustrates the importance of choosing the appropriate experimental paradigm and time of BrdU incorporation for neurogenesis studies.

Shors et al. (2001) have extended this work by testing the relationship between adult neurogenesis and hippocampal-dependent learning. This study also addresses a major limitation in the neurogenesis field of not being able to determine the function of the newborn cells in adult brain. A DNA-methylating agent, methylazoxymethanol acetate (MAM), that is toxic for proliferating cells was used to test the role of neurogenesis in hippocampal-dependent learning. In this study, administration of MAM produced a dose-dependent inhibition of neurogenesis in adult hippocampus that correlated with a decrease in trace memory. The study included several controls and additional tests suggesting that the effects of MAM were related to inhibition of neurogenesis and not side effects of the cell cycle inhibitor, although it is difficult to completely eliminate the possibility of nonspecific side effects. This approach will have to be combined with other approaches, such as the development of targeted transgenic mice that express recombinant cell cycle inhibitors selectively in neural progenitor cells, to confirm a functional role of cell proliferation in adult brain.

Studies of voluntary exercise demonstrate that activity on a running wheel, in the absence of other components of

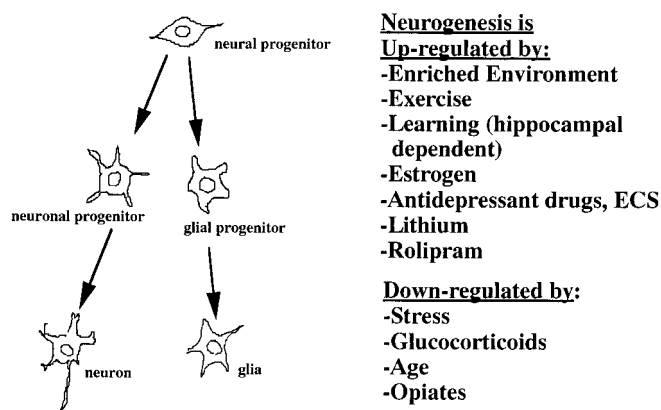


**Fig. 2.** BrdU immunohistochemistry and characterization of cell phenotype in hippocampus. a, shortly after the proliferation stage (2 h after BrdU administration) BrdU-positive cells are often found in clusters and are localized to subgranular zone (SGZ) between granule cell layer (GCL) and hilus. At this stage, the nuclei of BrdU-positive cells are dark and irregular in shape. b, high-power magnification of BrdU-labeled cells 2 h after BrdU administration. c, 4 weeks after BrdU administration the cells migrate into the granule cell layer and take on the characteristics of mature neurons. d through k, colocalization of BrdU with either NeuN, a neuronal marker (d–g), or S100 $\beta$ , a glial marker (h–k) by fluorescence confocal microscopy. Scale bars: a and b, 200  $\mu$ m; c through e, 10  $\mu$ m.

enriched environment, is sufficient to increase proliferation and recruitment of granule cells into the dentate gyrus (see van Praag et al., 1999). Although the exact mechanism underlying the exercise-induced up-regulation of neurogenesis has not been identified, exercise is reported to increase the expression of certain trophic factors, which have also been shown to increase neurogenesis during development or in adult brain. These factors include brain-derived neurotrophic factor (BDNF) and fibroblast growth factor-2 (Gomez-Pinilla et al., 1997; Cameron et al., 1998; Zigova et al., 1998; Rosello-Neustadt et al., 1999). The possibility of a direct relationship

between these factors and increased neurogenesis in response to exercise requires further experimentation.

It is also important to acknowledge that the influence of an enriched environment may represent a reversal of an impoverished environment. What is commonly referred to as standard housing for laboratory animals is actually a condition where the environmental stimulation is significantly reduced relative to that encountered in a normal environment. This point is critical when trying to interpret the effects of environmental, endocrine, and pharmacological stimuli on brain function and highlights the need for appropriate design of animal models in a more naturalistic setting.



**Fig. 3.** Adult neurogenesis in hippocampus can be up- or down-regulated. The proliferation and survival of cells in adult hippocampus can be influenced in both a positive and negative manner by a variety of stimuli. This includes administration of pharmacological agents, such as antidepressants and opiates. ECS, electroconvulsive seizure.

### Regulation of Neurogenesis by Stress and Adrenal Glucocorticoids

Another environmental factor that exerts a potent effect on neurogenesis is stress. In this case, the rate of neurogenesis is decreased, not increased, by stress (Fig. 3). Gould et al. (1998) have demonstrated that exposure of adult nonhuman primates to intruder stress decreases the rate of granule cell proliferation in the hippocampus. Decreased neurogenesis appears to result from the stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, particularly elevation of glucocorticoids. Administration of glucocorticoids decreases the proliferation of granule cell precursors in adult rat hippocampus, as well as during development (Gould et al., 1992). Prolonged exposure to glucocorticoids over the lifespan of an animal, as a result of normal aging, also accounts for decreased neurogenesis in aged animals (Cameron and McKay, 1999). Removal of adrenal steroids can restore



the rate of neurogenesis to that observed in young adult rats, demonstrating that aged animals retain the capacity for a rate of neurogenesis that is observed in young animals.

These findings raise the possibility that decreased neurogenesis contributes to the damaging effects of stress reported to occur in mood disorders, including depression and post-traumatic stress disorder. These disorders are often precipitated or worsened by stress and can be associated with elevated hypothalamic-pituitary-adrenal axis function. Recent brain imaging studies demonstrate that the volume of hippocampus is decreased in patients with depression or post-traumatic stress disorder (Bremner et al., 1995; Sheline et al., 1996, 1999; Duman et al., 1997, 2000). Hippocampus is one of several limbic structures that could contribute to the cognitive and vegetative abnormalities observed in patients with mood disorders. It is conceivable that decreased neurogenesis in response to prolonged stress and elevated glucocorticoids contribute to the reduced volume of hippocampus observed in these patients. Direct counting of cell number in post-mortem hippocampus of patients with mood disorders will be necessary to test this possibility. However, a role for neurogenesis in mood disorders is very speculative at this time, and additional studies will be needed to directly test this hypothesis in experimental animals.

In addition to adrenal steroids, sex steroids are also reported to influence the rate of neurogenesis. Granule cell proliferation is increased by administration of estrogen and is transiently regulated during the estrous cycle in rodents (Tanapat et al., 1999). The role of altered neurogenesis in normal and dysfunctional behavioral responses to sex steroids remains to be determined.

## Regulation of Neurogenesis by Psychotropic Drugs

Regulation of neurogenesis by environmental stimuli, stress, and sex steroids, factors that have been implicated in the pathophysiology of psychiatric illness, has led to a series of studies examining the actions of psychotropic drugs on neurogenesis. The results of this work have been interesting and suggest that regulation of neurogenesis could contribute to the therapeutic effects of different classes of psychotropic drugs, as well as the unwanted side effects of others (Fig. 3).

**Antidepressant Treatment Increases Hippocampal Neurogenesis.** Previous studies have demonstrated that repeated antidepressant administration increases the expression of BDNF in hippocampus (see Duman et al., 1997, 2000). In contrast, stress decreases BDNF expression in this brain region. These studies, combined with the finding that stress causes atrophy of hippocampal neurons and decreased neurogenesis, have contributed to a neurotrophic hypothesis of depression and antidepressant action (Duman et al., 1997, 2000). According to this hypothesis, depression may result, in some cases, from increased exposure to stress or other aversive stimuli that cause damage or death to hippocampal neurons. Over time, the accumulation of these damaging effects can result in certain behavioral and endocrine abnormalities observed in depressed patients (e.g., altered cognition, learning, and elevated HPA axis). Antidepressant treatment may block or even reverse these effects of stress via increased expression of BDNF.

To further test this hypothesis, the influence of antidepressants on hippocampal neurogenesis has been examined (Mal-

berg et al., 2000). Repeated antidepressant administration was found to increase the number of BrdU-labeled cells in hippocampus. This increase is dependent on long-term antidepressant administration (2–4 weeks), consistent with the time course for the therapeutic action of antidepressants. In addition, up-regulation of BrdU-labeled cells occurs in response to repeated administration of different classes of antidepressant drugs, including serotonin and norepinephrine-selective reuptake inhibitors. Others have confirmed the induction of neurogenesis by antidepressant drugs (Jacobs and Fornal, 1999; Manev et al., 2001). In addition, up-regulation of hippocampal neurogenesis is induced by electroconvulsive seizure treatment (Madsen et al., 2000; Malberg et al., 2000) as well as long-term lithium administration (Chen et al., 2000). These findings suggest that increased granule cell number is a common cellular action of antidepressant treatment.

The influence of antidepressant administration on granule cell proliferation, survival, and phenotype was also examined. Analysis of labeled cell number 2 h after BrdU administration (to determine proliferation) demonstrated an increase in the number of BrdU-labeled cells. Four weeks later, the majority of the newborn cells expressed neuronal markers relative to glial markers, and the ratio of neurons to glia was similar to that in control animals (Malberg et al., 2000). In addition, the number of BrdU-labeled cells at this time point was significantly increased, indicating that antidepressant treatment significantly increases the total number of neurons in hippocampus.

Induction of neurogenesis is one mechanism by which antidepressants could block or reverse the atrophy and loss of hippocampal neurons that occurs in response to stress. Additional studies are necessary to analyze the number of granule cells in post-mortem brain of depressed patients who were either drug-free or taking antidepressant medication at the time of death to directly examine this possibility.

**Regulation of Neurogenesis by Antipsychotic Drugs.** In the Malberg et al. (2000) study, repeated antipsychotic drug administration did not influence neurogenesis. However, there are two studies reporting regulation of granule cell proliferation by chronic administration of haloperidol. One of these studies reports an increase (Dawirs et al., 1998) and the other a decrease (Backhouse et al., 1982) in hippocampal cell proliferation. There are several important differences between these reports, including dose and time course of drug treatment, species and age of test animals, and the BrdU-labeling protocol. In the study reporting no effect (Malberg et al., 2000), the dose and time of haloperidol treatment were consistent with the therapeutic treatment regimen, and the BrdU-labeling protocol was the same as that used for the antidepressant studies. In one of the other studies, gerbils were used as the test animal and the dose was extremely high (Dawirs et al., 1998). In the other study, the influence of haloperidol on neurogenesis during early postnatal development was determined (Backhouse et al., 1982). Once again, the reports of different results occurring with the same drug demonstrate the importance of the experimental protocol used for studies of neurogenesis. However, the results also demonstrate that under certain conditions antipsychotic drugs can influence hippocampal neurogenesis. The relevance of these effects to either the therapeutic actions or

side effects of antipsychotic drugs will require further investigation.

**Regulation of Neurogenesis by Drugs of Abuse.** Drugs of abuse, including opiates and psychostimulants, are known to have long-term effects that are mediated by alteration of synaptic plasticity. In addition to their addictive properties, repeated use of these drugs can influence cognition, learning, and memory. Given the potential role of neurogenesis in learning and memory, the influence of opiates on hippocampal neurogenesis has been studied (Eisch et al., 2000). This study demonstrates that repeated administration of morphine decreases the proliferation of granule cells in adult rat hippocampus. A similar effect was also seen after self-administration of heroin. This unforced or volitional self-administration of the opiate is a more accurate model of drug use by opiate addicts. Repeated administration of opiates as well as other drugs of abuse is known to activate the HPA axis, raising the possibility that increased levels of adrenal-glucocorticoids could account for decreased granule cell proliferation. This point was addressed by demonstrating that opiate administration decreased hippocampal cell proliferation even in the absence of a glucocorticoid surge (i.e., adrenalectomy plus glucocorticoid replacement). Studies are currently being conducted to examine the influence of psychostimulants and determine whether decreased neurogenesis is observed with other classes of drugs of abuse (E. J. Nestler, unpublished observations).

These findings in adult brain add to previous studies demonstrating a significant effect of opiates on the proliferation of cultured progenitor cells or during early development. Selective mu and delta opiate receptors influence the proliferation and synthesis of DNA in cultured cerebellar progenitor cells, indicating the presence of opiate receptors on these cells (see Eisch et al., 2000 for discussion; Hauser et al., 2000). Currently, there is no direct evidence for or against the expression of opiate receptors on hippocampal progenitor cells. Another possibility is that the down-regulation of granule cell proliferation occurs via an indirect action of opiates on other cells that then release a factor that influences the progenitor cells.

### Development of Novel Therapeutic Agents That Regulate Neurogenesis.

The demonstration that neurogenesis in adult brain can be regulated by psychotropic drugs opens the door for the development of agents that are designed to directly influence this process. Identification of the neurotransmitters and growth factors, as well as the intracellular signal transduction pathways, which control neurogenesis in adult brain, will provide vital information toward this goal. Although the factors that control neurogenesis in embryonic and early postnatal development have been studied (see Cameron et al., 1998), much less is known about the regulation of neurogenesis in adult brain. Some of these possibilities, based on current information in adult brain, are briefly discussed. As the factors and signaling pathways that control neurogenesis are elucidated, the number and type of drug targets that influence neurogenesis will continue to be enriched.

**Regulation of Neurogenesis by Serotonin (5-HT).** A role for the 5-HT neurotransmitter system in the regulation of adult neurogenesis has been demonstrated using several

different approaches. Lesion of the 5-HT system or inhibition of 5-HT synthesis has been shown to decrease the proliferation of granule cells in the hippocampus (see Brezun and Daszuta, 2000). These investigators have also demonstrated that grafts of fetal raphe 5-HT neurons reverse this deficit. Lesion of 5-HT neurons does not completely eliminate adult neurogenesis in hippocampus, indicating that other factors also contribute to the basal rate of neurogenesis.

Induction of neurogenesis by the 5-HT system has also been demonstrated using a pharmacological approach. Administration of an agent that releases 5-HT, *d*-fenfluramine, increases the number of BrdU-labeled cells in the hippocampus (Jacobs et al., 1998). In addition, *d*-fenfluramine induction, as well as the basal rate, of granule cell proliferation is blocked by pretreatment with a 5-HT<sub>1A</sub> antagonist, WAY 100,635. In contrast, administration of a 5-HT<sub>1A</sub> agonist, 8-hydroxy-2-dipropylaminotetralin, increases the number of BrdU-labeled cells. These findings provide additional evidence that the 5-HT system exerts a positive effect on adult neurogenesis and demonstrate that this effect is mediated, at least in part, by the 5-HT<sub>1A</sub> receptor subtype.

It will be interesting to determine whether the 5-HT<sub>1A</sub> receptor mediates the induction of granule cell proliferation, which has been observed in response to administration of a 5-HT-selective reuptake inhibitor (Malberg et al., 2000). This possibility is consistent with previous studies demonstrating that repeated antidepressant treatment up-regulates 5-HT<sub>1A</sub> receptor function in hippocampus (Haddjeri et al., 1998). However, other 5-HT receptor subtypes may also regulate neurogenesis. Some interesting possibilities are activation of the 5-HT<sub>7</sub> receptor, which is positively coupled to the cAMP cascade, and inhibition of the 5-HT<sub>2A</sub> receptor, which has been shown to down-regulate expression of BDNF in the granule cell layer of hippocampus (see Duman et al., 1997, 2000).

There are currently no studies that directly examine regulation of adult neurogenesis by other monoamines, such as norepinephrine and dopamine. The influence of these neurotransmitter systems and their specific receptor subtypes on neurogenesis could also be interesting.

**Regulation of Adult Neurogenesis by N-Methyl-D-Aspartate (NMDA) Receptors.** Given the role of glutamate as the major excitatory neurotransmitter in the brain, it isn't surprising that this system has been shown to regulate neurogenesis in adult hippocampus. Studies to date have focused on one glutamate receptor subtype, the NMDA receptor (Cameron et al., 1995). Proliferation of granule cells in adult hippocampus is decreased by systemic administration of NMDA and increased by administration of an NMDA receptor antagonist. In addition, lesion of the entorhinal cortex, which provides a major glutamatergic input to the hippocampus, increases granule cell proliferation. Taken together, these results demonstrate a role for endogenous glutamate and activation of NMDA receptors in maintaining a normal rate of neurogenesis in adult hippocampus. However, it will be important to directly examine the role of glutamate neurotransmission in hippocampus, as systemic administration of an NMDA agonist or antagonist could influence neurogenesis indirectly via actions in other brain regions. It will also be important to examine other types of glutamate receptor subtypes in the regulation of neurogenesis.

**Regulation of Adult Neurogenesis by the cAMP Cascade.** Another approach to consider for development of agents that influence adult neurogenesis is via regulation of intracellular signal transduction cascades. Although there are very few such studies in vivo, one system that has been examined is the cAMP pathway. This has been examined using pharmacological and conditional transgenic approaches. First, levels of cAMP can be increased by pharmacological inhibition of cAMP phosphodiesterase 4, a subfamily of enzymes that metabolize cAMP. Chronic, but not acute, administration of rolipram, a selective phosphodiesterase 4 inhibitor, increases the proliferation of granule cells in hippocampus (Kim et al., 2000). In this study, rolipram treatment also increased levels of phosphorylated cAMP response element-binding protein (CREB), the activated form of this transcription factor. This demonstrates that rolipram treatment activates the cAMP cascade and suggests that CREB-mediated gene expression could contribute to the up-regulation of neurogenesis by rolipram. The second approach tested this possibility directly using an inducible transgenic strategy to overexpress a dominant negative mutant of CREB in hippocampal granule cells (Kim et al., 2000). Neurogenesis was significantly decreased in mice expressing the dominant negative mutant, providing additional evidence that the cAMP-CREB cascade exerts a positive effect on adult neurogenesis. Phosphorylated CREB is colocalized with BrdU and markers of maturing neurons, indicating that CREB may also be involved in the differentiation and maturation of newborn cells (Nakagawa et al., 2000). This possibility is supported by studies in cultured cells demonstrating that activation of the cAMP cascade increases the differentiation of cells into neurons (Palmer et al., 1997).

These findings indicate that activation of the cAMP-CREB cascade, either via receptors directly coupled to this second messenger system or via regulation of intracellular sites, could increase the proliferation as well as differentiation of granule cells in hippocampus. One possibility mentioned above is the 5-HT<sub>7</sub> receptor that is expressed in the granule cell layer and is directly coupled to the cAMP pathway. It is also possible that 5-HT<sub>1A</sub> receptor induction of neurogenesis is mediated by CREB. Although this receptor is known to inhibit cAMP and couple to other effector systems, depending on the cell type examined, recent studies also demonstrate that 5-HT<sub>1A</sub> receptors activate the mitogen-activated protein kinase cascade, which can also lead to phosphorylation of CREB (Mendez et al., 1999). Further characterization of the intracellular pathways that mediate the actions of 5-HT<sub>1A</sub> receptors, as well as the role of other intracellular pathways, in adult neurogenesis are required to answer these questions.

**Regulation of Neurogenesis by Growth Factors.** Another major area of interest has been the role of growth factors in the regulation of adult neurogenesis. Most studies to date have been conducted in cultured cells or during development (see Cameron et al., 1998), but there are a few reports in adult brain. In addition to the study of BDNF (Zigova et al., 1998), insulin-like growth factor-1 (IGF-1) is reported to increase hippocampal neurogenesis (Aberg et al., 2000). Surprisingly, IGF-1 administered peripherally has been found to enter the brain (Carro et al., 2000) and to increase granule cell proliferation (Aberg et al., 2000). Peripheral IGF-1 levels are also increased by exercise, and this

leads to increased binding of IGF-1 in hippocampus. This suggests that IGF-1 could mediate the induction of neurogenesis in response to exercise (Kemperman et al., 1997). These studies demonstrate a role for growth factors in the regulation of neurogenesis and the potential for using specific growth factor receptors, or their intracellular pathways, as targets to alter this process. However, progress must be made in generating small molecules that act at neurotrophic and growth factor receptors, an endeavor that has proven largely unsuccessful to date.

## Summary

The possibility that new neurons can form in adult and even aged brain has now been clearly established. Increased cell birth is associated with learning, memory, exercise, and antidepressant treatment, and decreased rates of cell proliferation are seen in response to stress and during aging. In addition, drugs, as well as hormones and growth factors, can regulate the rate of cell proliferation. These findings raise the possibility of developing agents that specifically influence cell proliferation in hippocampus and that influence the behaviors controlled by this brain region. However, the exact role of new neurons in the function of adult hippocampus has not been established. Approaches are currently being designed to directly inhibit granule cell proliferation to address this question. In addition, it is likely that the function of new neurons must be placed in the context of a particular behavior or stimulus. This implies that drug-induced regulation of neurogenesis should be combined with behavioral therapy to direct the function of new neurons. In spite of these uncertainties and complexities, the potential for therapeutic intervention involving specific regulation of neurogenesis is a powerful and exciting possibility for the future.

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