Adult Neurogenesis: From Precursors to Network and Physiology

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Abrous, Djoher Nora, Muriel Koehl, and Michel Le Moal. Adult Neurogenesis: From Precursors to Network and Physiology. Physiol Rev 85: 523–569, 2005; doi:10.1152/physrev.00055.2003.—The discovery that the adult mammalian brain creates new neurons from pools of stemlike cells was a breakthrough in neuroscience. Interestingly, this particular new form of structural brain plasticity seems specific to discrete brain regions, and most investigations concern the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampal formation (HF). Overall, two main lines of research have emerged over the last two decades: the first aims to understand the fundamental biological properties of neural stemlike cells (and their progeny) and the integration of the newly born neurons into preexisting networks, while the second focuses on understanding its relevance in brain functioning, which has been more extensively approached in the DG. Here, we propose an overview of the current knowledge on adult neurogenesis and its functional relevance for the adult brain. We first present an analysis of the methodological issues that have hampered progress in this field and describe the main neurogenic sites with their specificities. We will see that despite considerable progress, the levels of anatomic and functional integration of the newly born neurons within the host circuitry have yet to be elucidated. Then the intracellular mechanisms controlling neuronal fate are presented briefly, along with the extrinsic factors that regulate adult neurogenesis. We will see that a growing list of epigenetic factors that display a specificity of action depending on the neurogenic site under consideration has been identified. Finally, we review the progress accomplished in implicating neurogenesis in hippocampal functioning under physiological conditions and in the development of hippocampal-related pathologies such as epilepsy, mood disorders, and addiction. This constitutes a necessary step in promoting the development of therapeutic strategies.

I. NEUROGENESIS IN THE ADULT BRAIN: A NEW PARADIGM FOR STRUCTURE-FUNCTION RELATIONSHIPS

Developmental biology teaches us that brain functioning requires neurons of appropriate types to be generated in appropriate places and numbers, and then to migrate to their final positions and refine their synaptic contacts to a high degree of precision and avoid aberrant connections. Finally, the brain must accommodate growth of the organism and new behavioral or cognitive capacities. For decades, neurobiologists have known that neuronal network organization as well as individual performances are altered by environmental events, experience, or internal signals such as hormones, aging, and various injuries (322).

The adult brain faces two seemingly contradictory challenges. On the one hand it must maintain behavior
and thus preserve the underlying circuitry, and on the other hand, it must allow circuits to adapt to environmental challenges. This dilemma between stability and plasticity is a subject of debate. It has been suggested that the structural changes underlying plasticity exist at the levels of dendritic spines, dendrites, and axons (28, 287, 297, 383, 408). However, direct observation of dendrites and spines in the adult brain supports two divergent view points, i.e., both long-term dendrite stability (192, 368) and experience-dependent synaptic plasticity (541). Data suggest that dendritic spines are heterogeneous in shape and structure and can be roughly divided in two groups: large spines that survive for months and even years and small spines that are motile, change form rapidly, and either disappear or transform into large spines (for review, see Refs. 132, 216, 255). During long-term potentiation (LTP) of synaptic transmission, which is known to involve modifications in dendritic spine morphology (297, 337, 586), small spines are both generated and eliminated (143, 338). Given the structure-stability-function relationships, it has been suggested that the small spines are generated during activity-dependent processes and acquisition of memories, providing an inexhaustible source of new synapses, and that the large spines are the structural basis of memories in the long-term (255). For many authors, all these local and cellular phenomena have been labeled as neuroplasticity, a concept used to account for the functional observations.

In the search for structure-function relationships, great enthusiasm followed the discovery that embryonic neurons, for instance, dopaminergic neurons, could be transplanted into adult brains, survive and alleviate some postlesion behavioral alterations. Although it generated a considerable amount of data, this approach has not proven very successful so far in living up to functional and therapeutic hopes (157, 217). However, it has generated a considerable amount of data on the growth and the migration of these neurons, and on their ability to make synapse with the surrounding neurons of the host and to influence the activity of their target neurons. However, the turning point has been the demonstration that the adult mammalian brain is also capable of mitosis and that the generated cells differentiate into neurons that migrate to integrate circuitries. Interestingly, this particular new form of structural brain plasticity seems specific to discrete brain regions and most investigations concern the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampal formation (HF), although an interesting debate developed in the wake of the proposition that neurogenesis occurs in the neocortex of the adult primate.

In brief, the discovery of neurogenesis in the adult brain confronted the persistent assumption that adult neurons did not undergo proliferation and that structure could not be changed in this way. In the 1960s, Altman’s first observations of adult neurogenesis (11), followed by the studies of Kaplan and Hinds (for a historical view, see Ref. 249), were followed by negative reactions and critical publications that did not confirm the existence of newborn neurons in adults (452). Ironically, at about the same period, data were published on neurogenesis in birds related to the appearance of seasonal song, a discovery that gave rise to new thoughts on brain plasticity and adaptation (for review, see Ref. 410).

Overall, two main lines of research have emerged since the discovery that neurogenesis persists in discrete regions of the adult brain. The first aims to isolate neural stem cells and understand their fundamental biological properties, the ultimate objective being to manipulate them and enhance repair and regeneration. The second focuses on understanding its functional relevance, especially in the DG. We will thus approach these two lines of research in the following chapters. As we will see, it has now been unambiguously demonstrated that persistent neurogenesis occurs in the SVZ and DG of adult rodents. Most of our knowledge on the birth, proliferation, migration, and death of adult-born cells results from studies performed in the SVZ paradigm that easily allow the dissection of the properties of the stem cells (and their progeny) and the integration of the newly born neurons into preexisting networks. Once the existence of this phenomenon had been established, the important step was to understand what triggers and inhibits it, and how it is regulated. Identifying the functional roles of these adult-born neurons in the context of structure-function relationships will be the main focus of the review. The considerable evidence supporting the participation of these new neurons in brain functioning has been collected in the DG, and their role in information storage and hippocampal-related pathology will be examined. On the assumption that the DG transforms entorhinal inputs to facilitate storage in CA3, adult neurogenesis may allow the DG to adapt to new flows of relevant information while at the same time preserving it for the processing of old memories. In more general terms, we will ask whether neurogenesis acts on memory consolidation in a specific and functional manner or whether it prepares the HF for general experiences and challenges.

II. FROM NEWLY BORN CELLS TO NETWORK

A. Definitions

The recent interest in adult neurogenesis studies has led to a promiscuous use of the term stem cell and a broadening of its definition. However, based on their functional properties, one can distinguish “stem” cells from “progenitor or precursor” cells. Thus a stem cell is currently defined as an undifferentiated cell that exhibits
the ability to proliferate, to self-renew, and to differentiate into multiple yet distinct lineages (356, 570). In the adult brain, most stemlike cells are in a quiescent stage, except in the two neurogenic zones considered here, the SVZ and the DG of the HF, where they have a very slow dividing turnover (a few weeks). In contrast, progenitor cells are mitotic cells with a faster dividing cell cycle that retain the ability to proliferate and to give rise to terminally differentiated cells but are not capable of indefinite self-renewal; they are more committed than stemlike cells, and their multipotentiality is still a matter of debate. Finally, when the cell type being studied is not clear, as is the case in vivo, both stem and progenitor cells are referred to as precursor cells. For an overview of the characteristics that can be used to differentiate neural stem from progenitor cells, and of the controversies that still persist around definitions, we refer the reader to reviews by Gage (160), Geuna et al. (167), and Seaberg and van der Kooy (494).

B. In Vivo Evaluation of Adult Neurogenesis: Methodological Issues

1. Original studies: DNA synthesis staining and BrdU utilization

The original studies on in vivo cell proliferation relied on the use of [3H]thymidine ([3H]dT), which is incorporated into the cell during DNA synthesis, i.e., the S phase of the cell cycle. Mitotically active cells are subsequently revealed by autoradiography. More recently, BrdU, an analog of thymidine, has been used in lieu of [3H]dT (412). These markers have comparable availability times and labeling efficiencies (411). Although [3H]dT presents the advantage of good stoichiometry (allowing determination of the number of mitotic events after labeling), BrdU revealed by immunohistochemistry is more frequently used as the cost is lower, section processing is faster, and stereological analysis is possible. In addition, BrdU is easier to combine with other cellular markers, which makes it possible to phenotype the newly born cells by double or triple immunohistochemistry.

The original protocols used for BrdU labeling, established by Gage and colleagues (268, 267), consisted of a dozen BrdU injections (50 mg · kg\(^{-1}\) · day\(^{-1}\) ip). Since this pioneering work, with the flurry of data, protocols have diversified. As we will see in section II, the number and the doses of BrdU injections as well as the frequency of administration vary considerably from one experiment to another depending on the area of interest (fewer injections are required for studying the SVZ compared with the DG), the species, the phenomena being examined (a decrease or an increase in neurogenesis), and the subject type (young intact subjects, impaired or old individuals). To make the picture more complex, BrdU is sometimes administered in mice via drinking water (1–1.5 mg/ml for 2–4 wk) to minimize animal pain and distress (80, 332), with the disadvantage that individual daily intake is unknown.

Following BrdU injections, the killing of the animal is adjusted depending on the step of neurogenesis that is being investigated: cell proliferation, cell determination toward a given phenotype, cell migration and differentiation, or cell death. In this way, to study proliferation, animals are killed following the last BrdU injection within a short time lapse ranging from a few hours to a few days. As early as 2 h after a single injection, mitotic figures can be seen, and this time point has been proposed as a true measure of proliferation. However, in the case of senescent rats, multiple injections are required as cell proliferation and the frequency of labeled clusters are too low. Furthermore, when animals are killed within a short time after the last BrdU injection, newly born cells are small, irregular, and clustered (the clusters being more numerous with multiple BrdU injections), which renders quantitative analysis challenging. For this reason, a washout protocol of a few days allowing cells to migrate is applied in many experiments. Cell migration is followed by killing the animals at different days (1–10 days) after BrdU injection, in which case it is necessary to limit the number of BrdU injections (208). Survival of the newborn cells is usually studied by killing the animals after a long delay (several weeks) following BrdU administration, when the phenotype of the cells has been determined. Less frequently, retroviral tracing has also been used for “developmental” studies (81, 123, 439, 501). However, because retroviral infection is not controlled, labeling is quite variable and is thus unsuitable for quantitative analysis between different experimental groups (556). Finally, in most pharmacological and behavioral studies, the treatment under investigation is interrupted after BrdU pulses, and animals are killed after a “chase” period of several weeks. It is important to be aware that in this case, the influence of the treatment on cell determination and cell survival is not addressed. To do so, the “treatment” should be prolonged over the “chase” period time (for example, see Refs. 267, 335).

2. Phenotyping cells

One of the most important tasks is to phenotype cells, and markers specific to the dividing cells or the state of maturation of newly born cells are available for in vivo studies.

A) PRECURSOR CELLS. So far, no marker that is exclusive to stemlike cells has been identified, which certainly reflects the divergence in opinion on the identity of the stemlike cell population. However, markers of stem-cell candidates or simply dividing cells are now available and can be divided into two categories depending on the purpose
of the studies. The first category covers “universal” or general molecular markers that are used to define the biological characteristics of stemlike cells (for an extensive review, see Ref. 440). Among these markers, the most widely used is nestin, which defines classes of intermediate filament proteins; it was first described as the product of a gene whose expression distinguishes precursors from more differentiated cells in the neural tube (156) and was found to be specifically expressed in neural progenitor cells (307). More recently, a family of molecular markers has been identified, which includes mouse-Musashi1 (m-Msi1), a neural RNA-binding protein expressed predominantly in proliferating neuronal and/or glial precursor cells but not in newly generated postmitotic neurons and oligodendrocytes (246, 479).

The second category of markers is more specifically used in research aimed at uncovering the functional role of adult neurogenesis and consists of antigens present in cycling cells that are used as proliferative markers. They are often used in combination with BrdU, since incorporation of BrdU alone identifies cells undergoing DNA replication but does not formally provide evidence that these cells are actually capable of division. They include Ki-67, a nuclear protein expressed in dividing cells for the entire duration of their mitotic activity, the expression of which is neither linked to DNA repair nor to apoptotic processes (142, 259). Although less frequently used, proliferating cell nuclear antigen (PCNA) allows assessment of cell proliferation as well. It is an auxiliary protein of DNA polymerase δ which is increasingly expressed through G1, peaks at the G1/S interface, and decreases through G2 (146, 162, 294). This marker has been used successfully to study cell proliferation and cell cycle length in conjunction with BrdU cumulative labeling (39). Finally and much less used are P34cdc2, a key player in the initiation of mitosis (415), and phosphorylated Histone H3 (HH3) expression, which is confined to cells in the G2 and M phases of the cell cycle. In general, cells in the G2 phase exhibit punctuate HH3 nuclear staining that changes to a more condensed pattern once they enter the M phase (214).

B) IMMATURE NEURONS. The mammalian RNA-binding protein Hu, the homolog of the Drosophila neuron-specific RNA-binding protein Elav, is exclusively expressed in postmitotic neurons (7, 479) and thus is frequently used as an early neuronal marker (31, 414). Other markers include neuron-specific class III β-tubulin (TuJ1), a cytoskeletal protein expressed in all postmitotic neurons (360, 361), doublecortin (DCX), which encodes a microtubule-associated protein expressed in migrating neuroblasts (155, 168, 389), TOAD 64 (turned on after division, also called TUC-4, CRMP4, PULip1), a cytoplasmic protein expressed transiently by postmitotic neurons (366), and the polysialylated-neuronal cell adhesion molecule PSA-NCAM (277, 498). Another candidate for immature neuronal markers is the basic helix-loop-helix (bHLH) transcription factor NeuroD, which seems to be expressed from a very early stage to the stage where new neurons develop dendrites (496, 497). Some of these markers stain nonneuronal cells, and some of them (TOAD 64, PSANCAM, DCX) are present in the brain, for example, in the piriform cortex, independently of neurogenesis (113, 389, 392, 423, 498, 560), which makes the interpretation of results more difficult.

C) MATURE NEURONS. The most frequently used specific markers for mature neurons are neuron-specific enolase (NSE), neuronal specific nuclear protein (NeuN) (386), and microtubule-associated protein (MAP-2). However, the identification of the neuronal phenotype of newly generated cells using these markers is considered ambiguous as they also label other types of cells (189, 411, 453, 454). An alternative approach consists in demonstrating that adult-born cells do not express any marker for glial cells such as 2',3'-cyclic nucleotide (CNP) for oligodendrocytes, and calcium binding proteins (S100, S100β) or glial fibrillary acidic protein (GFAP) for astrocytes.

3. Current identified pitfalls

In addition to the problem of neuronal identification, several methodological pitfalls have been identified. First, embryonic and postnatal injections of BrdU have been reported to induce physical, morphological, and behavioral abnormalities (282, 495). Although only a few studies have addressed the deleterious effects of BrdU injections in adulthood, a neurotoxic effect has been reported in aged rats, loss of body weight, and deterioration in the state of the coat being induced by repeated administrations of 150 mg · kg⁻¹ · day⁻¹ of BrdU for 5 days (130). Together with its early teratogenic effects, this suggests that incorporation of BrdU into mitotically active cells may inhibit cell formation and affect stemlike cell population. This raises the as yet unresolved problem that BrdU may alter the functioning of the labeled cells. Second, changes in neurogenesis detected by variations in BrdU staining may be related to a modification in BrdU availability through an alteration in blood flow or in blood-brain barrier permeability. To rule out such confounding parameters, using endogenous markers of the cell cycle (Ki67, PCNA, HH3, and P34cdc2) and evaluating changes in different brain regions may be helpful. For behavioral studies, adequate control groups should be used to measure the involvement of a potential modification of blood flow by exercise. Third, pharmacological treatments or behavioral and environment-induced changes in neurogenesis may exert their effects by modifying the length of the cell cycle, the number of proliferating cells, or both. However, in most “functional” studies, evaluating the cell cycle parameters is difficult if not impossible, and one might question the relevance of this
“exercise.” Finally, BrdU or [³H]dT labeling might produce false negatives and positives. The most commonly used dose of BrdU (50 mg/kg) labels only a fraction of the proliferating cells (75), which may result in an apparent absence of effects of a given experimental setting on cell proliferation. However, multiple injections of this low dose may overcome this problem. Furthermore, because the precursor cell population most certainly does not divide synchronically, labeling all dividing cells with a single injection of BrdU, whatever the dose, appears utopian. On the other hand, higher doses of BrdU associated with an enhanced sensitivity of immunohistochemical methods might lead to labeling apoptotic cells or nonproliferating cells that synthesize DNA for repair (594), and thus to false positives. This problem is particularly relevant for studies on lesions, epilepsy, or ischemia, where a high rate of cell death is observed. There are several possible ways to rule out BrdU labeling of nonproliferating cells: 1) observing mitotic figures a few hours after BrdU or [³H]dT application; 2) using other endogenous cell cycle markers; 3) using electronic microscopy; 4) analyzing a time course of BrdU labeling (95); 5) combining BrdU with markers of cell death, although in this case false negative results due to the downregulation of markers by dying cells cannot be ruled out; 6) using retroviral labeling (81, 123, 439, 501, 556), which is not devoid of disadvantages (the type of cells incorporating the virus may not be fully controlled, and stereotoxic injection, known to cause injury, is required); and 7) visualizing neurogenic sites in nestin-promoter-GFP transgenic mice (577).

4. Summary

This last decade has been marked by tremendous advances including the development of BrdU labeling methods, the discovery of selective markers that differentiate neurons from glial cells, and the use of new molecular and genetic tools to follow the “development” of the newly born cells. As we will see in the following sections, this technical progress has led to a lot of research and data that have confirmed observations made in the early 1960s and considered at that time by many specialists as nonconclusive. However, researchers are now aware of problems and agree that proper interpretations depend on accepted rules: 1) [³H]dT and BrdU labeling are not sufficient to unambiguously differentiate old neurons from newborn cells, 2) the neuron-specific markers presently used must be complemented by appropriate controls to detect false positives and by electrophysiological recordings to assert real neuronal phenotypes, and 3) the maturation and the integration of the adult-born neurons must be followed up.

C. Integration of the Newly Born Cells into Neurogenic Site Networks

1. The SVZ

a) Identification of the Stemlike Cells. The SVZ, located throughout the lateral wall of the lateral ventricle, harbors the largest population of proliferating cells in the adult brain of rodents (12, 448, 511), monkeys (178, 179, 182, 248, 286, 434), and humans (47, 144), and it has been estimated that 30,000 cells are generated bilaterally daily in the mouse SVZ (317). This region exhibits a rostrocaudal gradient of proliferative activity: proliferation is higher in the dorsolateral corner of the rostral ventricle and falls caudally, moving from the striatum towards the HF (511). This gradient has been correlated with the capability of the constitutively proliferative cells to divide and to form neurospheres (381, 493) and certainly reflects the existence of two populations of dividing cells, one of quiescent stemlike cells and one of rapidly proliferating progenitor cells (381, 382). Four cell types have been described in the SVZ: 1) ependymal ciliated cells (type E) facing the lumen of the ventricle, whose function is to circulate the cerebrospinal fluid; 2) proliferating type A neuroblasts, expressing PSA-NCAM, Tuj1, and Hu, and unsheathing migrating type A neuroblasts; and 4) actively proliferating type C cells or “transit amplifying progenitors” expressing nestin, and forming clusters interspaced among chains throughout the SVZ (15, 55, 122, 124, 166, 220, 474).

Although it has been proposed that the stemlike cells may be ependymal type E cells, which were shown to divide in vivo and to differentiate into neurons in the OB (242), this view has been challenged by van der Kooy and colleagues (85) who have found that ependymal cells generating spheres do not have the ability to self-renew or to produce neurons, and by Capela and Temple (79) who have shown that ependymal cells do not form neurospheres. It is more likely that the type B cells represent a stemlike cell type (123, 124). Indeed, after 1 wk of intracerebroventricular (icv) infusion of the antimitotic drug cytosine-β-D-arabinofuranoside (AraC), type C and type A cells are eliminated, whereas type B cells continue to divide. Between 1 and 2 days after treatment cessation, type C cells reappear, followed 2 days later by type A cells, suggesting that type B cells give rise to type C cells, which in turn generate neuroblasts (125). Furthermore, following the targeted introduction of a retrovirus into GFAP-positive cells, which include type B cells, labeled cells migrate towards the OB where they give rise to new neurons (123). Thus the lineage progression B → C → A has been proposed (123, 124). However, Doetsch et al. (126) have also shown that after exposure to high concentrations of EGF, the type C cells retain stemlike cell...
properties (126). In fact, there are opposing views on whether neurospheres are derived from GFAP-positive cells (type B cells) or from fast proliferating type C cells (225, 380) (see Fig. 1).

B) MIGRATION OF THE NEWLY BORN CELLS THROUGH THE ROSTRAL MIGRATORY STREAM. The newly generated cells originating from different levels of the SVZ migrate in chains rostrally, up to 5 mm in rodents and to 20 mm in monkeys, to reach the OB (122, 286, 317). This migration, which follows the rostral migration stream (RMS) and requires 2–6 days in rodents (220, 317, 325), involves PSA-NCAM (100, 220, 418, 474, 540) and a chemorepulsion mechanism through Slit-Robo signaling (574). Although a role of the OB as a chemoattractant structure has been suggested, its involvement in proliferation and guidance of the newly born cells still remains unclear. Indeed, whereas OB removal (275), transection of the olfactory peduncle (231), or unilateral nostril occlusion (96) does not prevent SVZ precursors from proliferating and migrating towards the OB, a transection through the RMS (9) or a removal of the rostral OB (314) impedes neuroblast migration. It has thus been proposed that a diffusible attractant is secreted in specific layers in the OB, including the glomerular layer (314).

After reaching the middle of the OB, the newborn cells detach from chains, migrate radially, and progress into one of the overlying cell layers whereupon they undergo terminal differentiation. Neuroblast detachment from chains is initiated by Reelin and tenascin, whereas radial migration depends on tenascin-R (198, 478).

C) LIFE AND DEATH OF THE NEWLY BORN CELLS. Massive cell death has been observed during the first 2 mo after a BrdU pulse (572). This elimination mechanism is prominent in the OB compared with the RMS and the SVZ (39, 50, 439) and may maintain constant OB cell number by a continuous cell turnover, as was suggested during earlier development (419). The number of newly born cells that survive 1 mo after a BrdU pulse (50 mg · kg$^{-1}$ · day$^{-1}$ of BrdU for 4 consecutive days in 2-mo-old female wistar rats) has been estimated to be 60,000 per granule cell layer in one study (50) and 120,000 in another (572). In the latter case, it was shown that 50% of the newly generated neurons (~80,000 per granule cell layer and ~500 per periglomerular layer) that survived the initial period of cell death survived for at least 19 mo (572), confirming earlier work (253). With the use of retroviral labeling of precursors in the SVZ, it was confirmed that one-half of the labeled cells died shortly after their arrival in the OB (between 15 and 45 days after neuronal birth) and that most dying cells were mature, harboring dendritic arborization, and receiving connections (439). It was further shown in this study that survival of the newly generated granule cells depends on sensory input.

D) DIFFERENTIATION OF THE NEWLY BORN CELLS IN THE Olfactory BULb. The newly born cells differentiate mainly into neurons that grow dendritic trees and differentiate into two types of intrabulbar interneurons. Most of the cells (75–99%) differentiate into GABA granule cells (GCs), whereas a smaller number (1–25%) differentiate into periglomerular cells expressing GABA and/or tyrosine hydroxylase (38, 81, 256, 326, 439, 475, 572). Several months after their birth, 10,000 GABAergic granule neurons, 100 periglomerular GABAergic neurons, and >60 new dopa-
minergic periglomerular neurons have survived and are added per bulb daily (572).

The different maturation stages of adult-born granule cells have been observed using retroviral labeling of precursors in the SVZ (439). Five different classes of adult-born cells were distinguished according to their morphology and location: 1) migrating neuroblasts in the rostral extension of the RMS (days 2–7), 2) neuroblasts migrating radially (days 5–7), 3) GCs with dendritic processes that do not extend beyond the mitral cell layer (days 9–13), 4) GCs with a nonspiny dendritic arborization in the external plexiform layer (days 11–22), and 5) mature GCs with extensive dendritic arborization (days 15–30).

The newly born GCs and periglomerular neurons appear to be synaptic integrated into the existing circuitry as they are labeled following an injection (within the piriform cortex) of a green fluorescent protein (GFP) expressing pseudorabies virus (PVR GS518) known to be transported along the neurons and to cross synapses (80).

E) Functional Properties of the Newly Born Cells. The temporal sequence of electrophysiological changes in the adult born neurons has been observed by coupling live imaging (the newly born cells being labeled with a replication-defective retrovirus expressing eGFP) and patch-clamp recordings (81). Migrating neuroblasts [class 1 and 2 according to the description of Petreanu and Alvarez-Buylla (439)] were found to be silent, action potentials appearing later. Class 1 cells expressed functional GABA<sub>A</sub> receptors and AMPA receptors, and when they started migrating radially (class 2), they expressed functional N-methyl-D-aspartate (NMDA) receptors. Synaptic (GABA<sub>A</sub>nergic then glutamatergic) inputs were present in nonspiking neurons (class 3 and 4) while they were growing their dendrites. Spiking activity was the last property acquired by class 5 neurons, their electrophysiological characteristics being indistinguishable from those of older GCs.

The functionality of adult-born neurons was further examined using the induction of the immediate early gene product c-Fos, a marker for mapping activated neurons (222). With this approach, it has been shown that 1) in mice, 3- to 7-wk-old adult-born periglomerular neurons respond to physiological (odor) stimuli (80); and 2) in hamsters, sociosexual cues are able to activate the adult-born neurons localized in both the accessory and the main OB (221).

2. The DG

A) Identification of the Stemlike Cells. Cell proliferation was first demonstrated in the DG of rodents 40 years ago by autoradiography in a germinal zone which is not, in contrast to the SVZ, located close to the walls of the ventricle. This subgranular zone (SGZ) is located at the interface between the granule cell layer (GCL) and the hilus of the DG, deep within the parenchyma (12, 13). This cell proliferation is also a feature of monkeys (178, 182, 284) and humans (144). The nature of the proliferating cells is still a matter of debate. According to work carried out in Alvarez-Buylla’s laboratory (501), the stemlike cells may correspond to a subpopulation of GFAP-positive cells, equivalent to the type B cells described in the SVZ, since 2 h after their birth a large majority of newly born cells express GFAP (501). While the number of these cells decreases in the following days, the proportion of dividing GFAP-negative cells increases. These small dark cells, originally described by Kaplan and Bell (251), are called D cells. Chronic treatment with the antimotic AraC eliminates most dividing cells, D cells, and astrocytes, but soon after treatment cessation, some surviving B cells begin to divide, whereas D cells reappear only at 4 days. Type B cells, specifically labeled with an avian retrovirus, give rise to granule neurons with mossy fibers reaching the CA3 subfield. These results indicate that B cells act as stemlike cells, regenerate D cells, and function as transient precursors, and give rise to new granule neurons. This hypothesis is reinforced by the observation that astrocytes retain expression of m-Msi1 (480). On the basis of the analysis of nestin-promoter GFP transgenic mice, two classes of cells have been identified: type 1 cells (GFAP<sup>+</sup>, S100<sup>β</sup>, DCX<sup>+</sup>, PSA-NCAM<sup>+</sup>), which correspond to type B cells, and are the putative stem cell, and type 2 cells (GFAP<sup>−</sup>, S100<sup>β</sup>, DCX<sup>−</sup>, PSA-NCAM<sup>−</sup>) supposed to be the type D cells (154, 159, 289, 521). Because there is no overlap between glial and neuronal markers in type 2 cells, an observation inconsistent with Seri et al. lineage (501), it has been proposed that either type 1 cells divide asymmetrically and thus generate a neuronal lineage-restricted progenitor cell (type 2) and a glial lineage-restricted progenitor cell, or alternatively that a transient stage (lacking glial and neuronal markers) exists between the type 1 and type 2 cells (521). On the other hand, Palmer et al. (424) reported that very few proliferating cells in the adult DG are GFAP-positive (424), a discrepancy as yet unexplained. Finally, the assumption that the DG harbors stemlike cells has been challenged by showing that this region contains progenitor cells with restricted properties rather than stemlike cells with self-renewing properties (475, 493) (see Fig. 2).

B) Life and Death of the Newly Born Cells. Following a single pulse with <sup>3</sup>HdTh or BrdU, the number of labeled cells doubles within 24 h and then roughly doubles again over the next 2 days in rats (204, 416) and tree shrews (177), indicating that cells continue to divide during this time lapse (416). These data are in agreement with the measured length of the cell cycle estimated to be ~24 h in 3-mo-old rats (75). It has been proposed that the progeny continue to divide further during the first week after their birth as the number of labeled cells continues to rise (204). Recently, it has been confirmed that the cells are still in the cell cycle 3 days after their initial division (521).
The clusters of dividing cells have been shown to include several cell types: neural precursors, committed neuroblasts, glial precursors, and endothelial precursors (angioblasts representing 37% of proliferative cells) that are proximal to vessels (256, 363, 424). This suggests that neurogenesis most probably occurs within the context of vascular recruitment. Within this niche, endothelial cells may constitute a critical regulator for self-renewal of stemlike cells (505) through the release of trophic factors (see sect. II D2 C and Table 1).

In the rodent DG, the vast majority of newly born cells are postmitotic and at least partially differentiated between 3 and 7 days (111, 416), since during this period BrdU-labeled cells express NeuroD (417). The cells that do not terminally differentiate die within 1 wk of their generation, a process affecting 60% of the newborn cells (111, 204). In the macaque, a similar time course has been demonstrated with an initial rise in BrdU-labeled cells followed by a fall after 5 wk (182). Interestingly, the GCL harbors a much lower number of proliferating cells compared with the SVZ. Thus, in a study performed in 9- to 10-wk-old rats, ~9,000 newborn cells were found to be generated per day (75). In a more recent study, only ~4,000 new cells, out of which 3,000 were found to be new neurons, were shown to be added daily in the DG of 4-mo-old rats (456); this discrepancy in the number of newborn cells is certainly linked to the difference in the age of the animals used in the studies, as the production of new neurons in the DG diminishes with age (see sect. III C1 A). In the macaque, the number of newborn cells per day (~200) represents 0.004% of one GCL (284).

C) FATE OF THE NEWLY BORN CELLS. The newborn cells differentiate mainly into neurons in the GCL. In his original study using electron microscopy, Kaplan and co-workers (249–252) reported that the newborn cells exhibit the ultrastructural characteristics of neurons. Then, they were shown to express neuronal markers. During the first 2 days after their birth, most BrdU-labeled cells express nestin (95), then TuJ1 (75) or TOAD-64 (75, 536) in the following 3 days, and DCX between 1 and 14 days after their generation (65). DCX-BrdU-colabeled cells show features of progenitor cells as some of them coexpress Ki-67 and are thus still able to divide (58, 154, 521). This rapid postmitotic status of new cells has been confirmed with calretinin, a calcium binding protein that is transiently expressed by postmitotic cells, the expression of which is observed as early as 1 day after labeling dividing cells, a peak being reached after 1 wk (58). Although newly born cells express NeuN as early as 72 h after their generation (58, 193), only half of the BrdU-IR cells expressed NeuN over 3 wk (65). Using NSE, half of the newly born cells were found to be neurons 2 wk after their birth (78). Calbindin is expressed later, by 3 wk after birth (180, 290, 536, 537). A low percentage of adult-born cells differentiates into astrocytes (GFAP/S100β), and rare are those that adopt a microglial or oligodendrocyte phenotype (521). It should be emphasized that even 4 mo after their labeling a nonnegligible proportion of BrdU-IR cells have an unknown phenotype. In monkeys, most newly generated cells are claimed to be neurons as well since they express TuJ1, TOAD-64, NeuN, NSE, and calbindin, and rarely markers of astrocytes (GFAP) or oligodendrocytes (CNP) (178, 182, 284). It was first thought that the newly born cells did not survive, thereby constituting a continuously refreshed pool of neurons (189). However, it was
### TABLE 1. Extrinsic factors regulating in vivo cell proliferation and neurogenesis in the subventricular zone/olfactory bulb system and the dentate gyrus of the hippocampal formation under physiological conditions

<table>
<thead>
<tr>
<th>Factors</th>
<th>Subventricular Zone/Olfactory Bulb</th>
<th>Dentate Gyrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormones and peptides</td>
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<tr>
<td>Corticosterone</td>
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<td></td>
</tr>
<tr>
<td>Suppression (adx)</td>
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<tr>
<td>Cort. treatment</td>
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<td>✓</td>
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<tr>
<td>Estradiol</td>
<td></td>
<td></td>
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<tr>
<td>Estrus cycle</td>
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<td>✓</td>
</tr>
<tr>
<td>OVX</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>OVX + acute 17β-E</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>OVX + chronic 17β-E</td>
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<tr>
<td>17β-E</td>
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<tr>
<td>Prolactin</td>
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<td>PACAP</td>
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<td>Neurosteroids</td>
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<td>DHEA</td>
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<td>PregS</td>
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<td>Allopreg</td>
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<td>Glutamate</td>
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<tr>
<td>Ent. Cx. lesion</td>
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<tr>
<td>NMDA activation</td>
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<td>mGluR II blockade</td>
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<td>Serotonin</td>
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<tr>
<td>5-HT depletion</td>
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<tr>
<td>Lesion + graft</td>
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<tr>
<td>Reuptake inhibition</td>
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<tr>
<td>5-HT1a blockade</td>
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<tr>
<td>5-HT2A activation</td>
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<tr>
<td>Depletion</td>
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<tr>
<td>Reuptake inhibition</td>
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<tr>
<td>No donor</td>
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<td>BMP</td>
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<tr>
<td>Noggin</td>
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<tr>
<td>Vitamins and retinoids</td>
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<tr>
<td>Vitamin E</td>
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<tr>
<td>Deficiency</td>
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<td>✓</td>
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<tr>
<td>Supplementation (tocopherol)</td>
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<td></td>
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<tr>
<td>Retinoic acid (chronic</td>
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<td></td>
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<tr>
<td>treatment)</td>
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</table>

Adx, adrenalectomy; Cort, corticosterone; OVX, ovariectomy; 17β-E, 17β-estradiol; proE, proestrus; PregS, pregnenolone sulfate; Allopreg, allopregnanolone; Ent. Cx., entorhinal cortex; Hpx, hypophysectomy; 0, no change; blank case, not determined; ✓, increase; ✓, decrease.
more recently found that they do not have an ephemeral existence and survive for at least 11 mo (263).

Recently, it has been shown that beside glutamatergic granule neurons, a small percentage of newly born cells (14%) differentiate into GABAergic basket cells (316). Furthermore, under specific circumstances (ischemia or neonatal kainate administration), pyramidal cells in Ammon's horn are also generated (see also sect. int H; Refs. 128, 399, 489). However, the progenitors responsible for the regenerating pyramidal neurons originate from the periventricular region rather than from the SGZ (399).

D) INTEGRATION OF THE NEWLY BORN CELLS. The newborn cells integrate the GCL 4–10 days after their generation. As they form dendrites, they receive synaptic contacts and extend axons into the CA3 region, suggesting that they make synapses long before being fully mature (75, 204, 252, 344, 517). Indeed, using virus-based transynaptic activity neuronal tracing (PVR GS518), the neurons generated in the DG have been shown to be synaptically integrated into the preexisting circuitry 4–8 wk after their birth (80), whereas they reach a mature morphology (soma size, total dendritic length, dendritic branching, and spine density) only 4 mo after their birth (513).

E) FUNCTIONAL PROPERTIES OF THE NEWLY BORN CELLS. When still located at the interface of the hilus, the newly born cells exhibit electrophysiological properties characteristic of immature neurons: they are completely unaffected by GABA_A receptor inhibition, and they exhibit paired-pulse facilitation, have a lower threshold for induction of long-term potentiation (LTP), and display robust LTP (564). With the use of nestin-promoter GFP transgenic mice, type I cells (putative B cells) were found to have low input resistance values, whereas the type II cells (type D cells, expressing PSA-NCAM) exhibited higher input resistance and voltage-dependent sodium currents (159). Recently, PSA-NCAM expressing cells have been shown to differ from mature neurons in their passive (input resistance) and active membrane properties (such as calcium spikes that boost fast sodium action potentials) and in their enhanced ability to develop LTP (490). One month after their birth, the “newborn” neurons, labeled by a retroviral vector expressing GFP, exhibit some electrophysiological properties (input resistance, threshold potential for spiking and firing) similar to those of mature granule neurons (556). The stimulation of the perforant pathway, the main excitatory afference to the DG, elicits responses in “newborn” GFP-labeled cells, indicating that they receive functional synaptic inputs and are therefore functionally integrated into the preexisting network. However, a depression of synaptic currents is evoked in these cells following the paired-pulse stimulation of the perforant pathway, whereas a facilitation response is observed in mature cells, a discrepancy attributed to differences in presynaptic release properties (556). The functionality of the adult-born granule cells has been further demonstrated by showing, using c-fos, that 40% of 1-mo-old newborn granule neurons were able to respond to various chemoconvulsants (232, 234). In contrast, following a more physiological form of hippocampal stimulation consisting in a hippocampal-dependent learning task, only 3% of cells are activated (232). Finally, besides these functional excitatory adult-born granule cells, it has been shown that GABAergic newborn basket cells form functional inhibitory synapses with the granule cells (316). This discovery raises the question of the mechanisms and factors involved in the production of excitatory granule neurons versus inhibitory interneurons.

3. The cortex

Although very controversial, the existence of adult neurogenesis in the cortex deserves attention. Indeed, Altman and co-workers in the 1960s (11, 12, 14) and Kaplan in the early 1980s (247–249) reported evidence for neurogenesis in the cortex of rats and cats. More recently, neurogenesis has been described in the rat anterior neocortex (182) and in the prefrontal, inferior temporal, and posterior parietal cortex of macaques (179, 182). Within 2 wk after BrdU injection(s), the number of BrdU-labeled cells increased and then fell after 5 wk, indicating that a large number of newly born cells died. The surviving cells were assumed to be neurons since they extended axons locally and expressed markers of immature (TOAD 64, TuJ1) and mature (38–52% of BrdU-NeuN cells) neurons but did not express GFAP or CNP. Although the site of origin of the newly born neurons remains unclear, it has been proposed that they may be generated into the SVZ and migrate to neocortical areas through the white matter or may be recruited from local quiescent stemlike cells (179, 182, 284).

In contrast to these data, other groups have reported a complete absence of neurogenesis in the mouse (134, 332) and monkey cortex (281, 453). Neurogenesis was observed in the neocortex of mice only following a targeted apoptotic degeneration of corticothalamic neurons (332), and in this case, endogenous neural precursors migrated and differentiated into neocortical neurons in a layer- and region-specific manner and reformed appropriate long-distance corticothalamic connections; thus they appeared to reconstruct the lesioned circuits. In the primary neocortex, although cell proliferation occurs, the newly born cells do not seem to differentiate into neurons (285). These discrepancies may be due to differences in housing conditions, the animals’ histories, and genetic background, and other technical considerations (survival times, BrdU dosage and administration mode, immunohistochemistry...), but generally, the existence of this cortical neurogenesis is still a matter of debate (411).
NEUROGENESIS IN THE ADULT BRAIN

4. Summary

Following a century of doubt and controversies, there is now a consensus that neurogenesis occurs in the adult brain in at least two regions, the SVZ and the DG. In both structures, stemlike cells proliferate, migrate, and differentiate mainly into granule neurons that will synthesize GABA in the OB and glutamate in the DG. Although less studied, a small proportion of adult-born cells differentiate into other types of interneurons (the periglomerular in the OB and the basket cells in the DG). Altogether, this adult neurogenesis leads to the birth of ~30,000 new neurons per day in the SVZ and between 3,000 and 9,000 in the DG of young adult rats, depending on their age. The reasons for which, 1) the SVZ and the DG harbor adult neurogenesis, 2) neurogenesis is curtailed in the DG compared with the OB, and 3) genesis rates are much lower in primates, are currently unknown. Furthermore, because these structures do not grow in size, a homeostatic compensatory equilibrium must be attained through an increase in cell death that must be equivalent to the initial addition of neurons. This poorly understood phenomenon, in particular in the DG, deserves more attention for it is an important partner of neurogenesis. Finally, recent evidence indicates that the adult-born neurons of the OB and the DG are functional and thus play a physiological role. Although these findings suggest a relevant contribution of these newly generated neurons to the bulbar or hippocampal function, further studies are needed to confirm these reports and fully unravel their fundamental consequences on the animals’ behavior (see sect. III).

D. Factors Regulating Adult Neurogenesis

Although various factors that affect the division, migration, and differentiation of neural precursor cells have been isolated, the precise mechanisms that control neuronal fate in the adult nervous system remain largely unknown. Both cell intrinsic programs and extracellular/environmental factors, which we will review below, are at play.

1. Intrinsic programs controlling neurogenesis

Two fundamental decisions are involved in order for a precursor cell to generate a neural cell. The first one is to decide whether to self-renew or to undergo mitotic arrest, and the second is to interpret mitotic arrest, using cell autonomous cues that direct toward a particular fate. Much of our current understanding of these cell intrinsic programs comes from work performed when neurogenesis is at its best, i.e., during development. However, it is important to emphasize that the rules that govern neuronal specification during development may not be the same in the adult brain. Furthermore, since a description of the detailed molecular mechanisms involved in cell proliferation and determination is beyond the scope of this review, we here propose only a brief overview of the intrinsic factors involved, with a special mention whenever their involvement has been extended to the adult neurogenic zones.

A) Control of cell proliferation. Among the cell cycle factors regulating cellular proliferation, Rb (retinoblastoma) and its related proteins (p107, p130), necdin, and the E2F protein families are key players (for a review, see Ref. 580). Thus, during the G1 phase, Rb predominates in a hypophosphorylated form that can bind to E2F, a positive regulator of the cell cycle (403), thereby repressing its transcriptional activity and preventing the cells from entering the S phase. In cycling cells, phosphorylated Rb accumulated during the late G1 phase releases E2F, thus allowing S phase entry. This phosphorylation depends on the activation of cyclin-dependent kinases (CDK) acting sequentially. Early to mid-G1, cyclins of the D class (D1, D2, and D3) activate CDK4/CDK6, and in late G1, cyclin E activates CDK2, leading to hyperphosphorylation of the Rb protein. Two families of CDK inhibitors (CDKIs) that can suppress cell proliferation by inhibiting Rb phosphorylation have been identified (566): members of the inhibitors of CDK4 family (INK4 family), including p15^Ink4b, p16^Ink4a, p18^Ink4c, and p19^Ink4d, and members of the kinase inhibitory protein family (Cip/Kip family) such as p21^Waf1/Cip1, p27^Kip1 or p57^Kip2 (see Fig. 3).

It is now clear that the Rb and E2F protein families are differentially expressed in proliferative and postmitotic cells of the adult brain. Thus, in the two neurogenic sites, the DG and the SVZ, Rb immunoreactivity is high in proliferating neuronal precursors and reduced during terminal differentiation (415), which implies that the transient increase in the level of Rb is an important step in the initiation of terminal mitosis in neuronal progenitors. Moreover, the Rb protein family was found to be essential for the development of a neural lineage and the exit from the cell cycle, whereas it does not seem to be involved in the maintenance of postmitotic neurons (69, 89, 228, 299). The role of E2F1 as a key factor in regulating adult neurogenesis has been further emphasized in a recent study performed in mice lacking E2F1. These mice, when adult, exhibit a lower level of cell proliferation and a reduction in the number of neurons generated in adult neurogenic areas (94).

The role of cell cycle regulators in the control of neuron production has been studied as well. Transgenic mice that lack p27^Kip1 expression display a higher rate of cell proliferation versus differentiation in the SVZ, leading to an increased number of type C cells, a reduced number of type A neuroblasts, and no change in the number of type B cells (127). Finally, distinct functions for CDK inhibitors, either in the control of cell cycle exit and differentiation during neurogenesis (respectively, p27^Kip1...
and p19\textsuperscript{Ink4d}) or in the maintenance of a quiescent state in neural progenitors (p18\textsuperscript{Ink4c}) or neurons (p21\textsuperscript{Cip1}) in adults have been underlined (303).

**B) CONTROL OF CELL FATE.** Data from developmental biology have clearly indicated that a core genetic program involving multiple bHLH transcription factors is required for both neuronal differentiation and determination. These factors, deriving from proneural and neurogenic genes, antagonistically control the switch from cell proliferation to neural differentiation: cascades of neuronal bHLH genes promote differentiation, whereas antineuronal bHLH genes repress them under the control of Notch and keep cells at a precursor stage (for recent general reviews, see Refs. 358, 473).

Two classes of proneural genes can be distinguished: the determination factors, such as Mash1 (mammalian achaete-scute homolog), Math1 (mammalian atonal homolog), and Ngns (Neurogenins) including Ngn2, expressed early in mitotic neural precursor cells, and the differentiation factors, including NeuroD, NeuroD2, and Math2, expressed later in postmitotic cells (for reviews, see Refs. 49, 379). It was recently determined that these proneural genes are downstream effectors of Pax6 (for a review, see Ref. 174), a transcription factor that promotes neurogenesis (210).

These proneural genes are components of a cell-cell signaling mechanism whereby a cell that becomes committed to a neuronal fate inhibits its neighbor from doing likewise. This process of lateral inhibition, which restricts the domains of the proneural gene activity and involves neurogenic genes, is mediated by the Notch pathway (for reviews, see Refs. 23, 32, 161, 244, 491, 567). Among the effectors of Notch, three families of negative regulators of the bHLH transcription factors are known: the HES, Id, and HES-related (HESR or HERP) families (226, 413, 481, 545). These effectors repress neuronal determination and differentiation in those cells not destined to become neuroblasts. Paralleling this regulation of neuronal proliferation by inhibiting neuronal fate, Notch was also found to be involved in determining glial fate (186, 398, 467).

Recent studies have highlighted that some of these factors, which are at play during development, may be involved in neuronal cell fate during adulthood. Thus Notch1 and Hes5 were found to be expressed at high levels and with a similar pattern in both the adult SVZ and DG, whereas numb and numblike, which negatively regulate the Notch signal transduction, were not, suggesting an active Notch signal in the adult neurogenic zones (523). Similarly, the mRNA expression of several bHLH factors was found to be present at various degrees in the adult HF, ranging from the restricted expression of Mash1 within the proliferative SGZ, which is consistent with its role in maintaining a precursor cell phenotype, to the widespread profile of Hes5 throughout the HF (140).

In conclusion, despite the considerable advances achieved recently with the development of molecular tools, the complex intrinsic machinery that controls and coordinates proliferation and differentiation is still poorly understood. As is the case for hematopoiesis, for which much progress has been achieved, understanding the gene expression patterns of progenitor cells and their progeny will be a critical step in elucidating the mechanisms underlying cell fate.
2. Extrinsic factors regulating neurogenesis

A) Hormones and neurosteroids. I) Adrenal corticosteroids. Historically, corticosteroids were the first factors to be studied for their influence on adult neurogenesis (354). Corticosteroids are released into the blood circulation following the activation of the hypothalamo-pituitary-adrenal (HPA) axis, primarily by stress (351). Corticosterone, the main corticosteroid in rodents, regulates its own secretion through negative feedback, by interacting with two receptors (the mineralocorticoid MR, the glucocorticoid GR), present in the DG. Suppression of corticosterone secretion after bilateral adrenalectomy (adx) increases glial and neuronal births in the DG (71, 176), whereas mitotic activity in the SVZ remains unchanged (465), suggesting a site-specific inhibitory influence of corticosteroids. It was further shown that proliferation increases within 24 h after adx and remains constant over the 6 subsequent days; the newly generated cells survive for at least 4 wk in the absence of corticosterone, indicating that their survival is corticosterone independent (465). Cell death is also enhanced, but the populations of cells undergoing mitosis or apoptosis are distinct: immature cells divide at the interface of the hilus, whereas more mature neurons, located at the interface of the molecular layer, die (72). These alterations are prevented by corticosterone replacement (176, 465). The respective roles of MR and GR on adrenalectomy-induced structural modifications have recently been examined (374). Treatment with a low dose of the MR agonist, aldosterone, prevents adrenalectomy-induced increase in cell death, whereas a higher dose is necessary to normalize cell proliferation. Furthermore, treatment with a GR agonist, RU 28362, at doses that should fully occupy this receptor prevents both adrenalectomy-induced cell death and birth. Thus the stimulation of both MR and GR mediates the effects of corticosterone on cell proliferation and protects mature cells from cell death.

This inhibitory action of corticosterone on neurogenesis has also been found in other models: 1) acute corticosterone administration [2 × 40 mg/kg administered 1 h before a single [H]dT injection (71, 76); implantation of a 200-mg corticosterone pellet followed by 1 × 200 mg/kg BrdU (17)], 2) chronic corticosterone treatment [1 × 40 mg/kg for 21 days; BrdU 10 × 50 mg/kg every 12 h on days 17–21 (212)], 3) stress (see sect. mB1), and 4) interindividual differences in the activity of the HPA axis (305; see sect. mB2).

The mechanisms by which corticosterone hampers cell proliferation remain unknown. In vitro, glucocorticoids block the G1 phase of the cell cycle, notably through repression of cyclin D1, cyclin D2, Cdk4 and Cdk6, and induction of the CDKIs p21^Cip1 and p27^Kip1 (438, 585, 592). However, it remains to be determined which of these mechanisms is involved in vivo. Because only a small minority of precursor cells express corticoid receptor (77), corticosterone may not act directly on proliferating precursors, although it cannot be excluded that immature forms of receptors that are not recognized by current antibodies are involved. Corticosterone could act on neighboring glial or neuronal cells expressing corticosteroid receptors, which could control the cell cycle by releasing growth factors (515). Alternatively, corticosterone could regulate proliferation indirectly by increasing glutamate release in the DG (520) (see sect. nD2h) as the effects of adx or high levels of corticosterone can be blocked by NMDA receptor activation or inactivation, respectively (76). Corticosterone may also inhibit cell proliferation by downregulating the production of insulin-like growth factor I (IGF-I) (585, 592).

II) Gonadal hormones. The influence of female gonadal hormones has been investigated in the DG since estrogen replacement therapy seems to reduce the risk of age-related cognitive impairments (213). Although cell proliferation in the GCL (and not the hilus) is higher in female than in male rats, the newly born cells do not survive, which explains the lack of sex differences in the number of BrdU-IR cells 2 wk after labeling (536). Sex-dependent proliferative activity involves the stimulatory influence of estrogens, since the number of BrdU-labeled cells is highest during the proestrus phase of the estrus cycle, when circulating levels of estrogens are highest (536), and acute administration of 17β-estradiol reverses the ovariectomy-induced decrease in cell birth (29, 536).

In contrast, using TOAD-64 and calbindin as early and late neuronal markers, it has been shown that estradiol does not alter neuronal differentiation (536).

However, discrepant results have been reported following chronic administration of estradiol to ovariectomized adult female rats (435), spontaneous hypertensive rats (436), or adult wild meadow voles (163). Cell proliferation in the GCL (measured 24 h after a single intraperitoneal injection of [3H]dT) is lower in female meadow rats (436), or adult wild meadow voles (163). Cell proliferation in the GCL (measured 24 h after a single intraperitoneal injection of [3H]dT) is lower in female meadow voles captured during the breeding season, when estradiol levels are high, compared with reproductively inactive females. A similar relationship has been confirmed in laboratory-reared female meadow voles (421).

These apparently contradictory results might be explained by a complex regulatory mechanism. In fact, a single administration of estradiol initially enhances (within 4 h) and subsequently suppresses (within 48 h) cell proliferation in the DG of ovariectomized female rats or meadow voles (420, 421). The increase in cell proliferation is mediated by serotonin (29), whereas the decrease is prevented by adx (422), suggesting an involvement of corticosterone. However, although corticosterone-induced regulation of cell birth certainly involves NMDA receptors, estradiol influences on cell proliferation are not mediated by these receptors (420). Finally, estrogen may act directly on estrogen receptors subtype α (ER α)
present on hippocampal precursors (435) or through IFG-I receptors (see sect. mD2c).

III) Neurosteroids. Neurosteroids represent a subclass of steroids synthesized de novo in many regions of the brain, independently of peripheral sources (36). They are synthesized in the HF, by glial cells mainly, and influence hippocampal-mediated functions (349). Two principal families can be distinguished according to their pharmacological properties (333). In particular, dehydroepiandrosterone (DHEA) and pregnenolone sulfate (Preg-S) act as allosteric antagonists of the GABA_{\text{A}} receptors while allopregnanolone (AlloP) is a positive modulator of these receptors.

Chronic treatment of young adult male rats with subcutaneous pellets of DHEA (200 mg/pellet for 12 days) has been shown to stimulate hippocampal cell proliferation measured 24 h after BrdU injections (50 mg \cdot kg\(^{-1}\) \cdot day\(^{-1}\)) given on the last 4 days of the steroid treatment (254). After an additional 16 days of treatment, the newly born cells survive and express the neuronal marker NeuN. Interestingly, DHEA treatment is also able to reverse the suppressive effect of corticosterone on neurogenesis, suggesting that it may act as an antistress neurohormone.

Recently, we have shown that icv infusion of Preg-S (2 \times 12 nmol/7 \mu l) increases cell proliferation in the DG of young adult rats within 24 h, whereas treatment with AlloP (2 \times 6.3 nmol/7 \mu l) decreases it (348). The newly generated cells survive for at least 1 mo and differentiate mainly into neurons (expressing NeuN). These actions of Preg-S are mediated by GABA_{\text{A}} receptors present on hippocampal precursors, since icv administration of muscimol, a GABA_{\text{A}} agonist, blocks Preg-S-induced cell proliferation. Furthermore, a direct action of Preg-S was suggested as this compound stimulates in vitro the proliferation of spheres derived from the adult SVZ (348).

Altogether, the reported effects of DHEA and Preg-S are congruent with the observations that in vitro activation of GABA_{\text{A}} receptors inhibits cortical cell proliferation, whereas GABA_{\text{A}} receptors antagonists stimulate it (21, 318, 327).

B) NEUROTRANSMITTERS AND NEUROREGULATORS. I) Glutamate. Destruction of the perforant pathway, the main glutamatergic afference to the DG arising from the entorhinal cortex, increases cell proliferation, thus indicating that under these experimental conditions glutamate exerts an inhibitory influence (73). As expected, blockade of NMDA subtypes of glutamate receptors by injection of a noncompetitive antagonist (MK801) increases cell genesis within a few hours in rats (1 mg/kg; Refs. 73, 76), tree shrews (1 mg/kg; Ref. 177), gerbils (3 \times 3 mg/kg; Ref. 46), and ovariectomized adult female meadow voles (30 mg/kg; Ref. 420), and treatment with the competitive NMDA receptor antagonist CGP 37849 (5 mg/kg) increases both cell proliferation and granule neurons density (73). Conversely, administration of NMDA (30 mg/kg) decreases cell proliferation in several species within hours (73, 76, 420), which is congruent with the antiproliferative properties of glutamate on in vitro cortical cell proliferation (318). However, it should be mentioned that an inhibitory influence of glutamate receptor blockade on neurogenesis has been reported in stroke-damaged brains (see sect. mD1b). The newly born cells induced by the inactivation of NMDA receptors differentiate into neurons, expressing DCX, TOAD-64, NSE, or NeuN markers (73, 177, 390, 417). Furthermore, the MK-801-induced neurons are functional as they respond to NMDA stimulation, measured by the phosphorylation of extracellular signal-regulated kinase (ERK), 29 days after their birth date (417). This indicates that newly born neurons acquire components for the intracellular signal transduction cascade linking NMDA receptors to phosphorylation of ERK (114).

The mechanisms by which precursor proliferation is inhibited by glutamate in vivo through NMDA receptors remain unknown, but they most probably do not involve collateral deleterious effects as treatment with CGP 43487 upregulates cell birth without inducing cell death or astrogliosis (300).

Because glutamate also acts onto AMPA receptors, detected in neural progenitors (165), the influence of potentiators of AMPA receptors such as LY451646 has been evaluated on hippocampal mitotic activity. Acute administration of LY451646 (0.025, 0.05, 0.125 mg/kg) does not influence the number of newly born cells observed 24 h after BrdU pulse (4 \times 75 mg/kg every 2 h). However, the median dose increases the number of cells per cluster (64%) and the number of clusters (45%) (27). Furthermore, chronic treatment (21 days) with LY451646 (0.05, 0.125, 0.500 mg/kg) enhances the number of proliferating cells, of cells per cluster, and of clusters in a dose-dependent manner (27).

Altogether, these results suggest that glutamate exerts a complex influence on hippocampal cell proliferation, increasing it through activation of AMPA receptors, and inhibiting it through activation of NMDA receptors. Finally, the recent discovery that GABA and glutamate are cotransmitted at the mossy fibers synapses adds further complexity to the respective role of each neurotransmitter in neurogenesis regulation (196).

II) Serotonin. In the 1970s, it was proposed that early forming serotonin (5-HT) neurons act as humoral signals governing neuronal development and neurogenesis in particular (26, 298). Since then, several approaches have shown that serotonin upregulates cell proliferation in the adult DG and SVZ (see also sect. mD2a). Inhibition of 5-HT synthesis (by subchronic injections of PCPA for 6 days) and selective lesions of 5-HT neurons of the raphé decrease the number of BrdU- and PSA-NCAM-IR cells in the DG and the SVZ (BrdU 50 mg/kg ip injected on the last 3 days of PCPA treatment at 1–2h intervals and animals killed 6 h after the last injection; Ref. 61). This upreg ula-
tion of hippocampal cell proliferation depends on serotonin as it is reversed by intrahippocampal grafts of embryonic 5-HT neurons (63). Partial lesions of 5-HT raphe neurons lead, after an initial deprivation of hippocampal 5-HT fibers, to a spontaneous reinnervation of this structure by 5-HT fibers. At this time, the fall of mitotic activity (and PSA-NCAM expression) is reversed (62). These actions of serotonin are mediated by 5-HT1A receptors in both the DG and the SVZ (30, 451) while 5-HT2A and 5-HT2C receptors are selectively involved in the regulation of cell proliferation in the DG and SVZ, respectively (30).

Finally, 4 wk after chronic treatment with 5-HT1A or 5-HT2A agonists (daily injection of 8-OH-DPAT or RO 600175 for 15 days, 75 mg/kg of BrdU injected twice on the last 8 days of the treatment), the newly born cells in the DG and/or the OB express NeuN (30).

III) Nitric oxide. Nitric oxide (NO), a free radical molecule synthesized from L-arginine by NO synthase, serves as a neurotransmitter in the brain (8). In the SVZ of adult mice, neuronal precursors have been found to be within the sphere of influence of a NO source and to express NO synthase at the sites of terminal differentiation (377). Administration of DETA/NONOate, a NO donor, to young adult rats significantly increases both cell proliferation and migration in the SVZ and the DG (587).

C) Trophic factors. Many trophic factors have been shown to have mitogenic actions in the adult brain neurogenic regions. Thus the proliferative effects of basic fibroblast growth factor (bFGF or FGF-2) have been clearly demonstrated for the SVZ. Chronic administration of bFGF intracerebroventrically (360 ng/day for 14 days, 50 mg · kg⁻¹ · day⁻¹ of BrdU being injected during the last 12 days of the treatment; Ref. 291) or intranasally (5 × 0.2 µg/day at 5-min intervals for 1 wk concurrent with 2 × 50 mg · kg⁻¹ · day⁻¹ of BrdU at 8-h intervals; Ref. 240) increases the number of mitotic nuclei in the SVZ within 24 h. This leads to an increased number of newly born neurons (expressing DCX in the SVZ; Ref. 240) that reach the OB where they express NeuN (4 wk after their birth; Ref. 291). In contrast, in the DG, a lack of effect (291) or a small but nonsignificant increase in cell birth (25%) following 3 days icv infusion of 10 µg/ml was reported (238). Consistently, in mice lacking bFGF, basal hippocampal neurogenesis is “normal,” suggesting that other factors may maintain low levels of precursor proliferation under resting conditions (582). However, overexpression of bFGF by gene transfer in wild-type and bFGF-deficient mice upregulates DG cell proliferation, which indicates that a robust and constant bFGF expression is a necessary condition for increasing cell birth in this structure (582).

Epidermal growth factor (EGF; 350 ng/day for 14 days, 50 mg · kg⁻¹ · day⁻¹ of BrdU being injected during the last 12 days of the treatment; Ref. 291) and heparin-binding EGF (HB-EGF; 3 days icv infusion of 10 µg/ml concurrent with 2 × 50 mg · kg⁻¹ · day⁻¹ of BrdU at 8-h intervals; Refs. 236, 238) stimulate cell birth in the SVZ (1 and 7 days after growth factor infusion, respectively) certainly via EGF receptors (381, 416, 502). However, these two factors probably have distinct actions, since HB-EGF increases the number of BrdU-IR cells expressing DCX or NeuroD in the SVZ as well as the number of newly generated cells reaching the OB (236, 240), whereas EGF reduces the number of newly born neurons reaching the OB (291). In the DG, HB-EGF (236, 238) but not EGF (291) increases cell proliferation by interacting most probably with EGF receptors expressed by the dividing cells (416). The adult-born cells express the neuronal marker NeuroD (236).

Transforming growth factor (TGF)-α is required for SVZ precursor proliferation. Indeed, icv administration of TGF-α (400 ng/day for 6 days) induces a dramatic increase in precursor proliferation (98), whereas TGF-α null mice show a decrease in proliferating cells in the SVZ, and in the total number of newly born cells within their target, the OB (543). No data on the influence of TGF-α on hippocampal precursors are yet available.

IGF-I is a growth-promoting peptide hormone that is produced in the CNS by neurons and glial cells (20) and exhibits neurotrophic properties in adulthood (405). Its influence on neurogenesis has been examined in hypophysectomized rats presenting low levels of circulating IGF-I (1). Thus peripheral administration of IGF-I (1.25 mg · kg⁻¹ · day⁻¹) induces an increase of cell proliferation in the dentate GCL and the hilus after 6 days. Long-term treatment (0.39 mg · kg⁻¹ · day⁻¹ for 20 days) increases both BrdU-labeled cell number and their differentiation into neurons as measured by the percentage of BrdU-IR cells expressing calbindin. In contrast, an IGF-I-induced increase in cell proliferation in nonhypophysectomized rats is not associated with an enhancement of their differentiation potential toward a neuronal fate (542). In vitro experiments (2) strongly suggest that IGF-I regulates cell proliferation in vivo by acting directly on IGF-I receptors expressed by newly born cells (542), but a recent study has also shown that estrogen receptors are necessary for the induction of in vivo hippocampal cell proliferation by IGF-I (435).

Brain-derived neurotrophic factor (BDNF) is a member of a family of related neurotrophic proteins, the function of which is to prevent neurons from dying during development. Chronic icv infusion of BDNF (0.012 µl/day for 12 days) increased BrdU-labeled cells in the SVZ and in the GCL of the OB (593). The newly born cells expressed TuJ1 or MAP2. Survival (169, 276) and/or differentiation (6, 22) of the neuronal precursors and their progeny rather than proliferation seem to be influenced by BDNF. Concerning the DG, it has been shown that heterozygous BDNF knockout mice exhibit reduced proliferation and survival of BrdU-labeled cells (301), sug-
sion of Shh in structures that project towards the DG and Ammon’s horn in the HF, and the high levels of expression of Shh in structures that project towards the DG support the assumption that Shh could be an endogenous protein that acts through Patched 1-Smoothened (Ptc1-Smo) receptor complex to trigger various events during CNS development, including determination of ventral neural phenotypes, induction of oligodendrocyte precursors, proliferation of specific neuron progenitor populations, and modulation of growth cone movements (for a review, see Ref. 346). In a comprehensive study, Lai and co-workers (295) investigated the role of Shh in the adult HF through antagonism of BMPs, which alter the fate of neural stemlike cells from neurogenesis to astroglial differentiation, whereas their antagonist Noggin promotes neurogenesis (311). It has been proposed that Noggin produced by the type E cells antagonizes the type B cells expressing BMP (311). Furthermore, BMP2, BMP4, and BMP7 have been found to activate a promoter of the gene for the HLH factor Id1, which is known to inhibit the function of neurogenic transcription factors such as Mash1 and neurogenin. Thus the switch from neuronal to astrocyte fate realized by BMPs certainly involves HLH proteins (578). These findings were extended to the HF. Indeed, a novel secretory factor, neurogenin-1 (Ng1), released by hippocampal astrocytes and dentate granule cells adjacent to progenitor cells, was found to promote neuronal fate in the adult HF through antagonism of BMPs, which alter the fate of neural stemlike cells from neurogenesis to astrogligogenesis by upregulating the expression of the negative HLH factors Id1, Id3, and Hes5 (548).

E) Regulation by Glial Cells. Regulation of adult neurogenesis by glial cells has been emphasized (500). Astrocytes, which play an important role as sensors of changes in their extracellular microenvironment, could regulate neurogenesis by secreting local signals (514). These signals, unknown so far, may be ionic fluxes, neurosteroids, cytokines, growth factors, and glutamate metabolites (48, 243, 245, 296, 486, 516, 579, 595). The observation that some proliferating cells in the DG express a receptor for S100β, a small acidic calcium binding neurotrophic protein released by astrocytes, reinforces the putative contribution of glial cells in the regulation of adult neurogenesis (339).

F) Regulation by Cell Death. An equilibrium between neurogenesis and cell death probably ensures a homeostatic balance in the adult brain. This hypothesis, based on the observation that the neurogenic structures do not grow in size, implies that cell death provides a stimulus for increased neurogenesis, a hypothesis reinforced by several lines of argument: 1) during development, there is a balance between the birth and death of granule cells (183); 2) mechanical lesions, aspiration, and transection stimulate neuronal precursor proliferation in the GCL and/or the SVZ (180, 242, 531, 546, 568); 3) proliferation in the SVZ is upregulated under inflammatory conditions (70); 4) adrenalectomy, limbic seizures, and ischemia enhance both cell death and cell birth; and 5) the apoptotic degeneration of corticothalamic neurons (by means of targeted photolysis) induces endogenous neural precursors to dif-

Vascular endothelial growth factor (VEGF) is a hypoxia-induced angiogenic protein that exhibits neurotrophic and neuroprotective properties (359). Given that neurogenesis occurs in close proximity to blood vessels and that clusters of dividing cells contain endothelial precursors (see sect. II C2b), VEGF may constitute the link between neurogenesis and angiogenesis. This is supported by the observation that icv administration of VEGF (0.24 μg/day for 3 days concurrent with 2 × 50 mg · kg⁻¹ · day⁻¹ BrdU) stimulates cell proliferation in the rodent SVZ and SGZ by 7 days after treatment onset (241). At that time, the newly born cells express DCX and NeuN markers. The neuroproliferative effect of VEGF is associated with an upregulation of cyclin D1, Cyclin E and cdc25, and an increase in the nuclear expression of E2F1, E2F2, and E2F3 (591), which is consistent with a regulation of the G₁/S phase transition of the cell cycle.

D) Morphogenic Factors. Sonic hedgehog (Shh), an important morphogen in development, is a signaling glycoprotein that acts through Patched 1-Smoothened (Ptc1-Smo) receptor complex to trigger various events during CNS development, including determination of ventral neural phenotypes, induction of oligodendrocyte precursors, proliferation of specific neuron progenitor populations, and modulation of growth cone movements (for a review, see Ref. 346). In a comprehensive study, Lai and co-workers (295) investigated the role of Shh in the adult brain and showed that it increased proliferation of cultured adult rat hippocampal progenitors in a dose- and time-dependent fashion. In vivo, the exogenous administration of Shh and the inhibition of its signaling increased and reduced cell proliferation, respectively. Finally, the presence of the Shh receptor Patched in the DG and Ammon’s horn in the HF, and the high levels of expression of Shh in structures that project towards the DG support the assumption that Shh could be an endogenous positive regulator of cell proliferation in the adult DG (295).

Another group of early neural morphogens, the bone morphogenic proteins (BMPs), belongs to the TGF-β superfamily, which includes TGF-β, activins, and the relatives named growth/differentiating factors (GDF). These glycoproteins play a crucial role in bone remodeling and in the regulation of dorsoventral patterning of the neural tube and cell fate during embryonic development. Furthermore, several BMPs, including BMP4, are involved in repression of the oligodendroglial lineage and generation of the astroglial lineage during brain maturation (190). Similarly, in the adult SVZ, BMPs (BMP2, BMP4, BMP7) inhibit neurogenesis and direct astroglial differentiation, whereas their antagonist Noggin promotes neurogenesis (311). It has been proposed that Noggin produced by the type E cells antagonizes the type B cells expressing BMP (311). Furthermore, BMP2, BMP4, and BMP7 have been found to activate a promoter of the gene for the HLH factor Id1, which is known to inhibit the function of neurogenic transcription factors such as Mash1 and neurogenin. Thus the switch from neuronal to astrocyte fate realized by BMPs certainly involves HLH proteins (578). These findings were extended to the HF. Indeed, a novel secretory factor, neurogenin-1 (Ng1), released by hippocampal astrocytes and dentate granule cells adjacent to progenitor cells, was found to promote neuronal fate in the adult HF through antagonism of BMPs, which alter the fate of neural stemlike cells from neurogenesis to astrogligogenesis by upregulating the expression of the negative HLH factors Id1, Id3, and Hes5 (548).

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differentiate into mature neurons in regions of the cortex undergoing targeted neuronal death (332). The induction of neurogenesis in these regions of the adult neocortex that do not normally undergo any neurogenesis (332) may result from the removal of a normal inhibitory influence or the loss of a secreted stimulatory factor produced by the dying cells.

G) SUMMARY. Adult neurogenesis within each neurogenic site is regulated differently by a growing list of “epigenetic factors” [to which should be added prolactin (506), norepinephrine (35, 292), the pituitary adenylate cyclase-activating polypeptide (PACAP) (362), ciliary neurotrophic factor (141), retinoic acid (99), and vitamin E (83, 86, 87); see Table 1 for a complete summary]. Although most of these factors modulate cell proliferation through unknown mechanisms, a regulation of Cyclin D1 and p27Kip1 expressions may constitute a common pathway (438).

The specificities of each neurogenic site may be related to differences in the intrinsic properties of the dividing cells (for example, their intrinsic ability to sense neural activity; Ref. 114) and/or in their microenvironment, which corresponds to the summation of local neurogenic signals expressed or synthesized “locally” by healthy neighboring cells or by dying cells. These signals may also be synthesized “peripherally” and released in neurogenic sites by neuronal affereces or by blood vessels. In this context, the role of the cerebral vasculature has gained importance as adult neurogenesis occurs within an “angiogenic niche,” and an alteration in the vascular microenvironment, or its ability to respond to changes in metabolic demands, may be responsible for a disruption in neurogenesis (371, 372, 460).

III. THE ANATOMO-FUNCTIONAL APPROACH

Complementary to the neuroanatomic studies that revealed some key biological properties of adult neural stemlike cells, the integration of the adult newborn neurons into preexisting networks has prompted the fundamental question of their functional relevance. So far, most studies have focused on the role of adult neurogenesis in hippocampal functioning. Parsimoniously, this region, included in the limbic-cognitive system, is part of an integrated network involved in learning and memory, attentional processes, motivational states, and emotion (68, 135, 136, 227, 352, 484). As pointed out by Hampton et al. (201), current theories of hippocampal function wrestle with two major conceptual issues: whether the structure encodes spatial, nonspatial, or both types of information (139) and whether the information encoded is episodic or semantic. Recent evidence infers that hippocampal neuronal networks could support the cognitive mechanisms of declarative memory by encoding features of experiences. Indeed, declarative memory involves a record of everyday experiences, which includes information about the content of that experience and the spatial and temporal context in which it occurred (episodic memory), woven together into the framework of the individual’s knowledge (semantic memory) (135). According to the memory space model of declarative memory, the HF plays a critical role in 1) representing experiences as a series of events and places and 2) linking memories together by identifying common features into a network that supports inferential expression. This said, the role of the HF in long-term memory is in dispute as the “standard theory” assumes that the HF becomes dispensable after the conversion of short-term memory into long-lasting memory, whereas the “multiple trace theory” makes the assertion that the HF is involved in the storage and retrieval of episodic memory regardless of the age of memory (394).

Although current advances do not yet allow the attribution of a specific role for newborn neurons in this conceptual framework, in the following sections we will review the progress achieved in implicating neurogenesis in both hippocampal functioning under physiological conditions and the apparition of hippocampal-related pathologies, which are mainly studied in preclinical animal models. Operationally, two approaches have been considered: the first consists of relating changes in hippocampal neurogenesis to changes in the ability to perform hippocampal-related tasks, and the second consists of studying the influence of hippocampal-related tasks on neurogenesis.

A. Environmental and Physiological Influences on Neurogenesis

1. Experience in enriched environments, neurogenesis, and learning

A) OLD PROBLEM, NEW DATA. Rosenzweig and co-workers (470–472) were the first to introduce the concept of an enriched situation, i.e., a complex environment enriched in sensorial stimulations, social experiences, and physical and cognitive exercises (555). This concept has given rise to a wealth of studies showing that exposure to such environments, which are considered as enriched for standard laboratory conditions, modifies brain functioning, learning, problem-solving ability, brain chemistry, and several structural aspects of brain organization. The consequence of environment enrichment on hippocampal neurogenesis was originally studied in “juvenile” female C57Bl/6J mice. Exposure to an enriched environment between 3 and 13 wk of age was found to increase survival of newly born cells (measured 4 wk after daily injections of 50 mg BrdU for 12 consecutive days from days 28 to 40 of enrichment), whereas cell proliferation (measured 1 day after the BrdU pulses) remained unchanged in the GCL and in the hilus (267). Similar results have been
obtained with adult female rats (after injecting BrdU 50 mg · kg⁻¹ · day⁻¹ on days 26–30 of enrichment) (407). These first reports led Kempermann and Gage (262) to further investigate the impact of environmental enrichment on mice, and it was later shown that cell proliferation in the GCL was increased only when mice were killed 3 mo after their removal from the enriched environment (262). In addition, a comparison of different strains of mice showed that enriched experience affects hippocampal neurogenesis differently according to genetic background: as reported above, it increases cell survival but not cell proliferation in C57BL/6 mice (267) which have a high baseline level of neurogenesis (268), whereas it increases cell proliferation in 129/SvJ mice (267) which have low levels of neurogenesis (268). This interesting finding indicates that environment has a differential impact depending on inheritable traits, an effect still unexplained. Finally, in most experiments, enrichment led to an improvement in spatial memory in the water maze, a learning task that requires the subjects to learn multiple extra-maze visual cues, allowing them to build a dynamic spatial representation of their surroundings to navigate to a platform hidden underneath the water surface (378). However, whether these genetic variations of neurogenesis have a functional significance in spatial memory remains unknown (266).

B) MECHANISMS INVOLVED IN ENRICHMENT-INDUCED NEUROGENESIS. Exposure to an enriched environment is known to influence brain chemistry, in particular neurotransmitters and trophic factors, which may underlie its action on neurogenesis (151, 370, 442, 471, 555, 584). In addition to these factors, several components of this environment may be involved in the regulation of neurogenesis.

Thus the effects of an enriched environment on neurogenesis and spatial memory may be related to an enhancement of motor exercise rather than learning. To examine this hypothesis, female mice were housed with a running wheel, injected with BrdU (50 mg · kg⁻¹ · day⁻¹ for the first 12 days) and killed 1 or 30 days after the last BrdU injection. In these conditions, cell proliferation and cell survival in the GCL, and performances in spatial learning tasks were enhanced, suggesting that “physical activity” is an important mediator of enrichment-induced neurogenesis (553, 554). Further studies proposed that the stimulatory effects of running are mediated by VEGF (148), blood-borne IGF-I (542), NMDA receptor ε1-subunit, and BDNF production (278).

In their early studies, Rosenzweig and Bennett (471) suggested that environmental changes or novelty were a major factor in the effects of enrichment on brain and behavior (471), which suggests that it could be a relevant factor involved in enrichment-induced neurogenesis as well. The observation that exposure to enriched conditions for an extended period of 6 mo does not add further benefit certainly lends weight to this hypothesis (262).

The social environment may also contribute to enrichment-induced neurogenesis. This hypothesis has been tested in rats reared for 4 wk in isolation and injected with BrdU (2 × 50 mg · kg⁻¹ · day⁻¹) on the last 3 days of the rearing treatment. Compared with group-reared rats, isolation decreases hippocampal cell proliferation within 24 h, the number of newly born cells surviving over an additional period of 4 wk of isolation, and the percentage of newly born cells expressing TOAD-64. Interestingly, the isolation-induced decrease in cell proliferation is reversed by a subsequent 4 wk of group housing (BrdU being injected on the last 3 days of the rearing period; Ref. 320). Together, these results indicate that social environment is a highly relevant factor underlying the anatomical effects of an enriched environment.

2. Reciprocal relation between learning and neurogenesis

A) LEARNING INFLUENCES SEVERAL ASPECTS OF NEUROGENESIS. The effect of learning on survival of newly born cells has been examined in the water maze and in eyeblink conditioning using a trace protocol (175). In this latter test, which requires an intact HF (385, 512), animals have to associate an auditory tone (conditioned stimulus) with a corneal airpuff or electrical stimulation (unconditioned stimulus) that is temporally separated by a trace interval (350). In both learning tasks, rats are tested 1 wk after a single BrdU injection (200 mg/kg). The number of BrdU-labeled cells is enhanced by learning immediately or 1 wk after the end of training, an effect accompanied by a decrease in cell death. In associative learning, the performance of individual rats positively predicts the life span of 1-wk-old BrdU-labeled cells, i.e., rats exhibiting the best behavioral scores possessed more newborn cells; this also suggests that learning (and not training) is a sufficient condition to increase the survival of adult-born cells (309). This learning-induced increase in cell survival was maintained 2 mo after acquisition of associative learning (309). The observed enhancement of cell survival is specific to learning because neurogenesis remains unchanged in rats exposed to unpaired conditions in eyeblink conditioning or in rats producing the same amount of motor responses in the water maze. Furthermore, learning-induced upregulation of neurogenesis may be specifically attributed to hippocampal functioning as delay-eyeblink conditioning, classically attributed to the cerebellum, and training on a cued test in the water maze, a hippocampal-independent type of learning, do not modify neurogenesis (508).

Although these experiments indicate an enhancing effect of learning in the water maze on cell survival, contradictions exist concerning cell proliferation. When animals are injected daily for the entire testing period (50 mg · kg⁻¹ · day⁻¹ for 12 days; Ref. 554) or the very last day of testing (200 mg/kg; Ref. 175), the number of BrdU-
labeled cells remains unchanged. However, when animals are injected at the end of the learning phase (50 mg · kg⁻¹ · day⁻¹ for 3 days), when an asymptotic level of performance is reached, then the number of newly born neurons in the GCL of the DG increases (306). To reconcile these data, we hypothesized that different phases of the learning process have distinct actions on cell proliferation. To test this hypothesis rats were injected with BrdU (50 mg · kg⁻¹ · day⁻¹) either during the initial phase (4 days, Fig. 4, A and E), characterized by a fast and large improvement in performance, or during the late phase (4 days, Fig. 4C), when an asymptotic level of performance was reached. We found that the early phase of learning does not modify proliferation (Fig. 4A), whereas the late one does (121). Indeed, the number of newly born cells increased contingently with the late phase of learning (Fig. 4B), and these new cells differentiated into neurons that persisted in the DG for at least 5 wk after the animals had acquired the task. In contrast, the late phase of learning induced a decrease in the number of newly born cells produced during the early phase (Fig. 4C).

Most importantly, the extent of BrdU cell loss was inversely related to learning performances: rats with the highest and lowest cell loss have the best and worst performances, respectively. This decline in adult-born cells most probably mirrors cell death, since learning induces an increase in numbers of pyknotic cells (129) and tunnel-positive cells (18). The observations that learning in the water maze increases the expression of proteins involved in apoptosis (82) and that intrahippocampal administration of anticaspases, which blocks cell death, impairs long-term but not short-term spatial memory (106), strengthen the hypothesis that cell death is an important component of hippocampal learning.

The mechanisms by which cell survival and proliferation are regulated during learning are so far unknown. A nonspecific effect of training or exercise on blood flow to the brain that may increase BrdU availability is unlikely, since neurogenesis is unchanged in control “swimmers” (121, 175). Trophic factors known to support the viability and function of many types of neurons are likely mediators of learning-dependent changes in the HF. Indeed, hippocampal bFGF and BDNF are increased during task learning and decreased once an asymptotic performance is reached (171, 271).

B) A BLOCKADE OF NEUROGENESIS DISTURBS LEARNING. The participation of neurogenesis in memory formation has been more directly tested by using the DNA methylating agent methylazoxymethanol (MAM; 5 mg/kg for 14 days concurrent with 75 mg · kg⁻¹ · day⁻¹ of BrdU on days 10–12–14). This treatment decreases the number of adult-born cells by 80% and alters trace conditioning, whereas delay conditioning, neuronal responsiveness (presynaptic neurotransmitter release and excitability), and synaptic plasticity are not disrupted (509). Three weeks after treatment cessation, the replenishment of immature neurons is associated with a recovery to acquire the trace conditioned response. Altogether, these results suggest that newly born cells are necessary for the formation of trace memories. Because MAM treatment for 6 days only is unable to affect trace conditioning, adult-born cells may not be “functional” until 1–2 wk after birth. Although it has been shown that newborn cells extend their axons in the CA3 subfield within 4–10 days after birth (204), a basic temporal study following their functional maturation (by means of electrophysiological properties or c-fos expression) is still lacking.
Recently, it has been reported that the formation of spatial memories may not be associated with neurogenesis. Indeed, the blockade of cell proliferation by the same 14-day MAM treatment as described above does not alter spatial learning in the water maze or contextual conditioning in fear conditioning (510). In our opinion, this lack of effect is due to the residual newly born cells that continue to be generated under MAM (<700/day for 3 BrdU injections), indicating that learning can occur with a very small percentage of new neurons. This assumption is based on the observation that some aged rats are able to acquire spatial navigation learning despite a very low number of newly born cells (~200/day for 5 BrdU injections; see sect. mC2, Fig. 6 (130)).

An emerging strategy to clarify the role of neurogenesis in learning consists of using ionizing-irradiation known to induce cognitive impairments in animals (37, 66, 119, 184) and in humans (372). X-irradiations (0–15 Gy) decrease the number of BrdU-labeled cells in a dose-dependent manner (following a 60 mg/kg injection of BrdU 1 h before death) and the number of cells expressing p34cdc2 or Ki-67 in the adult DG (369, 433, 532). Two months after irradiation, the production of adult-born neurons (expressing NeuN) is significantly reduced certainly as a consequence of an altered neurogenic micro-environment (369, 372). The reduction in hippocampal mitotic activity is accompanied 1 wk postirradiation by behavioral deficits in a hippocampal-dependent task of spatial short-term memory (place-recognition). In contrast, performances in the water maze remain unaffected 2 wk postirradiation, a time by which proliferative activity has resumed, albeit at a low level (~75 nascent cells daily in the dorsal DG; Ref. 330).

3. Discussion

Data accumulated in the past decade suggest that the birth of new cells is a necessary condition for the acquisition of memory traces (see also data on prenatal stress and aging). However, a higher reduction in cell genesis seems to be required for the appearance of deficits in learning spatial cues than in trace conditioning, a difference which may be related to task difficulty (508). Furthermore, hippocampal-dependent behaviors influence cell proliferation, cell survival, and cell death. The question of how adult neurogenesis participates in memory processing (i.e., encoding, consolidation, retrieval) is as
yet unresolved. Although speculative, the following framework is proposed (Fig. 5).

A) DO ADULT-BORN NEURONS PRODUCED BEFORE LEARNING HAVE A ROLE IN MEMORY PROCESSING? Since survival of the newly born cells whose birth predate the beginning of learning is increased during the learning task (175), the encoding of memory traces may be processed in these adult-born neurons. According to the “use it or lose it” principle, the cells that survived and integrated into the granule cells/mossy fiber system could function as postsynaptic detectors of correlated afferent activity and thereby update the bearing map, which is constructed in the DG from directional cues (229). After processing, which relies on pattern separation properties (270), episodic memories are stored temporally by virtue of the CA3 auto-associative system (468). The adult-born neurons could also be involved in temporal pattern completion: if a degraded version of a stimulus is presented after learning, the represented feature can be recalled from this incomplete input information.

The role of adult-born neurons in long-term storage and retrieval of remote memories is still elusive as the role of the HF and the DG is still a matter of debate. One view stipulates that the HF has a limited storage capacity as 1-mo-old memories do not require the HF, memory traces being transferred within cortical areas where they are consolidated and permanently stored (19, 56, 273, 274, 342, 573). In relation to this apparent time-limited role of the HF, it was originally thought that after encoding and conversion of short-term memory into long-lasting memory, the adult-born neurons were eliminated (189). However, adult-born neurons that have been recruited by learning survive for several weeks (121, 309) and thus outlive the time required for cortical transfer. This time-window is, in our opinion, hardly reconcilable with the “consolidation transfer hypothesis.” Furthermore, if one considers that adult-born neurons are integrated into the DG, which is no longer necessary for maintaining the memory traces, they may have adopted another, as yet undetermined, role (508).

However, the view that the HF becomes dispensable after the conversion of short-term memory into long-lasting memory is disputed, as it is supposed to be involved, together with neocortical areas, in the storage and the retrieval of remote memories (387, 388, 393–395). In particular, the HF seems important in the recall of old, detailed, episodic memories that are context dependent (469, 527). This scenario is consistent with the long-term survival of adult-born neurons (121, 309), which could act as binding detector cells capable of memorizing bindings between items (503) or could store a memory index, i.e., an index of neocortical areas that are activated by experiential events and contain no intrinsic meaning in terms of representation of external events (538). In these cases, adult-born neurons most probably do not store the memory traces themselves but may play a critical role in linking representations of distinct experience, and long-term storage of spatial representations could be achieved by synaptogenesis occurring within this CA3 (455). Alternatively, adult-born neurons could store the contextual information regarding the episode (the so-called contextual trace; Ref. 394).

B) A ROLE OF CELL DEATH IN STABILIZATION? A surprising phenomenon is that the death of old and/or newly born neurons also plays an important role in enabling hippocampal learning. This may constitute a trimming mechanism replacing the unnecessary old neurons and/or the immature neurons that have not established learning-related synaptic connections with freshly minted ones. This may serve the function of increasing the signal-to-noise ratio, thus consolidating the memory trace. This phenomenon, which on one hand is consistent with the “use it or lose it” principle and on the other hand goes against the classic assumption in learning-induced structural plasticity that “more is better,” recalls the regressive events observed during development (450).

C) IS LEARNING-INDUCED CELL PROLIFERATION INVOLVED IN MEMORY CLEARANCE OR FLEXIBILITY? Learning-induced cell proliferation is not correlated with learning performances, suggesting that these newly born cells do not directly sustain ongoing learning and may rather be involved in future behaviors. This phenomenon may represent a resetting process ensuring that the hippocampal system is available to process new memories. This hypothesis is strengthened by the observation that presenilin-1 knockout mice that present normal basal neurogenesis exhibit a reduction in enrichment-induced cell proliferation and better retrieval of contextual fear memory traces compared with control mice (153). Thus a deficiency in the upregulation of cell proliferation induced by the late phase of learning may prevent memory clearance, thereby impairing forgetting processes and disabling hippocampal functions. It is also possible that this alteration in cell proliferation alters the inferential use of past memories in a novel situation (flexibility) and thus leads to rigidity and perseverance.

B. Activation of the Stress Axis: Acute and Long-Term Effects on Neurogenesis

Exposure to stressful events prepares animals to engage in fight or flight responses, and a role of the HF in these defensive behaviors has been proposed (52, 205). Moreover, as stressful events have deleterious effects on hippocampal integrity (351, 352, 484), the influence of different types of stress on hippocampal neurogenesis has been studied.

1. Effects of stress on neurogenesis

In most studies, a decrease in cell proliferation has been observed after stressful events, but this effect ap-
pears highly dependent on the nature and the timing of stress.

In male but not female rats, exposure to the odor of a predator [trimethyl thiazoline (TMT), the major component of fox feces] downregulates the number of proliferating cells estimated 2 h, 24 h, and 1 wk after a single BrdU injection (100–200 mg/kg) (149, 537). However, this effect is transient as TMT-induced downregulation of BrdU cell numbers disappears 3 wk after labeling when dividing cells express NeuN or NSE (149). Likewise, in male tree shrews (Tupaia belangeri), a single exposure to an unknown congeners, which results in the establishment of a dominant/subordinate relationship, brings down the number of dividing cells (labeled by one injection of BrdU, 75 mg/kg) in the DG of the subordinate within 2 h (177). In the marmoset (Callithrix jacchus) however, a single exposure to a resident-intruder model of stress reduces the number of newly born cells (2 h after a 1 × 200 mg/kg BrdU injection before the stress) in the DG and not the SGZ, an effect that is normalized within 1 day (209). Chronic, unpredictable stress exerted over 3 wk reduces mitotic activity in the SGZ (and not the DG) as estimated by KI-67 staining. At this time point, another batch of animals was injected with BrdU (1 × 200 mg/kg), and 3 wk later, the effect of chronic stress on the survival of BrdU-labeled cells was examined. Because the BrdU cell number was unchanged, it was concluded that cell survival is not influenced by stress. Finally, male tree shrews subjected to a 7-day period of psychosocial stress exhibit reduced cell birth (evaluated 1 day after 1 × 100 mg/kg BrdU; Ref. 104). This stress-induced reduction in adult-born cells leads to a decline in neurogenesis, the neuronal phenotype being inferred by the expression of NeuN (441) or NSE (177, 181) 1 mo after labeling.

Because stressful events are known to activate the HPA axis, leading to a rise in plasma levels of corticosterone, and to activate the release of endogenous opioids, which are involved in defensive behaviors, the role of these two factors has been evaluated. To confirm the involvement of corticosterone, male rats were adrenalec-tomized, and low levels of hormone were restored. In these conditions, the prevention of the TMT-induced rise in corticosterone blocks the downregulation of cell proliferation (537). In contrast, administration of naltrexone, an opioid antagonist (5 mg/kg, 30 min before TMT exposure), does not attenuate the effect of TMT on cell proliferation, whereas the expression of defensive behaviors (defensive burying, stretch approach) is partially attenuated (219), which indicates a dissociation between TMT-induced cell proliferation and defensive behaviors (149). The activity of the HPA axis is known to be regulated by corticotrophin-releasing hormone and vasopressin, the roles of which have recently been evaluated. Thus, the chronic blockade of CRF1 and V1B receptors (by SSR125543A and SSR149415, respectively, 30 mg · kg⁻¹ · day⁻¹ for 28 days) starting 3 wk after the beginning of chronic mild stress (CMS for 21 days) reverses the stress-induced reduction of cell proliferation (measured 24 h following 1 × 75 mg/kg of BrdU injected at the end of the drug treatment period) (10). These antagonists are also able to prevent the stress-induced reduction of neurogenesis (by means of NeuN labeling) estimated 30 days after the cessation of the drug treatment and BrdU pulse (3.75 mg/kg every 2 h on the last 3 days of the CMS). The normalization of neurogenesis is furthermore associated with a prevention of the stress-induced hippocampal atrophy.

2. Interindividual differences in stress reactivity

Interindividual differences in behavioral reactivity to stress or to novelty and coping abilities have been evidenced in our laboratory in an outbred population of rats. Animals are split into two groups according to the median score of behavioral response, the behaviorally high-reactive (HR) rats, and the low-reactive (LR) rats (443, 446). Compared with the LR phenotype, the HR phenotype is characterized by a series of adaptive defects of a cognitive and motivational nature corresponding to a deficit in inhibition and control processes (115, 304, 443, 447). These interindividual differences have been related to differences in corticosterone secretion, HR rats displaying a hyperactive HPA axis (305, 443, 444, 446).

The behavioral trait of reactivity to stress has been shown to predict the level of neurogenesis in the GCL of the DG (305). Two weeks after their behavioral characterization, rats were injected with BrdU (3 × 50 mg/kg spaced by 4-h intervals) and killed the next day or 14 days later. Cell proliferation was found to be negatively correlated with locomotor reactivity to novelty, and when the group was split according to the median behavioral score into LRs and HRS, cell proliferation in LRs was twice that observed in HRS. Survival of nascent neurons was not influenced by this behavioral trait. A difference in the HPA axis reactivity most probably underlies the differences in neurogenesis, primarily due to differences in cell genesis. The functional significance of this difference in DG plasticity remains unclear but may reflect a disruption of behavioral inhibition in the HRs (187).

3. Perinatal manipulations

Studies on stress during the perinatal period have also led to intriguing observations. It is well documented from animal and human studies that during the perinatal
NEUROGENESIS IN THE ADULT BRAIN

During the prenatal period the development of an organism is subjected to complex environmental influences and that deleterious life events during development induce neurobiological and behavioral defects (207, 557, 569). Indeed, environmental changes and stimulations have the greatest impact when produced in the early stages of life and during developmental periods, leading to phenotypical orientations lasting throughout life. These environmental influences, which have been evidenced for decades, have been explored at structural and mechanistic levels only recently, revealing profound structural changes in the HF (see, for instance, Ref. 353). Stressful events during the prenatal period, such as prenatal stress which consists in stressing pregnant dams, or during the postnatal period in the rat, as for example maternal deprivation, i.e., long-lasting separations of the mum-pup dyad, alter brain chemistry, enhance emotional reactivity, produce cognitive impairments, and increase vulnerability to drugs of abuse (185, 482, 569). We and others have shown that stressful events during the prenatal (306) and the postnatal (300, 367, 431) periods downregulate hippocampal neurogenesis. In prenatally stressed rats, we found that cell proliferation (4 × 50 mg/kg of BrdU over 3 days) is reduced from adolescence to senescence, indicating an acceleration in the age-related decline of cell proliferation in the GCL. Survival of the newly born cells and neuronal differentiation (measured with NeuN) are not modified, and thus the net reduction in the number of adult-born neurons is associated with a decrease in the number of granule cells from adulthood to senescence. Given that corticosterone has been implicated in long-term behavioral changes induced by prenatal stress (280), this downregulation of neurogenesis may result from heightened corticosterone secretion (see sect. IIA2). Similarly, maternally deprived rats are characterized by reduced cell proliferation in the GCL both when infants (proliferation measured on postnatal day 21 following 7 days of maternal separation and 7 × 50 mg/kg BrdU) (300, 431) and when adults [proliferation measured at 2–3 mo of age following daily 3-h maternal separation bouts from postnatal day 1 to 14 and 1 × 200 mg/kg BrdU 2 h or 1 wk before death (367)]. In this recent study, the authors found that maternal deprivation reduced the number of immature, but not mature, neurons that are added to the DG in adulthood (367). Because maternal separation is reported to alter the activity of the HPA axis (482), it has been hypothesized that corticosterone mediates the effect of maternal separation on neurogenesis. In fact, it has been shown that the maternal separation-induced decrease in cell proliferation is abrogated by adrenalectomy plus corticosterone treatment aimed at restoring basal diurnal levels of the hormone. The authors proposed that hippocampal precursors might be hypersensitive to corticosterone, the diurnal basal and stress-induced levels of which are not modified in their paradigm (367). However, because maternal separation is known to decrease corticosterone-binding globulin (559), and thus enhance the free, and active, fraction of corticosterone reaching the brain, a higher level of “efficient” hormone could be responsible for the effects of maternal separation on hippocampal cell proliferation.

Given the involvement of the HF in learning and memory, we hypothesized that prenatal stress would lead to cognitive deficits via an inhibition of neurogenesis in the DG. Thus we have shown that prenatal stress is associated with an alteration in learning and memory performances, and more importantly, that the learning-induced increase in cell proliferation observed in control animals is blocked in prenatally stressed rats (306). This result is in accordance with our previous observation that cell proliferation is increased only when the animals reach an asymptotic level of performance, i.e., when the task has been mastered (see sect. IIA2A and Fig. 4). Furthermore, the behavioral deficits induced by prenatal stress, similar to those observed in animals exposed to X-irradiations (37), confirm an enabling role for hippocampal neurogenesis in cognition.

C. Aging and Longitudinal Observations

1. Neurobiology of the age-related decline in neurogenesis

A) INFLUENCE OF AGING ON NEUROGENESIS. Aging is a life-long process beginning with conception and ending with death. Within this developmental continuum, sexual maturity (2–3 mo in rodents) is used to define the start of adulthood. Although the definition of “how old is old” varies depending on species, genetic background, and experimental conditions, studies addressing the effects of age on biobehavioral parameters in rodents have identified at least three ages: adult (up to 10 mo), middle-aged, and “old” or “aged” or senescent (from 20 mo) (91, 92).

The persistence of neurogenesis in the DG of senescent rats was first reported by Kaplan and Bell (248, 250, 251). An age-related decline in DG neurogenesis has been confirmed in mice and rats using several BrdU labeling protocols (54, 208, 290, 306, 310, 499), whereas conflicting results have been reported for the SVZ (238, 253, 290, 543).

The decline in DG neurogenesis, which mainly occurs between 2 and 12 mo of age, does not seem to be related to 1) an alteration in adult-born cell survival as the percentage of surviving BrdU-labeled cells 1 mo after their birth is independent of the age of the animals (54, 208, 269, 310) or 2) a general change in metabolic conditions, as neuronal precursors respond to BDNF’s influence to the same extent throughout life (169). Rather, it may be a decline in proliferative activity that
underlies the age-related decline in neurogenesis, since
the number of newly born cells a few hours after label-
ing (54, 290) and the number of nestin-positive cells
(391) are lower in aged rats. However, it remains to be
determined whether the reduction in proliferative ac-
tivity is a consequence of a developmental lengthening
of the cell cycle time and/or of a shrinking of the
proliferative cell population. Finally, neuronal differen-
tiation seems to be delayed with advancing age as
suggested by the high percentage of cells that do not
express a neuronal or an astrocytic phenotype 1 mo
after their birth (54, 208, 264, 310, 391).

B) FACTORS REGULATING NEUROGENESIS IN THE SENESCENT BRAIN.
The progressive decline in cell proliferation could de-
rive from inadequate local environmental cues; the
aged DG may be under the influence of inhibitory fac-
tors and/or may lack stimulatory factors that sustain
division, differentiation, and/or survival of the newly
born cells.

I) Inhibitory factors. Corticosterone has received
particular attention since basal levels of this hormone,
known to inhibit cell proliferation in young rats (see sect.
II A), increase during aging (324, 484). Thus, 1 wk after
adrenalectomy in old rats, cell proliferation is upregulated
in the GCL (373) and in the DG (GCL and hilus, Ref. 74).
On the other hand, survival of the newly born cells is
unrelated to corticosterone levels (373), and most of them
differentiate into neurons expressing TOAD-64 or NeuN
(74, 373).

Glutamate is also known to inhibit DG neurogen-
esis in young rats (see sect. II A). Similarly, in middle-
aged and aged rats, a single injection of a NMDA recep-
tor antagonist (CGP-43487, 5 mg/kg 2 h after an injec-
tion of BrdU, 200 mg/kg) increases hippocampal cell
proliferation 5 days later. By 3 wk, despite a decline in
the number of newly born cells, more cells survived in
the treated aged rats compared with saline aged coun-
terparts (391).

II) Activating factors. The involvement of the neu-
rosteroid Preg-S in the hippocampal neurogenesis of old
rats has been examined. Indeed, 1) hippocampal levels of
Preg-S in the HF of senescent rats correlate with spatial
memory abilities, 2) intrahippocampal infusion of Preg-S
in cognitively impaired aged rats reverses memory distur-
bances (551), and 3) Preg-S stimulates neurogenesis in
the adult DG (see sect. II A). We have shown that icv
Preg-S infusion increases cell proliferation in the senes-
cent DG (348). This suggests that the age-related decrease
in Preg-S levels could lead to a loss of stimulation of
hippocampal neurogenesis and to subsequent cognitive
deficits. Finally, because Preg-S acts as a negative allostere-
ic modulator of GABA_A receptors, our results support
the inhibitory hypothesis of neurodegenerative disorders
such as Alzheimer’s disease and age-related brain impair-
ments, which postulates the involvement of abnormally
strong inhibitory GABAergic transmission (341).

Because adult neurogenesis can be enhanced by the
administration of growth factors (see sect. II A), the
responsiveness of the aged brain has been the subject of
investigation. In particular, a stimulatory effect of IGF-I
has been examined since 1) IGF-I brain levels decrease
with age (90), 2) IGF-I receptor mRNA is upregulated as a
function of aging and cognitive decline (522), and 3) IGF-I
infusion improves cognitive function in aged rats (345).
The chronic icv administration of IGF-I (1.2 µg/day for 14
days) in senescent rats elicits an approximately threefold
increase in BrdU-labeled cells (BrdU, 2 × 50 mg · kg⁻¹ ·
day⁻¹ on days 7–11) in the SGZ and the hilus (310). In a
4-wk survival study, IGF-I and saline-treated animals dis-
played a similar number of newly born cells (BrdU-la-
beled on days 7–11) compared with animals killed after a
short survival delay, indicating that IGF-I acts on cell
proliferation rather than on cell survival. Finally, in con-
trast to what was observed in adult rats (1), IGF-I did not
modify cell fate in old animals, with 20% of newly born
cells differentiating into neurons (inferred from NeuN
expression). Thus the threefold increase in BrdU-positive
cells elicited by IGF-I translated into a threefold increase
in adult-born neurons.

The influences of FGF-2 and HB-EGF were also ex-
amined in 20-no-old mice (icv infusion of 10 µg/ml for 3
days concurrent with 50 mg · kg⁻¹ · day⁻¹ BrdU). One
week after the cessation of the treatments, the number of
newly born cells was higher in both the SGZ and the SVZ;
these cells expressed DCX and occasionally GFAP (238).

More speculatively, it may be hypothesized that
proinflammatory cytokines such as interleukin 6 (IL-6)
also play an important role in age-related decline in neu-
rogenesis. Indeed, 1) it is involved in the appearance
of age-related cognitive disorders in humans (59, 120, 328,
355, 466, 565), 2) chronic stress increases its production
in aged patients (272), 3) IL-6 expression is increased in
the HF of senescence-accelerated mice (539), and 4) hip-
pocampal neurogenesis is reduced in adult transgenic
mice with chronic IL-6 production (552) or after adminis-
tration of bacterial lipopolysaccharide, a potent activator
of IL-6 (138, 372).

In summary, precursors that are able to enter the cell
cycle are still present in the aged brain, and low levels of
neurogenesis are linked to the accumulation of inhibitory
substances and/or the decrement of stimulatory factors.
The mechanisms of action of these modulators are still
unknown and, in particular, it remains to be determined
whether they act on the length of the cell cycle or on the
number of dividing cells. Whatever the underlying mech-
nanism, these results point to possible pharmacological
actions for preventing or treating age-related cognitive
impairments.

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2. Functional implications of age-related modulation of neurogenesis in memory

The hypothesis that the decline in neurogenesis may be responsible for age-related alteration in cognitive function has been tested by examining the influence of an enriched environment, and by taking advantage of the existence of interindividual differences in age-related cognitive functions. The influence of enriched environments on spatial memory and on neurogenesis (cell proliferation, cell survival, and neuronal differentiation) has been studied in 18-mo-old mice (269). Although the effects are not as strong as those observed in young animals, after 68 days of enrichment, neuronal differentiation is enhanced whereas cell proliferation and cell survival remain unchanged compared with nonenriched aged animals; performances in spatial memory are slightly improved. A similar conclusion was reached when middle-aged female mice were raised for 10 mo in an enriched environment (264), which indicates that as was found in younger subjects (see sect. IIIA1A), long-term exposure to an enriched environment in old subjects does not afford any further beneficial effect compared with short-term exposure as neither cell proliferation nor cell survival are increased. In this study, the improvement in learning parameters observed after enrichment was suggested to be aspecific, resulting from better adaptive skills in exploration, greater locomotor aptitude, and endurance following enrichment, leading the authors to suggest that the contribution of adult hippocampal neurogenesis to hippocampal plasticity might require a physically active pursuit as opposed to a passive sensory stimulation (264).

Large individual differences exist in cognitive abilities, and all aged rats do not exhibit cognitive disorders; some rats perform as well as young controls, whereas others present spatial memory impairments (457). The existence of such individual differences has been used as a naturalistic strategy to study the neurobiological correlates of cognitive aging, and it has been shown that the extent of memory dysfunction is related to the magnitude of changes occurring in the HF. Following a characterization of the cognitive abilities of aged rats in the water maze, we have shown (Fig. 6) that neurogenesis (i.e., cell proliferation, cell determination, and cell survival) is higher in cognitively unimpaired rats than in impaired ones (130).

Because age-impaired and -unimpaired rats did not differ in granule cell number, as previously described (458), it seems that the rejuvenation of granule cells rather than a change in their absolute number is the main factor sustaining memory formation. In other words, successful aging, i.e., preserved cognitive functions, is associated with the maintenance of a relatively high neurogenesis level, whereas pathological aging, i.e., memory deficits, is linked to exhaustion of neurogenesis. These differences in hippocampal neurogenesis may be due to differences in stimulatory and/or inhibitory substances, which may respectively prevent or accelerate pathological aging. We propose that the age-related increase in corticosterone levels, resulting from repeated stressful events, may lead, through life-span inhibition of neurogenesis, to defects in hippocampal networks that would be responsible for cognitive dysfunctions. The observation that lowering corticosterone levels in middle-aged animals significantly increases hippocampal neurogenesis and postpones the onset of spatial memory deficits supports this hypothesis (M. F. Montaron, E. Drapeau, C. Aurousseau, M. Le Moal, P. V. Piazza, and D. N. Abrous, unpublished data).

During pathological aging, alterations in hippocampal neurogenesis most probably mirror a more general failure in the mechanisms underlying neuronal plasticity (364). Similarly, it has been shown that lipofuscin deposits, an index of cell suffering, are higher in aged rats with impaired cognitive function (64) and are decreased in aged mice housed in an enriched environment (264). According to the hypothesis of the “Red Queen Theory” (5), during aging there is competition for the available plasticity to compensate for neuronal degeneration or to store new information. Thus, in pathological aging, hippocampal plasticity is exhausted, and the capacity of the old

![Figure 6](http://physrev.physiology.org/)
brain to acquire new information and to compensate for ongoing degenerating processes is impeded.

D. Involvement of Neurogenesis in Pathologies

1. Pathological conditions associated with an upregulation of neurogenesis

A) EPILEPSY. Human temporal lobe epilepsy (TLE) is associated with atrophy of the HF, an effect known since the beginning of the 19th century, and a loss of hippocampal and DG neurons (57, 384). In this pathology, the dentate granule cells give rise to abnormal axonal projection into the supragranular inner molecular layer of the DG and to an aberrant reorganization of the mossy fibers (MF) in the CA3 subfield of Ammon’s horn (529). It has been suggested that the sprouting of MF is relevant in the hyperexcitability of the TLE (426). This has led to investigation into whether newly born cells are important in the genesis of dentate MF sprouting (MFS) and participate in the reorganization of the network.

Kindling, an animal model of TLE, is characterized by a permanent epileptic state produced by a series of seizures induced by electrical stimulations made in various brain areas such as the hippocampus (42), the perforant path (396), and the amygdala (425, 492). Single and repeated kindling stimulations in the ventral CA1 region induce a facilitation of mitotic activity 2 wk later (42). Stimulation in the perforant pathway leads to a marked increase in the number of BrdU-labeled cells as early as 3 days after the last stimulation, whereas repeated kindling does not evoke further stimulation of cell proliferation (396). Cell division is in fact suppressed, suggesting a diminished efficiency of repeated or prolonged stimulation to elicit a neurogenic response. In the amygdala kindling model, it has been reported that during the early phases of kindling, when focal seizures are present, DG cell proliferation remains unchanged, whereas it is upregulated during the later phase of kindling, when motor seizures are present (425, 492).

In various chemoconvulsant (pilocarpine or kainate) models of human TLE, the induction of seizures is accompanied by a dramatic increase in neurogenesis in the SGZ of the DG (430). This increase is observed after a latent period of a few days following the status epilepticus (SE) and persists during the first week, before levels of neurogenesis return to baseline over the following weeks (396, 430). The recruitment of proliferative cells after kainate treatment occurs preferentially in GFAP cells, suggesting an increase in proliferative glial-like astrocytes (type B) (223). The expression patterns of multiple bHLH transcription factors have been studied following epileptogenesis; some were found to be increased (Mash1, Id2), some decreased (Hes5), and others unchanged (NeuroD, NeuroD2, Id3, and Math2), suggesting that molecules controlling cell-fate decisions in the developing dentate gyrus are also operative during seizure-induced neurogenesis (140). The newly born cells induced by seizures, which survive for up to 4 wk and differentiate into neurons (430), migrate to abnormal locations such as the CA3 cell layer, the dentate hilus and inner molecular layer (108, 430, 487). These ectopic granulalike neurons retain many, but not all, of their granule cell intrinsic properties (487). Incidentally, ectopic locations of SVZ-derived neuroblasts have also been observed in the striatum and the cortex following pilocarpine treatment (428).

Seizure-induced neurogenesis appears to precede MFS, suggesting that newly born cells could be important in the genesis of the ectopic innervation of Ammon’s horn by dentate fibers. This hypothesis has been tested by coadministrating kainate or pilocarpine with cycloheximide (CHX), a protein synthesis inhibitor known to abolish MFS (40). Contrary to what was expected, CHX did not influence chemoconvulsant-induced neurogenesis, indicating that its effect on MFS is independent of cell proliferation (97). Similarly, a single session of X-ray treatment, which attenuates pilocarpine-induced neurogenesis, fails to prevent MFS (427), indicating that neurogenesis upregulation is not sufficient to generate MFS. Thus the participation of the newly born cells in network reorganization and in the recurrence of epileptic seizure is still open to debate.

The mechanisms by which seizure activity stimulates neurogenesis are unknown. They may involve electrical activation since 1) physiological activity such as LTP-elicited mossy fiber stimulation is sufficient to increase neurogenesis in the DG (118), and 2) pure electrical activation, consisting in a continuous stimulation of the perforant path, elicits focal hippocampal seizure discharges and increases BrdU cell number 6 days later (430). Seizure-induced upregulation of neurogenesis may also involve 1) an alteration in excitatory amino acid transmission due to degeneration of the entorhinal afferences to the granule neurons of the DG (430); 2) activation by the dying cells (42, 50); 3) a change in bFGF, as kainate-induced upregulation of neurogenesis is blocked in mice lacking bFGF, an effect reversed by gene transfer (582); 4) changes in other neurotrophic factors (145, 164, 172, 583); and 5) activation of the 5-HT1A receptor as infusions of a 5-HT1A receptor antagonist (WAY-100,635) impede the increase in pilocarpine-induced neurogenesis whereas cell death, MF sprouting, and the onset of spontaneously recurring seizure are not prevented (451).

In summary, neurogenesis is altered by epileptiform status, and surprisingly maintenance of the newly born cells (in homotypic and ectopic locations) is systematically observed in the different models of epilepsy. The abnormal circuits may contribute to the unstable hippocampal circuitry and the development of chronic TLE. As proposed by Gray and Sundstrom (188), the DG could
“regulate as a gate with respect to controlling the propagation of seizure with granule cells controlling the throughput of epileptiform activity transition through the HF by virtue of a feed forward inhibitory pathway.” These alterations in neurogenesis may participate in the memory dysfunction associated with epilepsy (530). Although unverified, this latter finding suggests that the presence of more new neurons is not necessarily associated with a good memory and that neurogenesis and memory function may follow an inverted U-shaped curve.

B) ISCHEMIA. Until recently it was thought that the functional recovery that occurs in some cases following brain damage (such as stroke and trauma, see sect. mD1b) was related to the remodeling of neuronal pathways in nondamaged areas. The discovery of adult neurogenesis prompted researchers to investigate the role played by new neurons in recovery of lost functions.

Transient global ischemia, induced in gerbils and rats by bilateral common carotid artery occlusions, increases cell proliferation in the DG in a dose-dependent manner (260, 315, 575, 576). A peak in cell proliferation occurs 1–2 wk after ischemia, and a quarter of the newly generated cells die during the first month (315). During the first 2 wk after their birth, the newly born cells transiently express NeuroD and DCX (258). Those cells that survive differentiate into granule neurons expressing NeuN or MAP2 at 1 mo of age (258, 315). They acquire functional features of mature neurons as NMDA stimulation induces the phosphorylation of ERK in 34 and 61% of BrdU-NeuN labeled cells of 1 and 2 mo of age, respectively (258).

After focal cerebral ischemia, induced by unilateral middle cerebral artery occlusion (MCAO) (24, 237, 589, 590), cell genesis is increased to various degrees in the ipsilateral DG (as well as in the SVZ, OB, and cortex) over a period of several weeks. Increased BrdU incorporation in the DG (as in the others structures) contralateral to the infarct, occurring following a lapse of a few days, suggests a role for diffusible or humoral factors (237, 534). The newly born cells differentiate into granule neurons without any apparent degeneration (590). The enhancement of BrdU-labeled cells in the OB and the cortex has been attributed to the migration of neuronal precursors from the SVZ towards their normal target or towards the infarct, respectively (588). The presence of cell clusters in cortical areas, in the vicinity of blood vessels, also suggests the recruitment of resident quiescent stemlike cells or the infiltration of blood-borne cells (589). New cells arising from the SVZ also colonize the striatum after ischemia, 20% of which survive (representing 0.2% of the cell loss) and assume the phenotype of cells lost due to the lesion (24, 239, 429).

Neurogenesis upregulation in the DG has been attributed to cell death in the entorhinal cortex or the CA1 region (46), a hypothesis not always verified (25, 315). It has been proposed that the neuronal damage that follows ischemia involves the release of glutamate and overstimulation of glutamate receptors, which in turn upregulates neurogenesis. Indeed, the blockade of NMDA or AMPA/kainate glutamate receptors by specific antagonists (MK801; NBQX) inhibits the stroke-induced increase in neurogenesis (25, 46); however, these effects are surprising in light of the stimulatory influence of glutamate receptor blockade on neurogenesis in control brains (see sect. mD2b).

Mitotic factors such as bFGF are certainly involved as ischemia-induced upregulation of neurogenesis is blocked in mice genetically deficient in bFGF (582) and is increased following adenovirally mediated transfer of bFGF (347). Chronic icv infusions of bFGF and EGF greatly enhance the potential of endogenous progenitors to proliferate in response to ischemia and the cells then regenerate CA1 pyramidal neurons. The regenerated neurons are integrated into the neural circuitry as they receive afferences, project onto their normal target, the subiculum, and reverse the ischemia-induced deficits in cognitive functions (399). This constitutes the most impressive example of network reconstruction leading to behavioral recovery that takes advantage of adult endogenous neurogenesis. Infusion of VEGF (0.54 μg/day on days 1–3 of reperfusion concurrent with 2 × 50 mg · kg⁻¹ · day⁻¹ BrdU) increases the number of adult-born cells 1 mo (and not 3 days) after labeling, beyond the increase caused by ischemia (526). Ischemia-induced cell proliferation, measured within 3 wk post-BrdU pulse (2 × 50 mg · kg⁻¹ · day⁻¹ BrdU injected for 6 days starting 1 day after MCAO), is also increased by icv infusion of IGF-I and glial-derived neurotrophic factor (117).

Modulation of cell birth by ischemia also involves the production of several classes of inflammatory molecule metabolites. Administration of inhibitors of cyclooxygenase (Cox; i.e., acetylsalicylic acid, indomethacin, NS380), the rate-limiting enzyme for prostaglandin synthesis, curtails ischemia-induced proliferation after transient global ischemia in adult gerbils or rats (293, 485, 547). Mutation of one of the Cox isoenzymes, Cox-2, suppresses the ischemia-induced increase in hippocampal cell proliferation (485). Cox-2 may affect neurogenesis through the production of prostaglandin E₂ (547), which may act directly via prostaglandin EP3 receptors expressed in the GCL (397) or indirectly through bFGF (477). Alternatively, the production of NO, a key factor in inflammation, may be at play as administration of a NO donor (DETA/NONOate) to rats subjected to MCAO significantly increases cell proliferation in the SVZ and the DG, and significantly improves neurological recovery (587). This enhanced neurogenesis is certainly associated with the activation of inducible NOS since its inhibition (by amino-guanidine) prevents ischemia-induced neurogenesis. Furthermore, stroke-induced neurogenesis is abolished in mice lacking the iNOS gene (590). The participation of
leukotrienes, another key class of inflammatory molecules, has been postulated in cell proliferation induced by stroke (340). Finally, factors produced as part of the ischemia-hypoxic response such as the cytokine erythropoietin (507) and the recently discovered “stem cell factor” (SCF) acting through the c-kit receptor tyrosine kinase (235), may also modulate neurogenesis after ischemia.

In summary, enhancement of neurogenesis in the DG and other brain regions may compensate for disconnected circuits that occur after ischemia and contribute to the improvement of functional outcome. Exploiting this plasticity potential of the ischemic brain may provide a possible strategy for enhancing recovery in patients suffering from this injury.

C) HUNTINGTON’S DISEASE. Huntington’s disease (HD) is a neurodegenerative disease that leads to neuronal loss in the caudate-putamen. The discovery of progenitor cells in the human brain (144) has raised the possibility that progenitors from the SVZ may provide a source of replacement. Postmortem analysis of control and HD human brains has revealed that cell proliferation, evaluated with PCNA, is increased in the subependymal layer in HD brains as a function of the severity of the illness (103). The newly generated cells expressed neuronal and glial markers (TuJ1 and GFAP, respectively).

These pioneer data indicate that the diseased adult brain is still capable of neuronal regeneration, which opens new avenues in the treatment of neurodegenerative diseases.

D) OTHER TYPES OF CEREBRAL INJURY. Traumatic brain injury (TBI), the most common cause of death in developed countries, increases cell proliferation in the SGZ (as well as in the SVZ and the cortex; Refs. 107, 319, 459). The number of newly born cells increases as early as 24 h post-TBI; the cells accumulate over time and express TOAD-64 and NeuN (65%) 9 and 21 days, respectively, after the last BrdU injection (200 mg/kg for 9 days; Ref. 107). Similarly to what can be observed after ischemia, DETA/NONOate administration (for 7 days starting 1 day after TBI) to rats subjected to TBI significantly increases proliferation, survival, migration, and differentiation of neural progenitors in the DG (as well as other brain structures) and significantly improves neurological functional outcome (319).

2. Pathologies associated with a downregulation of neurogenesis

A) AFFECTIVE DISORDERS. In unipolar major depression, bipolar mood disorders, and other chronic diseases associated with affective disorders [posttraumatic stress disorder (PTSD), Cushing’s disease], brain imaging studies have consistently described hippocampal atrophy (60, 195, 329, 504, 518, 519, 550). Although a role for neurogenesis in mood disorders is speculative, it has been suggested that a fall in neurogenesis in the DG contributes, together with atrophy and death of hippocampal neurons, to hippocampal attrition.

I) Antidepressants. It has been suggested that biogenic amine deficiency underlies mood disorders, and indeed, treatments increasing extracellular levels of serotonin or norepinephrine are effective in the remediation of depressive symptoms. Because serotonin and norepinephrine stimulate cell genesis (see sect. nD2S and Table 1), and given that it takes weeks for antidepressants to take effect, it has been proposed that they may exert their action via a remodeling of neuronal networks by promoting either neurogenesis or the survival of hippocampal neurons believed to be endangered (131). Chronic (and not acute) treatments with different classes of antidepressants such as a selective serotonin reuptake inhibitor (fluoxetine, 5 or 10 mg · kg\(^{-1}\) · day\(^{-1}\) for 11–28 days), a selective norepinephrine reuptake inhibitor (reboxetine, mg · kg\(^{-1}\) · day\(^{-1}\) for 21 days), a monoamine oxidase inhibitor (tranylcypromine, 7.5 mg · kg\(^{-1}\) · day\(^{-1}\) the first week and 10 mg · kg\(^{-1}\) · day\(^{-1}\) the second week), and tricyclic antidepressants (TCAs, imipramine and desipramine: 20 mg · kg\(^{-1}\) · day\(^{-1}\) for 28 days) increase hippocampal cell proliferation evaluated 2 or 24 h after the last BrdU pulse (1 × 75 mg/kg or 4 × 75 mg/kg every 2 h) performed on the final day or 4 days after the treatment (335, 483). Three weeks after labeling, most of the newly born cells express NeuN (70%), independently of the classes of antidepressants (335, 483). It was further shown that a fluoxetine- but not an imipramine-induced increase in cell proliferation is mediated by 5HT\(_{1A}\) receptors, since it is abolished in mice lacking 5HT\(_{1A}\) receptors (483).

To determine effects of fluoxetine on cell survival, BrdU (4 × 75 mg/kg spaced by 2 h) was administered before intiation of the chronic treatment (5 mg/kg for 14 days). Neither survival nor maturation of the cells, evaluated 28 days after the last BrdU injection, was influenced by fluoxetine treatment (335). The preclinical contribution of hippocampal neurogenesis has been examined in the learned helplessness paradigm, a classical animal model for depression, and it was reported that inescapable stress induced a decrease in cell proliferation that was reversed, together with behavioral despair, by a 7-day course of fluoxetine (10 mg/kg) (334). A strongest link between “depression” and neurogenesis was evidenced following X-irradiation that blocked the effects of fluoxetine and imipramine on both novelty-suppressed feeding (another test used to assess chronic antidepressant effects) and neurogenesis (483).

Although the etiologies of mood disorders are numerous, ranging from various environmental factors to genetic vulnerability, several lines of evidence suggest that stress-induced cellular changes in the adult HF, mediated
by an upregulation of the HPA axis, contribute to the pathophysiology of these disorders, at least in animal models. The impact of antidepressants on neurogenesis was therefore tested in various stress models. In adult male tree shrews exposed to chronic psychosocial stress (see sect. II B1), chronic treatment with tianeptine (50 mg · kg$^{-1}$ · day$^{-1}$ for 28 days), a modified TCA, reversed the stress-induced decline in hippocampal volume, hippocampal activity, and hippocampal neurogenesis (104). In rat pups, fluoxetine treatment (5 mg/kg for 7 days concurrent with 50 mg/kg BrdU) counteracted the downregulation of cell proliferation observed in the DG of 2-wk-old maternally separated pups (300). In a chronic mild stress model (CMS; see sect. II B1), chronic treatment with the antidepressant fluoxetine (10 mg/kg for 28 days starting 3 wk after the beginning of the CMS) reversed the stress-induced reduction of cell proliferation and prevented the stress-induced reduction of neurogenesis and hippocampal atrophy (10).

II) Electroconvulsive therapy. Electroconvulsive therapy (ECT) is one of the most effective treatments for major depression, especially in subjects who do not respond to antidepressants, but its mechanisms of action remain largely unknown. Electroconvulsive seizure (ECS) differs from kindling and chemokonvulsant treatments in that it does not lead to an epileptiform state and does not cause cell death. ECS (one daily for 10 days) enhances (2 or 24 h after a BrdU pulse) cell proliferation in the DG, and the newly born cells survive for at least 3 mo, a time point when most of them express NeuN (75%) and few GFAP (13%) (331, 335). This proliferative effect is dose-dependent as a series of seizures further increases neurogenesis (331). The neurogenic efficacy of ECS has been investigated in conditions mimicking stress-induced depression, i.e., in animals with high corticosterone levels. The downregulation of neurogenesis (75%) in the GCL of rats chronically treated with corticosterone was eliminated by ECS performed at the end of the treatment (212). Because ECS upregulates several factors described as stimulating neurogenesis, such as bFGF, neuropetide Y, VEGF, BDNF, and Cox-2 (404, 406), these factors are likely mediators of the proliferative effect of ECS. Among them, BDNF deserves special mention as ECS-induced MFS is diminished in BDNF knockout mice (549).

III) Mood stabilizers. Chronic treatment with lithium can reverse the hippocampal attrition in sufferers of manic-depressive illness (MDI) and enhance the levels of N-acetylaspartate, a putative marker of neuronal viability (375, 376, 544). Chronic treatment of mice with lithium (2.4 g/kg during 3–4 wk) increases the number of BrdU-labeled cells by 25% 1 day after BrdU treatment (1 × 50 mg/kg over 12 days), two-thirds of which coexpress NeuN. Interestingly, the expression of bcl-2, an antiapoptotic regulator, is also increased suggesting that survival of the newly born neurons might be enhanced (84).

IV) Summary. These results suggest that antidepressants, ECT, and mood stabilizers may exert some of their therapeutic effects on mood disorders by stimulating neurogenesis. They also suggest that alternative strategies for stimulating neurogenesis may provide new avenues for the treatment of mood disorders. Since a fall in neurogenesis has been associated with an alteration in cognitive functions, further studies are needed to clarify the involvement of neurogenesis, and hippocampal function, in the development of cognitive deficits observed in depression in young and elderly patients.

B) SCHIZOPHRENIA AND NEUROLEPTICS. Developmental dysfunctions of the HF is thought to play a major role in the pathogenesis of schizophrenia (206, 488), and defects such as a reduction in hippocampal volume (53, 400, 558), hippocampal shape deformations (101), abnormalities in the GCL (357), the MF pathway (170), the hippocampal cell density (41, 150, 233) and orientation (93, 288), and in several cellular markers (203, 313) have been reported. These changes are believed to underlie the progressive deficit in cognition that is a hallmark of schizophrenia. Interestingly, models of aberrant neurogenesis (prenatal stress, X-rays, or MAM manipulations) are thought to reproduce some of the behavioral characteristics associated with schizophrenia (313).

The influence on neurogenesis of chronic treatment with the typical neuroleptic haloperidol, a mainstay in the treatment of schizophrenia, has been evaluated, leading to controversial results. Indeed, haloperidol has been shown to increase (110) or to have no effect (199, 335, 483, 562) on neurogenesis in the rodent DG. Differences in dosage, time course of drug treatment, species and age of the animals, and BrdU labeling protocol could account for the observed discrepancy. On the other hand, chronic treatment with atypical neuroleptics such as risperidone (0.5 mg · kg$^{-1}$ · day$^{-1}$ for 21 days) or olanzapine (2 mg · kg$^{-1}$ · day$^{-1}$ for 21 days) increases cell proliferation (24 h after 1 × 100 mg/kg BrdU on the 20th day of neuroleptic treatment) in the SVZ but not in the DG (562). A low dose of clozapine (0.5 mg · kg$^{-1}$ · day$^{-1}$ for 4 wk before a single injection of BrdU at 200 mg/kg) enhances the number of BrdU-IR cells in the DG; however, this effect is transient as the newly born cells do not survive 3 wk after labeling.

Although these data clearly show that further work is required to sort out discrepancies in results, they also suggest that schizophrenia may be associated with reduced neurogenesis in the DG, where normal levels could be reestablished by neuroleptic treatment, a hypothesis that awaits confirmation.

C) ADDICTION TO DRUGS OF ABUSE. Long-term neuroadaptive changes in the mesolimbic dopaminergic system have classically been observed in drug abuse (283, 402). A potential role of the HF in addiction has been suggested based on the observation that both a reduction in hippocampal volume and structural abnormalities are ob-
erved in patients with chronic alcoholism (4, 112, 357, 524) and in psychostimulant addicts (33). In animals, chronic treatment with different drugs of abuse leads to structural changes in the HF (308, 432, 461, 463), to cell loss (563), and to LTP alterations (109, 158, 191, 200, 202, 449). The possible involvement of the HF in addiction is supported by the observation that 1) inhibiting the activity of the calcium/calmodulin-dependent protein kinase II impairs drug conditioning (321, 535) and attenuates the dependence on morphine and relapse (321), 2) dorsal hippocampal lesions disrupt acquisition of cocaine conditioning (365), 3) electrical stimulation of the glutamatergic efferent pathway of the HF to the nucleus accumbens reinstates cocaine-seeking behavior (561) and α-amphetamine self-administration behavior (533), and 4) inactivation of the ventral subiculum decreases reinstatement of cocaine-seeking behavior (525). These results suggest that memory traces of the association between a specific context and the availability of the drug could be stored in the HF, and the hypothesis that drug addiction is an aberrant form of learning mediated by maladaptive recruitments of hippocampal-dependent memory system has recently gained interest (45, 147, 401, 462, 571). In this context, the involvement of neurogenesis in addiction has been studied by examining the influence of several drugs of abuse on adult hippocampal neurogenesis and by comparing neurogenesis in animals that differ in their vulnerability to developing drug abuse.

1) Opiates. Among the multiple actions of opiates in the brain, their influence on cognition and reward is without a doubt the most relevant parameter with regard to opiate addiction. Eisch et al. (137) were the first to demonstrate that the exogenous chronic (daily implantation of subcutaneous pellets containing 75 mg morphine during 5 days) and not acute (10 mg/kg) administration of morphine decreases cell proliferation in the GCL by 28% (measured 2 h after an injection of BrdU, 100 mg/kg, on day 6). When cells are allowed to mature for 4 wk, survival of BrdU-labeled cells is halved and 90% of the newly born cells differentiate into neurons. More relevant to addiction, the influence of heroin self-administration has been investigated. After 26 days of heroin intake (60 μg · kg⁻¹ · injection⁻¹), a downregulation of cell proliferation (~30%) is observed in both the GCL and the hilus while cell death is unaffected. The downregulation of cell proliferation is independent of corticosterone secretion (137), a hormone known to have a major influence in addiction (343). Finally, it was recently reported that in vitro reduced signaling through μ- and δ-opioid receptors present on adult hippocampal progenitors, although decreasing cell proliferation, leads to a net increase in the number of newly generated neurons (437).

2) Nicotine. The neuroactive compound nicotine is considered to be responsible for the development and the maintenance of tobacco addiction. To analyze the effects of nicotine abuse on hippocampal neurogenesis, rats were trained to self-administer nicotine (0.02, 0.04, and 0.08 mg · kg⁻¹ · infusion⁻¹) for 42 days. A profound decrease in hippocampal cell proliferation was observed for the two highest doses of nicotine 1 day after BrdU pulse (4 × 50 mg/kg on days 39–41). This effect is not simply due to a difference in blood flow leading to a decrease in BrdU availability, since nicotine intake does not affect cell proliferation in the SVZ. Half of the newly born cells in the DG were found to express NeuN. In parallel with the decrease in cell proliferation, cell death, measured by the number of pyknotic cells, was increased by the two highest nicotine doses (3). Similar results were obtained following imposed administration of nicotine (1 mg · kg⁻¹ · day⁻¹ for 3 days concurrent with 50 mg/kg BrdU) to adolescent rats (230). Although the mechanisms involved in the effects of nicotine have not yet been investigated, nicotine could act directly on nicotinic acetylcholine receptors that are present on immortalized hippocampal progenitor cells (44), or indirectly through an upregulation of the HPA axis (67) and/or a downregulation of the serotonergic system (43).

II) Alcohol. The possibility that alcohol brings about damage in the adult brain by disrupting neurogenesis has been examined. Thus both acute (gavage with 5 g/kg ethanol) and chronic (ethanol infusion 3 times/day over 4 days for a mean dose of 9.3 g · kg⁻¹ · day⁻¹) binge alcohol treatments halve cell proliferation in the SGZ of the DG within 5 h (409). Furthermore, after 28 days, chronic binge treatment decreases the survival rate of the newly born cells (409). In contrast, in animals fed for 6 wk with a moderate dose of ethanol (6.4% vol/vol), the number of newly born cells (labeled by 7 BrdU injections at 2-h intervals) is not reduced when animals are killed 1 h after the last BrdU injection (218). In fact, the number of nascent cells decreases only after 2 wk suggesting that, in this experimental model, cell survival, rather than cell proliferation, is affected by ethanol exposure, a hypothesis that was confirmed by showing that cell death was dramatically increased in the DG. Finally, these effects are specific to the DG as bulbar neurogenesis is not influenced by chronic alcoholism. Similar results were obtained with imposed injections of alcohol to adolescent rats (1 mg · kg⁻¹ · day⁻¹ for 3 days concurrent with 50 mg · kg⁻¹ · day⁻¹ BrdU), which led to a downregulation of hippocampal cell proliferation and increased cell death (230). Furthermore, coadministration of alcohol with nicotine reduces cell proliferation and cell death more potently than treatment with either one of the agents (230). Although these effects of ethanol could be mediated by NMDA or GABA_A receptors (224), oxidative stress might also play a role since the antioxidant ebselen, used in the treatment of cognitive disorders of alcoholic patients, prevents ethanol effects on hippocampal neurogenesis (218).
IV) Cannabinoid. Derivatives of cannabis sativa such as marijuana are acknowledged to be the most commonly used illicit drugs (336). The structure of their active components, the cannabinoids, the identification in the brain of the cannabinoid-1 (CB1) receptors, and the isolation of an endogenous substance acting on these receptors, the endocannabinoid anandamide, have been reported only recently. It has been suggested that the CB1 receptors that are expressed in the HF by the GABAergic interneurons are important in learning and memory (109). Chronic treatment with anandamide (5 mg · kg⁻¹ · day⁻¹ for 4 days) inhibits neurogenesis in the DG by decreasing the number of newly born cells that differentiate into granule neurons 12 days after the cessation of the treatment (100 mg/kg BrdU on day 2). Conversely, blocking the endogenous cannabinoid tone with SR144716 (1 mg · kg⁻¹ · day⁻¹ together with anandamide), an antagonist of the CB1 receptors, increases hippocampal neurogenesis by favoring neuronal differentiation (476). These effects are in accordance with a potentiality to modulate neural cell fate (197) in particular through the ERK signaling pathway (476). In addition to showing that endocannabinoids constitute a physiological system regulating neurogenesis, these data raise the possibility that cannabinoid addiction could be associated with an alteration in hippocampal neurogenesis.

V) Neurogenesis as a substrate for vulnerability to addiction. The high responder rats (see sect. wB2) and the prenatally stressed rats (see sect. wB3) characterized by curtailed neurogenesis share some similarities that are relevant to drug addiction. Indeed, the HRs spontaneously develop amphetamine (443, 445) or nicotine (528) self-administration behavior, and prenatally stressed rats exhibit greater sensitivity to the psychomotor-stimulant effects of nicotine (279) and amphetamine (215) and are more vulnerable to drug addictive effects as they develop intravenous amphetamine self-administration (116). Thus subjects starting off with the lowest neurogenesis may be most vulnerable to the development of addictive behavior. It is noteworthy that there is high comorbidity between drug abuse and many psychiatric illness, among which depression and schizophrenia (34, 152). Because hippocampal neurogenesis is reduced in preclinical models of these pathologies, it may be hypothesized that impaired hippocampal neurogenesis may constitute a potential substrate for vulnerability to drug abuse, depression, and schizophrenia (see also Fig. 8).

VI) Conclusions. The downregulation of hippocampal neurogenesis, together with other maladaptive plasticity processes, i.e., dendritic remodeling and synaptic plasticity modifications, are associated with permanent functional alterations in behavioral inhibition and learning, hallmarks of addiction. An alteration in hippocampal plasticity could be responsible for the cognitive deficits that appear in the course of the “illness” (88, 109, 133, 194) or during drug withdrawal (211). Because drug-seeking and -taking behaviors are sensitive to the presence of drug-associated stimuli (contextual cues), initially low hippocampal plasticity and a drug-induced decrease in hippocampal plasticity may be involved in the vulnerability and the maintenance of drug abuse. Changes in hippocampal activity could lead to a dysregulation of the dopaminergic mesoaccumbens reward system (283, 402) through the hippocampal glutamatergic output that gates information in the nucleus accumbens (173) and/or through the hippocampal glutamatergic output to the mesencephalic dopaminergic cell bodies. Indeed, stimulation of the ventral subiculum increases the firing of dopaminergic neurons (51) and enhances dopamine release within the nucleus accumbens (302). Finally, drug-taking in subjects vulnerable to drug addiction most probably further increases corticosterone secretion, stimulates the dopaminergic reward system, and decreases hippocampal neurogenesis, thereby constituting a pathophysiological loop.

IV. CONCLUSION

The discovery of neoneurogenesis in discrete areas of the adult brain has opened up an extremely interesting field of research in itself. Indeed, although it was initially confronted with the universal belief that after a certain period of development neither neural cell genesis nor divisions were any longer possible, it now constitutes a prototypic model of developmental neuroscience. The study of the mechanisms that preside over cell proliferation, migration, differentiation, and survival is essentially performed in the SVZ-OB paradigm as these phenomena occur in distinct compartments (cell proliferation in the SVZ, migration in the RMS, differentiation in the OB) thus rendering their analysis easier. However, the evaluation of their behavioral consequences is most frequently examined in relation to the HF (with the exception of Ref. 464).

To conclude, the main issues raised by neurogenesis are as follows.

1) From a cellular point of view, a true characterization, and thus definition of stemlike and progenitor cells in the adult brain is still lacking. Although immense progress has been achieved in the description of different cell types (and their properties) in the SVZ, no consensus has been reached. Furthermore, although it has been suggested that adult neural stemlike cells could behave similarly to embryonic stem cells as far as the generation of neurons and differentiation into any neuronal type are concerned, little is known regarding their intrinsic properties and the nature of the signals that lead to the generation of neurons in the adult brain. It is expected that functional genomic technologies may help in defining the
transcriptional program of neural progenitors and the changes in gene expression in progenitors over time (105, 323).

2) Future studies will have to better characterize the early development of adult-born neurons to dissect the steps that are influenced by experience and vulnerable to traumatic conditions (stress, drug, epilepsy...). This raises the problem of tracking the fate of adult-born cells using BrdU, [3H]dT, or a retrovirus, which may alter the functioning of the adult-born neurons under investigation.

3) Undoubtedly one of the most interesting sets of data accumulated concerns the factors that powerfully regulate neurogenesis. However, the mechanisms by which these regulations are exerted in the normal and pathological brain remain obscure.

4) Although most work is focused on the generation of glutamatergic excitatory granule neurons in the adult DG, the recent discovery that GABAergic inhibitory neurons are also produced raises the important possibility that external factors (from molecules to modifications in animals’ environments) might diversely affect the genesis of either type of neurons and thus have distinct functional consequences.

5) Functional studies are too often restricted to the evaluation of a single parameter; they should be extended to the different components of neurogenesis (basal neurogenesis and experimentally induced cell proliferation, cell death, and cell survival) and to other parameters related to the anatomical (e.g., the presence of synaptic contacts) and functional (electrophysiological properties, ERK phosphorylation, immediate early gene activation...) integration of new neurons into the preexisting networks. Finally, the contribution of newly born neurons to the functioning of the HF and its output should be evaluated as well as their influence on the activity of other related structures (e.g., the frontal cortex).

6) Functional evaluation of hippocampal neurogenesis has, in fact, been the object of relatively few studies that have been restricted to a limited number of behavioral tasks (mainly the Morris water maze). A more complete diversified behavioral analysis is therefore necessary to understand the contribution of neurogenesis to hippocampal functioning.

7) Except for the elegant work of Shors et al. (509), which relies on the use of MAM which is unfortunately a highly toxic compound, most studies correlate changes in rates of neuron addition with behavioral changes, in particular cognitive functions. Thus it is not clear whether these changes are coincidental or coregulated. A more direct approach to address the existence of a causal relationship between behavioral changes and neurogenesis will probably depend on the development of inducible

![Diagram showing the relationship between hippocampal functioning and neurogenesis](image-url)

**FIG. 7.** Variation of hippocampal functioning as a function of hippocampal neurogenesis and associated pathological states.
transgenic models allowing a structure-specific blockade of neurogenesis at a given time during behavioral testing. However, this approach is a real challenge since it is based on the invalidation of the crucial genes controlling neurogenesis site specifically, which have not yet been identified.

8) From a phylogenetic point of view, the apparent reduction in neurogenesis, when moving from rodents to primates, raises the question of the associated evolutionary advantage.

9) From studies that have correlated changes in rates of neuron addition with hippocampal functioning, an inverted U curve shape can be proposed (see Fig. 7). Thus adult neurogenesis may be beneficial for adult brain functioning only within a physiological adaptive range. Beyond a critical level of neurogenesis, which is overpassed by stressful life events, aging, and drug abuse, dysfunction appears. On the other hand, the addition of adult-born neurons above a critical set point (i.e., following epileptiform status) may lead to pathological states and behavioral impairments most probably by introducing noise in the networks, as a consequence of ectopic connectivity.

10) Within this framework, it is not known whether an alteration in neurogenesis is the cause or the result of psychiatric illness (Fig. 8). Indeed, in preclinical studies it has been shown that phenotypes vulnerable to the development of affective or cognitive disorders are characterized by lower hippocampal neurogenesis. It may be hypothesized that these subjects who start off with impaired neurogenesis may be predisposed to the development of affective disorders, addictive behavior, and age-related cognitive disorders. Alternatively, the onset of pathology could produce changes in brain plasticity by acting through several mechanisms (for example, stress and HPA activity are important mediators in the genesis of cognitive impairment, depression, addiction). Repeated “traumatic” episodes, together with a genetic predisposition, may lead to reduced neurogenesis and participate in the worsening of the symptoms. Although these propositions are highly speculative, they highlight the fact that treatments that increase neurogenesis may help to cure or prevent the development of affective and/or cognitive disorders.

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