The neurogenesis field has made remarkable progress in the past two decades (82, 111, 124, 370). Less than 20 years ago, the neuroscience community’s appreciation of adult neurogenesis was quite limited. Despite having been characterized using a range of techniques, the fact that young neurons continue to be incorporated into the adult brain was not widely accepted until the mid 1990s (128, 150). Several factors precipitated this shift. First, immunohistochemical techniques for labeling dividing cells with nucleotide analogs [e.g., bromodeoxyuridine (BrdU)] and protein markers that were specific to neurons (e.g., NeuN), coupled with confocal imaging, allowed the confident identification of adult-born neurons. Second, these tracing techniques revealed that the incorporation of young neurons did not simply occur at a persistent low rate of residual development; rather, it was heavily regulated by behavioral factors such as stress, age, exercise, and enrichment. Finally, the positive identification of adult-born neurons in the human hippocampus, and even in aged individuals, demonstrated that this phenomenon was potentially relevant to human cognition (95).

Neurogenesis has been described in several brain regions of multiple species. It was identified early on in songbirds (253) and has since been observed in other bird species, reptiles, and fish (372). In mammals, neurogenesis appears to be considerably more limited, with robust levels limited to the dentate gyrus (DG) region of the hippocampus and the olfactory bulb (OB). New neurons have also been reported in other areas, with the neocortex (52, 120, 126) and hypothalamus (168) attracting the most attention, although the extent of neurogenesis in these areas remains controversial (273). It is worth noting that the existence of OB neurogenesis [in which maturing neurons migrate from a stem cell population in the subventricular zone (SVZ)] in humans is itself controversial, with differing reports having been presented in recent years (35, 75, 288). Human neurogenesis in the DG is generally accepted, although the levels of new neuron production have only been characterized in limited cases (95, 315).

Both a microcosm of neural development and a never-before-appreciated form of neural circuit plasticity, neurogenesis has recently attracted investigations from perspectives ranging from molecular neuroscience studies of the genetic regulation of young neurons to studies of the computational and behavioral implications of neurogenesis at circuit scales. In this review, we focus primarily on the process of DG neurogenesis (FIGURE 1), summarizing the full process from the origins of new neurons as neural stem cells (NSCs) residing in the subgranular zone (SGZ) through their maturation into fully functional granule cells (GCs). We describe the regulation of the neurogenesis process, which is
influenced by molecular, network, and behavioral sources. We then review the current view of the role of neurogenesis in the DG’s function in learning and memory, ending with a description of current and future techniques for studying new neurons.

A. Characterization of Neurogenesis

It is important to briefly summarize the methods for tracing new neurons, as the techniques used to label a dividing and maturing cell often influence the nature and interpretation of the experiments described below. The most common method for labeling dividing cells involves the incorporation of a traceable molecule into DNA. Because DNA synthesis is generally limited to mitosis, at least at measurable levels, it has been used as a marker of neurogenesis. The first neurogenesis studies utilized tritiated (³H) thymidine (10), permitting the radiographic tracing of cells that had been born at the time of injection. In the 1990s, another thymidine analog, BrdU, was developed because this molecule could be detected using immunohistology. This development was a vital advance since immunolabeling could permit fate identification of dividing cells through the colabeling of other markers, such as the neuronal marker NeuN or the glial marker glial fibrillary acidic protein (GFAP). BrdU labeling remains widely used, as are its sister molecules IdU and CldU (for its iodide and chloride equivalents, respectively), in part because it can measure both proliferation rates and survival. It is worth noting that BrdU, despite its usefulness, is not an ideal marker for several reasons, including potential toxicity, nonspecificity (damaged cells may incorporate it), and histological limitations (172).
Other immunohistological markers can be used to identify proliferating cells; for neurogenesis work, the most widely used is Ki67 (245). Similarly, there are several markers for specific stem cell phases, such as Sox-2 and nestin, which will be briefly described below. It is important to note that, while these protein markers are specific and do not require preceding injections, they are also transient and not particularly suitable for long-term labeling or quantification. Genetic tracing studies, such as cre-lox systems in which a transiently activated promoter permanently labels a neuron with a marker such as lacZ or green fluorescent protein (GFP), are increasingly used for long-term quantification (140, 250). Finally, the most common approach is retroviral labeling of dividing cells with a marker such as GFP. Retroviral labeling is fairly sparse, which limits quantification but can provide highly accurate birthdating, can show full morphologies of new neurons (371), and is suitable for imaging in live slices for electrophysiology studies (343). Several of these methods will be described at greater length later in the text.

B. Neurogenesis in Humans

For obvious reasons, measuring neurogenesis in humans is considerably more difficult. While human neurogenesis was originally confirmed using BrdU (95), the sample sizes were too low for thorough quantification, and subsequent studies using histological markers left some doubt regarding the overall levels of neurogenesis in humans (299, 300). Recently, Frisen and colleagues (315) described a technique whereby rates of new neuron birth in humans could be estimated by taking advantage of increased radioactive carbon in the atmosphere due to above-ground nuclear testing (315). For roughly 15 years, levels of $^{14}$C increased steadily above historic levels; thereafter, levels decayed steadily due to international agreements to limit above-ground testing. Upon entering food supplies, $^{14}$C incorporates into DNA in a manner similar to that seen in the original $[^3]$Hthyidine studies. This technique enables estimates of overall levels of neurogenesis, showing a turnover rate of GCs at $\sim$1.75% a year. Notably, subsequent studies with this technique did not observe new neuron turnover in the OB; however, it may be possible that new neuron incorporation rates there were not well suited for this technique. Interestingly, Frisen and colleagues (96) did observe new neurons in the human striatum, which has not been widely observed in rodents but has in other mammals such as rabbits (44, 206).

II. NSCs AND THE NEUROGENIC NICHE

The ability of NSCs to self-renew, proliferate, and differentiate into all cell types of the nervous system makes understanding these cells and the factors that regulate this process critical to developing potential therapies for a number of neurodegenerative diseases. In the adult brain, neurogenesis is well documented to continue throughout life in only two regions: the SVZ of the lateral ventricles and the SGZ of the DG. Although additional areas have been reported to support adult neurogenesis, we do not focus on those studies in this review. In both regions, NSCs give rise to neural progenitor cells (NPCs), which are limited in proliferation and differentiate into neurons or glia (111). NPCs from the SVZ migrate along the rostral migratory stream and supply newborn neurons for the OB; those in the SGZ migrate a short distance into the GC layer of the DG and integrate into the existing circuitry of the hippocampus.

There has been ongoing debate regarding which cell population within the SGZ is the “true” NSC population, if such a thing can be appropriately defined (41). Considerable evidence exists from experiments in mice leveraging promoters from genes such as Gap, Nestin, Sox2, and Mash to perform lineage analysis (93, 314, 321). These promoters each appear to label slightly different, though overlapping, populations of cells, including both radial glia cells (RGCs), often referred to as type 1 cells, and nonradial type 2 cells.

Several studies have demonstrated that RGCs can give rise to type 2 cells through asymmetric division; however, it is debated whether RGCs can replicate through symmetric division (41). It is also not known whether type 2 cells can revert to an RGC state. However, it is known that from type 2 cells, generates what appears to be a more fate-committed intermediate progenitor cell (IPC) population, sometimes referred to type 3 cells, which has a distinct expression profile, including transcription factors such as Tbr2, and appears constrained to a future neuronal fate (137). It is unclear when and at what rates astrocytes arise from this process as well, with competing hypotheses existing about whether glia are an end product of neuronal differentiation (93) or a separation differentiation path for multipotent cells (42), although it appears that the gliogenesis process may be equivalently complex (46). Notably, it does not appear that oligodendrocytes are a product of this process in vivo (42), although they may arise from a separate pool of progenitors (149).

Since which neurons in the above process are indeed self-renewing and multipotent is still contested, in this review we broadly define NSCs as those cells that are capable of self-renewal, albeit slowly, and broadly multipotent. Likewise, we refer to NPCs as those cells that proliferate quickly and remain capable of becoming glia and neurons but are limited in the number of progeny they can produce. In addition, with regard to neurogenesis and its regulation, it is often useful to consider three higher level processes: cell proliferation, neuronal differentiation, and cell survival (FIGURE 1). Each aspect is critical to the overall levels of neurogenesis. For example, NPC proliferation occurs in other regions of the adult brain, but the cells do not differentiate into neurons, becoming glia instead. However, NPCs isolated from these nonneurogenic regions, such as...
cortex and optic nerve, in the adult brain, retain the potential to become neurons in vitro following treatment with fibroblast growth factor fibroblast growth factor-2 (FGF-2), indicating that extrinsic factors play a major role in stimulating NPCs to differentiate into neurons (262). Further supporting the idea of a neurogenic microenvironment is the finding that the adoption of neuronal cell fates by NPCs under normal physiological conditions is limited in the adult brain to a few regions such as the SVZ and SGZ, but transplantation of NPCs from the SVZ into ectopic regions of the adult brain results in gliogenesis, in which NPCs become oligodendrocytes and astrocytes (298). In contrast, NPCs from a nonneurogenic region, such as the spinal cord, can differentiate into neurons when transplanted into the DG, supporting the idea that external cues from the local microenvironment promote neuronal differentiation of NPCs (306).

The SVZ and SGZ represent neurogenic niches or local microenvironments that permit and support neurogenesis. Studies have identified key components and factors of the neurogenic niche in a number of ways, including in vitro coculture experiments and in vivo manipulations of neurogenesis. While there are a number of underlying similarities between the two environments, in this review we focus on the critical elements relevant to the hippocampal neurogenic niche.

A. Microglia

The neurogenic niche supports and promotes neurogenesis through both secreted factors and cell-cell contact. Microglia are the resident macrophages and primary immune cells of the brain, and they have a multitude of functions, ranging from phagocytosis to neuroprotection. In the adult brain, microglia in the resting state continuously survey the local environment, with their dynamic processes interacting with a number of cell types, including astrocytes, neurons, and endothelial cells (249). In the adult DG, microglia are found distributed evenly in the hilus and along the border of the GC layer (360). In the adult hippocampus, resting microglia are associated with GC death (277); more specifically, interactions between microglia and NPCs regulate neurogenesis via phagocytosis (310). Most newborn cells in the adult DG fail to survive past the first week (159, 310); eventually, the number of surviving newborn cells stabilizes after 3–4 wk (159, 310, 326). The decline in newborn cells within the first week is largely due to apoptotic cell death, which is mediated by microglia, as their processes are localized and engulf apoptotic BrdU-positive cells (310). These studies investigating the behavior of resting and unchallenged microglia demonstrate the active role these cells play in monitoring and maintaining the local environment of an unperturbed adult brain.

The contribution of microglia to the neurogenic niche appears to depend largely on their secretion of cytokines and chemokines. Additionally, it is the balance of proinflammatory and anti-inflammatory signaling that is critical to whether or not the environment supports or prohibits neurogenesis (28, 49, 61). Classically activated microglia secrete the cardinal proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), that can inhibit NSCs from differentiating into neurons in favor of astrocytes (61). However, depending on the environment, individual cytokines can exhibit proneurogenic or antineurogenic properties. For example, transforming growth factor-β (TGF-β) is a cytokine secreted by microglia that is known to activate both proinflammatory and anti-inflammatory pathways, and its expression has been shown to both induce (28) and inhibit neurogenesis (48), illustrating its ability to positively and negatively regulate this process.

Depending on the mode of activation, activated microglia can release proinflammatory or anti-inflammatory cytokines, modulating the immune response supporting or suppressing neurogenesis (49, 233). In in vitro studies, microglia are most commonly activated with lipopolysaccharide (LPS). LPS is an endotoxin from the gram-negative bacterial cell wall that activates microglia through Toll-like receptor-4 to induce both morphological changes and release of cytokines and nitric oxide (37, 243). Coculturing LPS-activated microglia or conditioned media (CM) with NPCs decreases neuronal differentiation of NPCs (49, 233). In contrast, both IL-4- and interferon (IFN)-γ-stimulated microglia induce neuronal differentiation of NPCs, illustrating the potential proneurogenic effects of activated microglia (49).

Interestingly, it is not only the mode of activation but also a temporal component that determines whether activated microglia induce or inhibit neurogenesis. For instance, coculturing acutely activated microglia with LPS (after 24 h of LPS treatment) or CM with NPCs decreases neuronal differentiation and increases glial differentiation (50, 233). NPCs also exhibit increased TUNEL staining when cocultured with CM from LPS-activated microglia, illustrating the potent effects of acutely activated microglia on NPC activity (233). However, CM from chronically activated microglia with LPS (after 72 h of LPS treatment) does not significantly decrease neuronal differentiation of NPCs (50), likely due to a difference in secreted cytokine profiles from acutely and chronically activated microglia. While acutely activated microglia release more proinflammatory cytokines, including IL-1α and IL-6, chronically activated microglia release more anti-inflammatory proteins, like IL-10 and PGE₂ (50).

Further studies have evaluated the activity of microglia by manipulating levels of neurogenesis. Two of the most common ways to increase neurogenesis are through running and exposure to an enriched environment (EE), which appear to
increase neurogenesis via different mechanisms enhancing cell proliferation and cell survival, respectively. The effect of running on cell proliferation is robust, as depicted in the increase in the numbers of BrdU cells in the DG with running (257, 350). However, exercise does not result in an increase in the overall number of microglia in the DG or increase the percentage of activated microglia, illustrating that the overall microglia profile remains unaffected by running (257). Additionally, running does not modulate the expression of MHCII or cytokines in the DG, suggesting that the effect of running on neurogenesis is not mediated by microglia (257). While running does not increase the number of total microglia or level of gene expression, running does influence the interaction between microglia and NPCs. Microglia isolated from runners induce more neurosphere formation of NPCs in vitro compared with microglia isolated from nonrunner mice (350). Fractalkine, whose receptor CX3CR1 is found on microglia (21), is critical in maintaining microglia in the resting, inactivated state (60). In vivo, CX3CR1-deficient mice, which have disrupted fractalkine signaling, exhibit decreased numbers of BrdU-positive cells as well as Dcx-positive cells, indicating a decrease in overall neurogenesis (21, 350). Infusion of a blocking antibody targeting CX3CR1 in running mice generated fewer neurospheres compared with wild-type runner mice, suggesting that the resting state of microglia is necessary for normal NPC activity and is an integral component of the neurogenic niche (350).

Studies have also used aged animals to investigate the role of microglia in the neurogenic niche. While hippocampal neurogenesis occurs throughout adulthood, there is a significant decline in the rate of neurogenesis with increasing age. Aged animals have significantly less NPC proliferation, neuronal differentiation, and newborn neuron survival compared with younger animals (43, 134, 173). Age-related changes are also seen in microglia, and increased levels of inflammation and oxidative stress, both of which strongly inhibit neurogenesis, are observed in aged animals (182). In addition, microglia profiles of aged animals include more activated microglia as well as higher levels of TNF-α and IL-6 secretion upon activation, resulting in elevated levels of proinflammatory cytokines in the brain (203, 251).

Aged animals have lower levels of anti-inflammatory signaling, such as the chemokine fractalkine. Intracerebroventricular infusion of fractalkine into aged animals restores hippocampal cell proliferation, suggesting that the presence of fractalkine helps to provide a noninflammatory environment supportive of neurogenesis. This phenomenon appears to be mediated by IL-1β, as blocking CX3CR1 increases IL-1β protein levels in the hippocampus (21). These data further highlight the sensitivity of neurogenesis to the balance of pro- and anti-inflammatory cytokines and chemokines.

In addition to inflammation, microglia play a key role in modulating levels of oxidative stress by producing the antioxidant glutathione (136, 198). Microglia from aged mice have significantly lower glutathione levels than microglia from young mice (251), and oxidation products are found in microglia in aged mice (133), suggesting that microglia are less effective in attenuating oxidative stress in aged mice. These data illustrate how changes in the activity of microglia with age potentially contribute to the age-related decline in neurogenesis by altering the neurogenic niche.

**B. Astrocytes**

In addition to microglia, NPCs interact with other cell types present in the neurogenic niche (Figure 1). Astrocytes represent one of the major contributors to the neurogenic niche, as coculturing with astrocytes, both directly and without contact, induces neuronal differentiation of NPCs (27, 256, 313). The relationship between astrocytes and NPCs depends on a number of factors, including gene expression of the astrocytes. Coculturing astrocytes with NPCs has revealed the importance of GFAP and vimentin in mediating cell-cell contact between these two cell types (358). Wild-type neurospheres differentiated into neurons at a greater rate when cocultured with astrocytes without GFAP and vimentin expression, indicating that these intermediate filament proteins negatively regulated neurogenesis in vitro (358). This inhibitory effect may be mediated by Jagged1 and Notch signaling pathways, as Jagged1 mRNA is significantly decreased in astrocytes without GFAP and vimentin (358). Other proteins in hippocampal astrocytes, such as ephrins, have also been identified to play a role in regulating neurogenesis through cell-cell contact (18). Specifically, ephrin-B2 expressed by hippocampal astrocytes induced neuronal differentiation of NPCs by activating β-catenin signaling, and shRNA-mediated knockdown of astrocytic ephrin-B2 resulted in reduced hippocampal neurogenesis (18).

The region of origin of astrocytes plays a significant role in their ability to promote neurogenesis of NPCs. Astrocytes isolated from the adult hippocampus but not spinal cord promote neuronal differentiation of NPCs in coculture through both soluble factors and cell-cell contact (313). While astrocytes derived from the hippocampus and cortex are able to induce neuronal differentiation, those isolated from the hippocampus are more effective, suggesting hippocampal astrocytes release more potent neurogenic factors (256).

Analyzing gene expression from astrocytes isolated from different regions of the central nervous system has identified a number of key factors. Two of these factors released by neurogenesis-promoting astrocytes are IL-1β and IL-6, which are proinflammatory cytokines that, in combination with other factors such as vascular cell adhesion molecule-1.
(VCAM-1) and interferon-induced protein 10 (IP-10), increase neuronal differentiation from NPCs (27). While pro-inflammatory cytokines generally inhibit neurogenesis, this inhibition depends largely on the concentration of the cytokines. More importantly, it is the combination of factors secreted by astrocytes into the local microenvironment that produces the neurogenic niche (27). In contrast, astrocytes isolated from the spinal cord express more insulin-like growth factor binding protein-6 (IGFBP-6), decorin, and enkephalin, which negatively regulate neurogenesis (27).

One factor that is continuously expressed in the hippocampus throughout adulthood is Wnt3a, representing a promising key factor of the neurogenic niche (197). Wnt3a is produced by astrocytes, and β-catenin signaling is stimulated in NPCs, indicating a possible pathway by which astrocytes directly regulate NPC activity (197). The Wnt signaling pathway has been shown to regulate NPC proliferation and differentiation in adult neurogenesis in vitro and in vivo (175, 197, 356). Wnt modulates adult neurogenesis by upregulating NeuroD1 expression, which is an essential transcription factor in the generation of GCs and neuronal differentiation from NPCs (175). In response to Wnt, NPCs increase expression of LINE-1, a retrotransposon that is important in NPC survival, demonstrating that Wnt regulates multiple facets of adult neurogenesis in the hippocampus (175). Other components of the Wnt pathway, including Dickkopf-1 and secreted frizzled-related protein 3, have been recently implicated in the regulation of neurogenesis by aging and behavior (143, 297).

Glial metabolism appears to be a key modulator of adult hippocampal neurogenesis. Selective inhibition of glial metabolism can be accomplished by treating cells with fluorocitrate, which inhibits glial aconitase, leading to reduced levels of glutamine (106). While it is unclear if there are direct effects on NPCs, fluorocitrate treatment results in a decrease in NPC proliferation both in vivo and in vitro, suggesting that a critical metabolite produced by astrocytes may be necessary for NPC proliferation (58). ATP from astrocytic metabolism serves as a signaling molecule to increase NPC proliferation in vitro and in animal models with defective astrocytic ATP release (58). Behaviorally, animals deficient in astrocytic ATP release exhibit depression-like behavior, which can be reversed with the administration of ATP (59), supporting the link between adult neurogenesis and depression-like behavior (283).

Studies have further investigated the role of astrocytes in the neurogenic niche using aged animals to evaluate how age-related changes in astrocytes correlate with their low levels of neurogenesis. The levels of hippocampal growth factors that are known to promote neurogenesis, including insulin growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF), are significantly reduced in aged rats compared with young rats (36, 305). While there are conflicting reports about whether levels of FGF-2 or its receptor are decreased in the hippocampus of aged rats, it is clear that this pathway is affected negatively with age (36, 305). The overall density of hippocampal astrocytes, which are immunopositive for GFAP, remains the same throughout adulthood; however, the density of astrocytes that express FGF-2 declines with age in the DG as well as other regions of the hippocampus, suggesting that the local environment for NPCs progressively becomes less supportive of neurogenesis with age (305). The altered profile of astrocytes in the aged hippocampus suggests that astrocytes are a major contributor to maintaining a neurogenic niche, and the decline in their secreted, proneurogenic factors correlates with the decline in hippocampal neurogenesis with age.

Interestingly, brain-derived neurotrophic factor (BDNF) is a neurotrophin that regulates hippocampal neurogenesis (194, 263). TrkB, a high-affinity receptor for BDNF, is expressed on NPCs (194), and levels of BDNF are highest in the hippocampus and hypothalamus in rat brains (154). Studies have demonstrated that hippocampal astrocytes minimally produce BDNF (281, 282), and BDNF is highly localized within the nucleus of GCs of the DG (154). While BDNF protein levels in the hippocampus do not decline with age, as do the previously discussed factors (38, 153, 178), its receptor TrkB decreases with increasing age in the hippocampus of rats (74, 311). There is also evidence that serum levels of BDNF in humans are correlated with hippocampal volume and memory, as illustrated by the lower levels seen in older people with smaller hippocampi and poor memory (94).

C. Vasculature

In addition to secreting factors to NPCs, astrocytes are tightly linked physically with endothelial cells, wrapping their endfeet around blood vessels (FIGURE 1). The vasculature represents an abundant source of extrinsic factors that can modulate adult neurogenesis. Illustrating this possibility, NPCs can be found in clusters in close proximity to blood vessels (264, 304). The physical location of NPCs in relation to blood vessels suggests that NPCs may receive critical factors from the vasculature to stimulate proliferation, neuronal differentiation, and survival (264). Endothelial cells secrete soluble factors that increase NPC proliferation, neurogenesis (303), maturation, and migration, in part by secreting BDNF (191).

The intimate relationship between NPCs and the vasculature is much more evident in animals with access to a running wheel. As discussed earlier, running is a potent stimulator of adult hippocampal neurogenesis, which is accompanied by an increase in cerebral blood flow in the DG (266). The number of blood vessels does not increase with running, but the surface area covered by blood vessels is significantly increased in the DG (344). The increase in the
density of blood vessels of runners is specific to the DG and not the entire hippocampus (66), a finding that is consistent with the increased cerebral blood flow observed in running mice (266). Although the number of blood vessels remains stable in the DG with running, there is evidence of angiogenesis in the molecular layer of the hippocampus with running, because newborn cells colabeled with an endothelial cell marker, rat endothelial cell antigen-1, are observed in this region (92). Angiogenesis and neurogenesis rely heavily on the circulating growth factors and nutrients supplied by the vasculature.

VEGF is a factor that has been shown to promote proliferation of not only endothelial cells but also NPCs (145). Expression of VEGF and its receptors is not limited to endothelial cells but also expressed in NPCs, neurons, and glial cells. VEGF mRNA and protein are expressed in the hippocampus, colocalizing with GFAP and NeuN (57, 196). VEGF-induced neurogenesis is mediated predominantly through the Flk-1 receptor rather than Flt-1. Expression of Flk-1, but not Flt-1, is robust in the SGZ, and Flk-1 colocalizes with BrdU-positive cells in the SGZ (57, 145). Adult rat NPCs express both VEGF and Flk-1, and VEGF stimulates neurogenesis via Flk-1 in vitro (292). In addition to local signaling, VEGF upregulation in the periphery is essential to the increase in neurogenesis induced by running, illustrating its broad range of influence in modulating neurogenesis (98).

III. DEVELOPMENT AND MATURATION PROCESS OF ADULT-BORN NEURONS

In the adult SGZ, NSCs give rise to NPCs, which in turn generate immature neurons. Immature neurons migrate a short distance to the granule cell layer (GCL). Adult-born neurons follow the same outside-in layering pattern as developmentally born neurons in DG, with adult-born neurons preferentially staying in the inner or middle of the GCL and cells born in embryonic and postnatal stages of development found in the outer GCL (219). Extracellular matrix molecules, such as the Reelin signaling pathway (45), tyrosine kinase receptors (70), and chemoattractant and repulsive signals (362), may play important roles in the migration of adult-born neurons. While the role of extracellular matrix molecules in neurodevelopment has been extensively studied, a recent study investigated the effects of manipulating Reelin and Disabled-1 (Dab1) on newborn cell migration and dendritic maturation in the hippocampus (327). In mice that overexpressed Reelin under the Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) promoter, 2-wk-old neurons displayed greater dendritic branching and arborization, indicative of faster maturation, compared with those found in wild-type littermates. Consistent with this finding, conditional deletion of Dab1 in newborn neurons resulted in aberrant dendritic extensions and shorter, fewer processes. Disturbed migration and integration are often identified in pathological conditions such as epilepsy, schizophrenia, and neurodegenerative diseases (63).

A. Morphological Maturation of Adult-Born Neurons

It takes roughly 2 mo for newborn neurons to reach morphological maturity (FIGURE 1). Although no significant structural differences are observed between fully mature adult-born and perinatal-born neurons, the maturation process is delayed in the adult (259, 371). It is likely that differences in the local niche between the adult and neonatal brain contribute to the different maturation rate. In the adult hippocampus, by roughly 1 wk after differentiation, newborn neurons can be seen extending their apical dendrite into the molecular layer, and spines are first detected in the dendrite at 16 days post division (dpd). Spine density significantly increases between 21 dpd and 28 dpd (371), which corresponds to a critical period for neurogenesis-related learning and memory (325). The number of spines appears to reach a plateau by 56 dpd. Furthermore, during these initial 2 mo, the spines remain highly plastic, dynamic, and regulated by neural activity (371).

Axon mossy fibers reach to the CA3 region before the first dendritic spine is detected (371) (FIGURE 2). The thin axon fibers are first found in the hilus at 10 dpd and continue growing until they reach to CA3 (371). Axonal boutons in hilus are significantly smaller than those in the CA3 region (330), consistent with what has been classically observed of mossy fibers. Synapses arising from adult-born neurons can be identified by electron microscopy (EM) as early as 17 dpd onto postsynaptic targets in the CA3 and the hilus (330). These axon boutons form synapses on thorny excrescences and dendritic shafts of neurons in CA3 and hilus, respectively. The overall size of these mossy terminals, the number of presynaptic vesicles, and the number of activated zones continue to increase until roughly 75 dpd, when they are comparable to those of perinatal-born neurons. In addition, some thin axon fibers form en passant synapses onto the dendrites of GABAergic interneurons. At a population level, synapses from 3- to 4-mo-old neurons were found to be functional by use of Channelrhodopsin-2 (ChR2) (330). A subsequent study using a similar optogenetic technique demonstrated that the maturation of mossy fiber axonal contacts onto CA3 from young neurons had a roughly similar time course as dendritic maturation, with newborn mossy terminals passing through several stages of increased plasticity (129).

Notably, the spine formation process of adult-born neurons appears to be different from that of perinatal-born neurons in that adult-born neurons preferentially target preexisting synapses, and little is known about the underlying mechanisms (332). One hypothesis is that glutamate spillover may play a chemoattractive role and induce filopodia growth.
towards active synapses (331, 332). Local synaptic activity may induce glutamate release and activate glutamate receptors in filopodia, which induce new filopodia to target the existing synapse (331). Recent evidence suggests that local astrocytes may also contribute to this targeting process (171). Three-dimensional EM analysis shows that both synaptic outputs (axon boutons) and inputs (dendritic spines) from adult-born neurons form intermediate structures on which multiple synaptic partners are shared at a single synaptic structure (i.e., multisynapse boutons) (330, 332). As neurons mature, the proportion of multisynapse bouton structures decreases.

This structural difference parallels the differences between the physiologies of immature and mature GCs. Newborn neurons display a high input resistance (97), receive less inhibition (193), and have been shown to exhibit considerably greater synaptic plasticity (115, 295). Because of these unique properties, young neurons are likely to be more excitable than mature neurons (97, 231, 232), and thus, in response to presynaptic inputs, the synapses formed by newborn neurons in the multisynapse boutons may be more dynamic than the existing synapses. The increased dynamics of young synapses may allow new protrusions to enlarge and subsequently lead to the eventual retraction of spines from the mature neurons (331).

**B. Physiological Maturation of Adult-Born Neurons**

For integration into the DG circuit, adult-born neurons follow the same process as perinatal neurons: silent synapse, GABA excitatory response, glutamatergic inputs, and GABA inhibitory response (97).

The amino acid GABA is the major inhibitory neurotransmitter in the adult brain (170). However, GABA plays very different roles in NPCs and their progeny (114). The presence of functional GABA receptors is observed as early as in type 2 progenitor cells (333). After neuronal fate is determined, yet prior to receiving synaptic inputs, adult-born neurons are tonically activated by GABA released at extrasynaptic sites (81, 114). GABA’s tonic effects are able to
regulate the excitability threshold for neurons (117, 351), including reducing its own tonic effect, enhancing long-term potentiation (LTP) and improving performance on hippocampus-dependent spatial learning (19, 69). In mature cortical and hippocampal neurons, α5 or σ subunits are primarily components of extrasynaptic GABA_A receptors (117, 351). Little is known about the major components of the extrasynaptic GABA_A receptors in adult-born neurons and about how GABA’s tonic effects regulate their maturation, integration, and function.

Functional GABA and glutamate receptors are detected in 3-day-old immature neurons (97), but GABAergic synaptic events are not detected until neurons are a week old. Similar to what is seen in type 2 progenitor cells and neurons in developing brain, high [Cl\(^{-}\)], and expression of Na\(^+\)-K\(^+\) transporter NKCC1 in immature neurons cause GABA to depolarize instead of hyperpolarize cells. This initial depolarizing GABA phenotype has been shown to be critical for the maturation of adult-born neurons (64, 114). Knocking down NKCC1 in immature neurons leads to significant defects in both GABAergic and glutamatergic synapse formation as well as in dendritic development of new neurons (114). Two possibilities may explain this phenotype: manipulation of NKCC1 expression may convert GABA from excitatory to inhibitory, which causes the defect in synapse formation and in morphology, or NKCC1 mutation changes the [Cl\(^{-}\)], inside of the cell, [Cl\(^{-}\)], is very critical for cell function (144). [Cl\(^{-}\)], may act as an intracellular messenger to directly regulate expression of genes such as the GABA_A receptor subunit (320) and KCC2 (141). Changing [Cl\(^{-}\)], itself may induce malformation of synapses and dendritic arborization. Different combinations of GABA_A receptor subunits are involved in GABAergic tonic and phasic response (117). Developmentally decreasing [Cl\(^{-}\)], in a cell might contribute to the switch of GABA from its tonic to phasic effects (261, 320) as well as the activation of the glutamatergic synapse (352).

Glutamatergic inputs are detected in 3-wk-old adult-born neurons. Interestingly, glutamatergic currents in immature neurons display a lower threshold for the induction of LTP facing stimulation of the perforant pathway (PP) (115) in a specific time window. Developmentally regulated synaptic expression of NR2B containing NMDA receptors partially contributes to the mechanisms for LTP induction in immature neurons (115).

Fast, perisomatic GABAergic inhibitory currents are recorded in neurons later than functional glutamatergic inputs. Adult-born neurons receive local inhibitory inputs from all three subregions of DG: molecular layers, GCLs, and hilus (193) (FIGURE 2). With maturation, inhibitory inputs to adult-born neurons gradually increase. This developmental change is due to an increase in the number of inhibitory synapses as well as their synaptic strength (193). This gradual increase of inhibition, together with the changes in the intrinsic physiology of young neurons and the onset of glutamatergic synapse formation in adult-born neurons, sets up a critical period that is a unique feature of young neurons during their maturation (193). This critical period is particularly important for the function of newborn neurons in memory encoding (6, 82).

IV. REGULATION OF NEUROGENESIS

As described above, the maturation of adult-born GCs generally mimics the neuronal maturation process observed in development, albeit at a slower time scale. The same holds true for the dynamics and regulation of gene transcription in maturing GCs. Many of the gene expression profiles observed in development are also evident in adult-born GCs, and these genetic configurations are integral to the regulation of the maturation process. Details concerning the transcriptional and genetic regulation of neurogenesis have recently been examined in depth elsewhere (100, 139, 156, 207, 237). These processes fit into the “control” processes underlying neurogenesis as described by Kempermann (156), and mechanistically would be expected to be similar between developmental and adult-born neurogenesis. Rather than fully repeating that information here, we refer the reader to those reviews and focus our discussion on several examples in depth, emphasizing the circuit and behavioral factors that regulate the proliferation and differentiation of new neurons that will be specific to the regulation and integration adult neurons.

The regulation of neurogenesis can be targeted at several steps of the overall process. As described above, stem cells pass through several distinct morphologically and genetically identifiable stages, most notably a slowly dividing RGC stage and a more rapidly proliferating neural progenitor stage. Although it is still somewhat a matter of debate whether RGCs truly self-renew, it appears clear that cells of the morphologically more compact progenitor population are essentially fate-committed to become neurons after a few rounds of amplification at most (88, 93, 321).

A. Network Level Regulation

The substantial regulatability of NSCs and NPCs suggests that local circuit activity likely can affect proliferation and differentiation while in the stem cell state (FIGURE 3). Recently, Song et al. (314) demonstrated that nestin-expressing RGCs in the SGZ were responsive to GABA through γ2 receptors (314). This GABA response appeared to be tonically active and originate from nearby, but not synaptically connected, parvalbumin (PV) basket cells. This GABA, presumably from spillover from GABAergic synapses, suppressed the symmetric proliferation of RGLs, instead biasing them to quiescence. Thus increased PV-basket cell ac-
tivity, which likely corresponds to overall increased DG activity, will lower proliferation of stem cells, whereas decreased DG activity will likely lower tonic GABA levels and increase proliferation.

This direct effect of regulation of RGCs by GABA, and presumably inverse correlation of proliferation with overall network activity, is notable with respect to another recent study by Dranovsky et al. (88) showing that exercise and EE increase the number of neurons that derive from the RGC population (while preserving overall numbers of RGCs), whereas social isolation significantly increases the density of RGCs. The model proposed by the authors is that EE increases the proliferation of the intermediate NPC population and that social isolation increases the RGC population directly.

As described by Song, the effect of GABA on RGCs is likely due to diffusion from nearby synapses. Diffuse GABA is...
only an indirect measure of network activity, and it is not long after progressing from the RGC stage that NPCs and very young new neurons begin to receive more direct GABAergic and glutamatergic inputs (through NMDA receptors) (80, 114, 215, 217, 260, 326, 333, 353). Responses to GABA progress from tonic to “phasic” GABA roughly around the time of activation of DCX as a label and, when tracked using retrovirus, within the first several days post injection (97, 114). Notably, the response to GABA is depolarizing in NPCs and young neurons due to the distinctly high reversal potential of $[\text{Cl}]^-$ because of the preferential expression of the NKCC1 ion pump as opposed to the KCC2 ion pump (114). In NPCs, this depolarizing preferential expression of the NKCC1 ion pump as opposed to hyperpolarizing. GABA mediates excitation in NPCs and subsequently induces calcium influx via voltage-gated calcium channels (333); in turn, calcium activity has been linked to the critical pro-neuronal differentiation gene NeuroD1 (80). Activation of local interneurons in hilus elicits a GABAA receptor-sensitive synaptic response in type 2 progenitor cells. High $[\text{Cl}^-]$ in type 2 progenitor cells makes the effects of GABA depolarizing as opposed to hyperpolarizing. GABA mediates excitation in NPCs and subsequently induces calcium influx via voltage-gated calcium channels, which promote NeuroD expression and lead differentiation towards a neuronal fate. Recently, it was reported that GABA released from PV-positive interneurons in the hilus promoted radial glia-like quiescent NSCs to actively divide (314). GABA can also provide a trophic effect in early development, such as promoting DNA synthesis in proliferating neurons (202) and cell motility of immature neurons (32, 216). Notably, the role of GABA in neuronal development has also been well characterized in the SVZ, where it is essential to regulating proliferation of progenitors (201).

Once differentiation is complete and neuronal maturation begins, the young neurons begin to develop considerably more sophisticated connectivity to the local circuit, which in response regulates the maturation process (97, 179, 215, 217). GABA remains depolarizing in young neurons until roughly 2 wk of age (114), which roughly corresponds to the time in which glutamatergic spines develop on immature dendrites (371). Glutamatergic inputs become more and more important as immature neurons develop. Initially the primary influence of glutamate is through NMDA receptors, which are notable for their permeability to $\text{Ca}^{2+}$. NMDA appears to be expressed in NPCs and has been shown in vitro to have a direct effect on internal calcium levels (80); however, physiology studies typically do not show any glutamatergic signaling in NPCs or very young neurons in slice (97, 333), which is not surprising given their localization to the SGZ, where few synapses are glutamatergic. By roughly 2.5 wk of age, young neurons do have dendrites with spines in the molecular layer and receive glutamatergic inputs. NMDA appears to be significant in these early stages of excitatory synapse formation (115, 326, 367), with NMDA activation involving the NR1 subunit being critical in the survival of young neurons passing through the 2- to 3-wk-old “critical period” (326). Notably, the requirement of NMDA for neuronal survival appears to be relative to the surrounding circuit; global blockade of NMDA in the circuit mitigates the effect of the conditional NR1 knockout to some extent (326). The NMDA-dependent survival of young neurons corresponds to the timing of EE’s effects on survival (325), as well as a particular period of increased plasticity in maturing neurons (115).

1. Neuromodulators and neurogenesis regulation

In addition to the local circuit effects on neurogenesis, several of the numerous modulatory systems that have effects on hippocampal function have been shown to affect the proliferation and differentiation of NSCs and NPCs and the maturation of adult-born neurons (FIGURE 3). Of these, serotonin (5-HT) has been the most heavily studied, in part due to its relationship with depression and stress (121, 283). Attention to serotonergic effects on the NSC population was stimulated by the observation that 5-HT-selective reuptake inhibitor (SSRI) antidepressants such as fluoxetine could directly induce increased levels of proliferation (209). Subsequent studies showed that the putative 5-HT-increasing effects of SSRIs were consistent with a direct effect of 5-HT on proliferation that likely arises through the 5-HT1A receptor, with possible contributions through the 5-HT1B and 5-HT2A receptors (25, 271, 289). Serotoninergic fibers project to the DG from two distinct regions, the median raphe (MR) nucleus and the dorsal raphe (DR) nucleus (188). MR projections are known to provide substantial inputs to the varied interneuron populations in the hilus and throughout the hippocampus, often onto the inosotropic (unique among the monoaminergic systems) 5-HT3 receptors (110, 345). In contrast, the DR projections to the DG terminate preferentially in the SGZ region, with fairly small axonal terminations that appear to be positioned for diffuse release of 5-HT (169). As a result, it appears that the DR projection is best positioned to impact the neurogenesis process directly; however, the MR may well regulate local circuit activity through an indirect effect via local interneurons.

The effects of the other major neuromodulatory systems [norepinephrine (NE), dopamine (DA), acetylcholine (ACh)] on neurogenesis are less well understood (346) (FIGURE 3). ACh is one of the more attractive candidates for a potential relationship, as the cholinergic systems are known to have a substantial relationship with the hippocampus and the DG in particular. Depletions of ACh (through lesions of basal forebrain cholinergic regions) lead to a reduction in neurogenesis (71, 148); however, high doses of self-administered nicotine also decrease neurogenesis (1). The $\alpha_7$-nAChR receptor was shown to be essential for the proper maturation of adult-born GCs, with decreased survival of new neurons and reduced synaptic development in...
those young neurons that survive in \(\alpha7\)KO mice (55). Like ACh, NE has substantial impacts on the DG (130), and the limited studies with reference to neurogenesis suggest that it is required for normal neurogenesis; selective lesions of NE-projecting fibers decrease neurogenesis considerably (174) and increases in NE through blockade of NE-axon autoreceptor inhibition lead to increased proliferation and survival (278). One recent study using an in vitro assay suggests that the effects of proliferation may be through \(\beta2\) receptors (218). Finally, the dopaminergic system, which is notoriously difficult to study in vivo, appears to have effects on neurogenesis as well; however, these studies have generally investigated the effects of antipsychotic drugs and Parkinson’s disease models on neurogenesis, so the direct effects are difficult to elucidate (346). It does appear that D2 receptors are involved in in vivo DG neurogenesis (365). Furthermore, it has been reported that DA can selectively impact the LTP potential of maturing neurons (238), so it is reasonable that DA could affect the maturation process of new neurons as well. Notably, these systems are all tightly coupled and known to be related to behaviors such as stress and exercise that are associated with neurogenesis modification (see below), so the absence of clear links is more indicative of the challenges in studying these systems in isolation in vivo than of an indictment of their functional importance.

B. Regulation by Local Signaling

In addition to the direct effects of neuronal activity on proliferation and differentiation, the DG network is capable of regulating neurogenesis through local astrocytes. Wnt signaling has previously been shown to play a critical role in the neuronal differentiation of NSCs (197). Wnt3a is secreted by astrocytes in the SGZ, whose factors have been shown to be capable of permitting neuronal differentiation (313). Through the canonical Wnt signaling pathway, \(\beta\)-catenin acts as a transcriptional activator of TCF/LEF transcription factors that in turn regulate the expression of Wnt target genes. Two such targets are the transcription factors NeuroD1 and Prox1 (112, 151, 175, 183).

Originally identified for its ability to activate the insulin gene promoter (246, 247), NeuroD1 has been shown to play a crucial role in neuronal differentiation (112, 183). NeuroD1 (also known as NeuroD and BETA2) is a basic helix-loop-helix (bHLH) transcription factor required for the survival and maturation of adult-born neurons. NeuroD1 null mice die from early-onset diabetes due to loss of expression of the insulin gene (246), so to study the role of NeuroD1 on adult neurogenesis, a transgenic mouse expressing a floxed NeuroD1 gene and ROSA26 YFP reporter was crossed with a tamoxifen (TAM)-inducible CRE recombinase under the transcriptional control of nestin (118, 177). A dose-dependent injection of tamoxifen allowed the conditional knockout of NeuroD1 in all nestin-expressing stem cells and their progeny. Compared with wild-type mice, in the NeuroD1 knockout animals, deletion of NeuroD1 did not alter the number of YFP-expressing recombined cells 6 days post TAM injection; however, 40 days after injection, the number of YFP-positive cells was significantly reduced (112). Morphological analysis revealed no difference in proliferation (YFP/Ki67) at 6 days, further supporting the idea that NeuroD1 is not required in early NPCs. However, reductions in both YFP/Prox-1 and YFP/DCX at 40 days post TAM injection indicate a critical role for NeuroD1 during the later stages of differentiation/maturation. The dendritic length of YFP cells in NeuroD1 knockout mice was reduced as well. These data together highlight NeuroD1 as a critical intrinsic transcription factor that regulates survival and maturation of immature granule neurons as they become mature granule neurons.

Another target of the Wnt signaling pathway is Prox1. Prox1 is unique in that its expression is restricted to the DG (180), whereas NeuroD1 is expressed in both neurogenic regions of the brain. Prox1 has previously been demonstrated to be a target of TCF/LEF signaling (151). In vitro analysis using ChIP as well as a luciferase reporter assay revealed two functional TCF/LEF sites within the Prox1 enhancer region. This finding was further confirmed in vivo. Knockdown of TCF/LEF transcription through a retrovirus expressing a dominant negative form of LEF (dnLEF) led to a reduction in colocalization with Prox1 compared with cells expressing a control GFP retrovirus. In addition, overexpression of a dominant active form of \(\beta\)-catenin induced significant ectopic expression of the Prox1 protein.

To determine the regulation of adult neurogenesis by Prox1, viral methods were used to either overexpress or inhibit Prox1 expression (151). In the knockdown studies, a lentivirus expressing an shRNA against Prox1 was injected into animals. Two weeks later, CldU was injected to label a newborn cell population, and 3 wk after CldU administration, a dramatic reduction in the number of CldU/DCX-positive cells was observed. Interestingly, however, the researchers did not observe a reduction in the number of NSCs expressing Sox2 and GFAP, indicating that Prox1 affected the number of newborn neurons (DCX) without altering the proliferative NSC populations. Overexpression of Prox1 into newborn cells, using a retrovirus, enhanced neuronal differentiation 1 wk after injection, as demonstrated by colocalization with DCX. To determine whether Prox1 played a role in the maintenance of mature GCs, animals were injected with BrdU to label a newborn cell population. Sixteen weeks later, when these neurons fully matured, the lentivirus shRNA Prox1 was injected to knock down Prox1 levels. Interestingly, there was no difference in the number of BrdU cells compared with the control animals. These data suggest that Prox1 is required for the neuronal differentiation of newborn neurons but not nec-
necessarily for the maintenance and survival of these neurons once they have reached maturity.

C. Regulation by Extrinsic Factors

Neurogenesis is a highly dynamic process and is regulated by many extrinsic factors as well as intrinsic factors (FIGURE 4). These external manipulations are known to both positively and negatively impact levels of neurogenesis throughout the life of mammals. Some of the most prominent positive regulators of hippocampal neurogenesis include EE, voluntary exercise, and diet (118, 234, 256, 257). In contrast, factors such as aging and stress have been shown to dramatically reduce levels of neurogenesis (126, 220). In either case, manipulations of these common elements of life play a significant part in the way that adult hippocampal neurogenesis is regulated.

One important observation is that in contrast to the molecular and circuit level regulatory factors described above, the extrinsic regulation of neurogenesis has been challenging to quantify with the same level of precision. While running

![Diagram of neurogenesis regulation by behaviors](http://physrev.physiology.org/)

**FIGURE 4.** Regulation of neurogenesis by behaviors. Neurogenesis is regulated by many behavioral factors as well. Running is one of the most potent inducers of neurogenesis, targeting the proliferation of neural progenitor cells. Enrichment has a complementary effect, increasing the survival of neurons at a critical stage of their maturation. In contrast, stress is a severe negative regulator of new neuron birth, suppressing proliferation. The effects of learning are more complex, suppressing the neurogenesis process at some stages while increasing it at other stages.
and stress were among the earliest known neurogenesis modulators and are among the most potent regulatory factors, studies focused on extrinsic regulation, particularly with regard to enrichment and diet, have been somewhat more challenging to interpret.

One reason is that the variability between individual animals and their responses to the manipulation can be quite substantial. For example, 40 mice were housed together in one large EE and their exploratory activity was recorded with radiofrequency identification transponders over 3 mo. Despite the similar genetic background of all 40 mice, there was huge variability in their exploration of the environment. Furthermore, levels of neurogenesis correlated with the amount of exploratory activity of each individual mouse (108). This variability in exploration can be extrapolated to other manipulations, including running, diet, and stress. An additional complication is the variability in experimental design, which makes cross-laboratory comparisons challenging. The following sections describe how the continuous process of neurogenesis is actively regulated by experiences we encounter on a daily basis, with the caveat that most of these findings remain phenomenological with only limited insight into the underlying mechanisms.

1. EE and Running

An EE can be defined as an environment that provides sensory, social, and motor stimulation. This environment may consist of an enlarged enclosure along with tunnels, huts, toys, running wheels, and other animals to provide more social interactions. One of the first studies to demonstrate the impact of EE on adult hippocampal neurogenesis labeled newborn cells with BrdU in mice that lived in an EE for 40 days. The researchers discovered that there was a small but significant increase in the number of surviving BrdU cells when animals were killed 4 wk later (160). Although there was no effect on cell proliferation in these animals, the mice that were exposed to the EE showed significantly higher numbers of total granule neurons in the hippocampus. In an effort to determine the long-term effects of EE, 10-mo-old mice were housed in an EE for roughly half of their life (10 mo) (158). The number of proliferating cells, as determined by BrdU 1 day prior to EE exposure, increased (although not significant), as did the neuronal differentiation of newborn cells. Cells labeled 4 wk prior to death were more than fivefold more likely to differentiate into a neuron in mice that lived in an EE compared with mice that were housed in a standard cage. In multiple instances, these enrichment-induced increases in neurogenesis levels were correlated with improved performance on hippocampus-dependent behavior such as the Morris water maze (MWM).

Living in an EE for 4 wk is associated with increases in neurotrophins and growth factors, including VEGF and BDNF, in the rodent hippocampus. In rats, there is a significant increase in VEGF mRNA expression after 4 wk of living in an EE (57). The increase in hippocampal neurogenesis with exposure to an EE appears to be dependent on VEGF expression, as shRNA-mediated knockdown of VEGF in hippocampal neurons inhibits EE-induced neurogenesis (57). In mice, expression of BDNF mRNA is upregulated, but VEGF, nerve growth factor (NGF), and epidermal growth factor (EGF) are not observed after exposure to an EE for 4 wk (176). Upregulation of BDNF is due to histone modifications of the BDNF promoter (increased histone H3K4 trimethylation of BDNF P3 and P6 promoters, and decreased histone H3K9 trimethylation of BDNF P4 promoter and histone H3K27 trimethylation of BDNF P3 and P4 promoters).

BDNF induces phosphorylation of mitogen/stress-activated kinase 1 (MSK1) (272). MSK1 is also activated by the ERK1/2 or MAPK signaling cascades and is involved in CREB and H3 phosphorylation, activation of the NFκB pathway, and the regulation of immediate early genes (for review, see Ref. 17). There is recent evidence that MSK1 is involved in regulating hippocampal synaptic plasticity and not only baseline but also EE-induced adult hippocampal neurogenesis (72, 152). In a mouse model without functional MSK1, MSK1−/− mice had less proliferation and fewer immature neurons in the DG compared with wild-type mice (152). After exposure to EE for 40 days, wild-type mice had increased cell proliferation and CREB phosphorylation, whereas these remained unchanged in MSK1−/− mice (152). These data suggest that MSK1 is part of a critical signaling pathway involved in regulating EE-induced hippocampal neurogenesis.

Running wheels were used in these particular EE paradigms, and it was later discovered that voluntary exercise, i.e., running, was one of the most salient features of the EE (340, 341). EEs are composed of multiple types of sensory enrichment and, although exposure to these environments has been shown to positively affect adult neurogenesis, it was unknown which component(s) contributed most to this effect. To answer that question, adult mice were assigned to random groups based on the different types of enrichment the animals experienced during exposure to an EE (341). These groups were termed learners, swimmers, runners, and enriched (without running wheel). Only the running and enriched groups of animals exhibited increased levels of neurogenesis. Consistent with previous findings, enrichment, even without a running wheel, had the biggest impact on the survival of newborn neurons 4 wk after BrdU labeling. Runners, however, displayed a dramatic increase in the number of proliferating cells, ultimately leading to an overall increase in the number of surviving newborn neurons at 4 wk post BrdU labeling. Both swimmers and learners showed no differences in either cell proliferation or cell survival compared with control animals. Interestingly, run-
Exercise (167, 239) can be attributed mainly to physical activity associated with an increase in neurogenesis observed with enrichment (340). More recently, several studies have suggested that the non-enrichment, the increase in neurogenesis and enhanced LTP enhancing LTP in the DG of the hippocampus (340). Like exercise, enrichment also had an influence on synaptic plasticity, specifically that the beneficial effects of EE are independent of hippocampal neurogenesis (225). Following complete ablation of hippocampal neurogenesis through x-focal irradiation, irradiated mice exposed to an EE performed as well as sham control enriched animals on the MWM, suggesting that the previously observed enhancement of enriched mice in MWM behavior was not attributable to hippocampal neurogenesis. Although the extent to which EE influences adult neurogenesis is debatable, one recent study has discovered that EE is aging. Aging is consistent with significant reduction in hippocampal neurogenesis (354).

Many studies have revealed the underlying molecular mechanism of regulating neurogenesis by exercise and enrichment. Running has been shown to increase thickness (12) and metabolic capacity (221) in specific regions of cortex. Neural plasticity and electrical activity have also been associated with exercise. Running and EE-associated motor activities increase cerebral blood flow (364), blood-brain barrier permeability (301), and glucose metabolism (348). These changes combined might increase hormone and growth factor level, such as VEGF (145), GDNF, and BDNF (286). BDNF in particular has received considerable attention in neurogenesis regulation. Proliferation of NPCs in vivo is boosted by exogenous BDNF injection into the hippocampus (293). Together with NT3, BDNF promotes the differentiation and maturation of adult NPCs in culture. The expression of BDNF is regulated by neural activity and plasticity. Increasing BDNF mRNA and protein has been found to be associated with memory acquisition and consolidation (33, 184). Interestingly, the signaling of BDNF promotes the survival and maturation of GABAergic inhibitory neurons. A recent study indicates that BDNF might potentially link neural activity and GABA-mediated effects on neurogenesis (354).

2. Aging

While there are clear benefits of manipulations such as exercise, enrichment, and diet, other external factors are consistent with a decline in hippocampal neurogenesis levels. The most dramatic and well-studied of these negative regulators is aging. Aging is consistent with significant reductions in cell proliferation, survival, and neuronal differentiation. With the use of BrdU to label dividing cells at a given time point, proliferation in the DG was significantly reduced in aged (21 mo) rats compared with middle-aged (6 mo) rats. Overall numbers of newborn neurons decreased roughly eight- to ninefold from middle-aged to aged rats, indicating the dramatic decrease in neurogenesis associated with aging (173). Another study observing cell proliferation in rats at broader time points indicated that this age-related decrease in neurogenesis was observed as early as 6 wk of life.
age (when compared with 2-wk-old rats) and persisted until 12 mo, where it began to level off. When DG volume was quantified in these animals, the volume increased from 2 to 6 wk and then remained relatively stable through 24 mo of age (134). When comparing cell proliferation and total DG volume during aging, the initial decrease in proliferation from 2 to 6 wk may be attributed to the fact that the brain has not yet fully developed, suggesting a peak in cell proliferation at this time. Similar observations have been noted in mice. Cell proliferation in mice decreases from 1 to 2 mo of age and progressively declines until 18 mo, where the significant decreases in proliferation are no longer observed (34, 43). Survival of these newborn labeled cells was dramatically reduced in 3- to 6-mo-old mice compared with 18-mo-old mice when BrdU was quantified 4 wk after labeling. Consistent with the decrease in cell survival, colocalization of BrdU and the neuronal marker NeuN was also substantially reduced in both 3- and 6-mo-old mice compared with 18-mo-old animals, indicating a decrease in neuronal differentiation with age. Interestingly, this decrease in neuronal differentiation was offset by an increase in glial differentiation (173, 344).

The age-related decline in hippocampal neurogenesis is likely a result of a reduction in NSC population and/or activity. The sex-determining region Y-box 2 (Sox2) and bHLH gene Hes5 have been identified as NSC markers (204, 293). In aged rats, the Sox2+ population remains unchanged but proliferation rates are decreased, suggesting that a decrease in NSC activity is the major contributor to the reduced hippocampal neurogenesis with age (131). In contrast, examination of Hes5+ cells in aged mice revealed an overall reduction in the total number of Hes5+ cells, indicating that there is a significant decrease in the NSC population with age (204). Specifically, the largest decrease was found in the proliferative horizontal Hes5+ cell population in aged mice. While it is unclear from these two studies whether the NSC population remains intact, there is a consistent finding of decreased proliferative activity of NSCs in aged animals. A combination of intrinsic and extrinsic age-related changes is likely to contribute to decreased hippocampal neurogenesis in aged animals (see Ref. 185 for review). A recent study identified that Wnt signaling from astrocytes regulates the expression of survivin, which is important for cell proliferation, in NPCs (229). Additionally, as discussed earlier in this review, the neurogenic niche is altered with increasing age; microglia and astrocytes transition from anti-inflammatory and antioxidative to proinflammatory signaling pathways.

Exercise and enrichment are capable of increasing the number of newborn neurons in the DG fivefold, and aging is associated with an eight- to ninefold decrease, demonstrating the extent to which external manipulations are capable of affecting adult hippocampal neurogenesis. Given the breadth with which changes in the external environment influence neurogenesis, it is interesting to see how these external factors interact with one another. The most widely studied interaction is how enrichment and exercise affect aging in animals. As previously mentioned, when 10-mo-old mice (a time when decreased neurogenesis is already present) lived in an EE for 10 more months, they exhibited (at the age of 20 mo) a fivefold increase in the number of newborn neurons compared with control animals (158). The improvement in neurogenesis levels was consistent with performance in the MWM task. Interestingly, even at advanced stages of aging, when the reduction in neurogenesis and cognitive decline are most severe, voluntary exercise has been shown to mitigate many of these deficits. Animals, 18 mo of age, that were allowed access to a running wheel for 45 days demonstrated a remarkable increase in cell survival and neuronal differentiation, ultimately leading to an increase in the total number of new neurons (344). Aged enriched animals exhibited improvements in the MWM.

3. Stress

Along with aging, another extrinsic factor negatively regulating hippocampal neurogenesis is stress (FIGURE 4). In the laboratory setting, there are various types of stressors used to observe the effects of stress on adult neurogenesis. Acute stressors are defined as a single stressful event, and chronic stressors are multiple stressful events over a period of time; however, both types of stressors vary based on their method of application (physical stressor, social stressor, and odor stressor) and duration (for a review, see Ref. 296). Despite the variability in how stress is applied, the general consensus of these studies is that stress has a substantial effect on DG cell proliferation. Tree shrews were subjected to acute psychosocial stress, for 1 h, when two males were placed together to establish a dominance/subordinance hierarchy that caused stress for the subordinate animal (125). After the stressful experience, animals received a BrdU injection and were killed 2 h later to determine cell proliferation. Tree shrews that were exposed to psychosocial stress exhibited significantly reduced numbers of proliferating BrdU-positive cells. Similarly, adult marmoset monkeys were subjected to a resident-intruder psychosocial stress model and, from this one stressful experience, intruder monkeys demonstrated a significant decrease in cell proliferation (127). There is one study, however, that did not observe an effect of stress on cell proliferation. Rats placed in a similar acute psychosocial situation did not exhibit a decrease in cell proliferation but rather a decrease in neuronal survival (328). Experiments utilizing a chronic psychosocial stressor method have observed similar decreases in cell proliferation in tree shrews, rats, and mice (76, 77, 104). Just like acute stressors, one study has also identified an inverse effect of chronic stress on cell survival (268). Interestingly, some studies have found that when animals are put through behavioral tasks, such as the MWM, the stress associated with either extended training paradigms (20) or even the novelty
of the task itself (91) is sufficient to reduce levels of neurogenesis. Animals that were exposed to multiple mild stressors such as cage tapping/tilting, auditory exposure to predators, cages with water or damp bedding, or housing with temporary light fluctuations were assessed for levels of neurogenesis. Survival of BrdU cells was significantly decreased in response to chronic exposure of two random mild stressors, across multiple mouse strains. This decrease in cell survival was consistent with a decrease in neuronal differentiation as quantified by BrdU and NeuN colocalization (228). Although slight differences in stress application and species of animal may account for the variability observed, the effects of stress on cell proliferation and cell survival have been well documented.

Stress is associated with a release of glucocorticoids into the bloodstream, which have been shown to modulate neurogenesis (53, 123) and potentially relate neurogenesis to its hypothesized role in mood (186). Following performance of an adrenalectomy to reduce circulating adrenal hormones in the bloodstream of rats, an increase in the number of GFAP-positive cells in the DG was observed (123). This increase was reduced when corticosterone was introduced through the drinking water, although not to the level of sham treated animals. In a similar experiment, rats were given subcutaneous injections of corticosterone and a significant decrease in the number of proliferating cells in the DG was observed (53). An adrenalectomy of these animals dramatically increased the number of proliferating cells. These experiments suggest a role for circulating glucocorticoids in the bloodstream and their negative regulation of adult neurogenesis, hinting at a possible mechanism by which stress can affect adult neurogenesis. Interestingly, one of the most potent positive regulators of adult neurogenesis, exercise, is also associated with an increase in glucocorticoids in the bloodstream; however, these seemingly contradictory occurrences could be partially explained by the massive upregulation of circulating growth factors accompanying exercise. This influx of circulating growth factors may overshadow the negative effects of stress-induced glucocorticoids. Finally, expression of glucocorticoid receptor (GR) is robust in the GCL and colocalizes with both NeuN and DCX (98). Additionally, glucocorticoids may act directly on NPCs, as treatment with a GR agonist reduces cell proliferation in vitro. shRNA-mediated knockdown of glucocorticoid receptors in the hippocampus disrupted migration and positioning of newborn cells in the GCL, suggesting a critical role for glucocorticoid receptors in mediating the integration of newborn cells into the existing circuitry (105).

4. Diet

Lifestyle choices such as exercise and diet have a tremendous impact on many aspects of brain function, including mood, energy metabolism, behavior, cognitive function, and hippocampal neurogenesis. Many studies have investigated which specific foods or compounds can provide a cognitive advantage or disadvantage. Rodents on a diet that lacks essential vitamins or minerals exhibit decreased hippocampal neurogenesis, which is accompanied by impaired learning and memory (for review, see Ref. 316). In contrast, rodents on a diet supplemented with polyphenols or omega 3 fatty acids show increased hippocampal neurogenesis as well as improved performance on cognitive tests (for review, see Ref. 316).

While there is an overwhelming amount of literature on diet and cognitive function, there are many factors that need to be considered before drawing conclusions about the effect of diet on cognitive function. Many of these studies do not take into account which compounds are being absorbed, how they are metabolized, and which specific metabolite acts directly in the brain to improve cognition. Furthermore, it is unclear how studies in rodents can be applied to humans, specifically with regards to dose and if humans can consume a comparable amount of the compounds. For these reasons, it is even more difficult to comment on in vitro studies in which compounds are directly applied to neurons to determine the effects of cell survival or function. Given the complicated nature of these studies, here we review a handful of studies that focus on specific flavonoids or flavonoid-rich diets in rodents and humans to provide a preview of a burgeoning field of cognitive neuroscience research.

Flavonoids are a subset of polyphenols that are found in many different foods such as berries, parsley, green tea, and cocoa (for review, see Refs. 31, 210). Epicatechin, found in high concentrations in tea and cocoa, has been shown to improve spatial memory retention in adult mice (342). When epicatechin is combined with running in adult mice, the cognitive improvement is more robust, and this combination is associated with an increase in spine density, suggesting that a synergy exists between diet and exercise (342).

Identifying individual compounds that are effective in enhancing neurogenesis and cognitive function is of particular interest to developing dietary interventions, particularly with the elderly population. Cognitive functions decline with increasing age, and a number of studies have investigated the potential use of flavonoid consumption to prevent this decline. In elderly humans (70–74 yr old), consumption of chocolate, wine, and tea is associated in a dose-dependent manner with better test scores on a battery of cognitive tests (254). A 10-yr study examined the cognitive function of subjects aged 65 or older as assessed with psychometric tests. Those with the greatest amount of flavonoid intake performed better at both the beginning and end of the study compared with those with the lowest intake. While the lowest flavonoid group scored 2.1 points lower compared with their baseline, the
highest flavonoid group scored 1.2 points lower on the Mini-mental status examination (MMSE), suggesting that flavonoid intake positively correlated with cognitive function (189). However, not all diets with high flavonoid content attenuate the cognitive decline associated with increasing age. Over a 5-yr study, subjects aged 65 or older who adhered to a more Mediterranean diet high in fruits and vegetable consumption exhibited a slower decline as assessed by the MMSE but not by other cognitive measures (102). In human studies, there are countless variables that cannot be controlled from one individual to the next, which makes it extremely difficult to determine the most influential factors in improved cognitive functions. As mentioned above, lifestyle choices, including physical and mental exercises, have a tremendous impact on cognitive performance and can vary extensively in these subjects. Additionally, individuals in these studies not only consumed different amounts of flavonoids but also from a variety of sources. While flavonoid intake is self-reported from an individual’s diet, the person is typically unaware of how the processing of food alters the quantity of flavonoids in a given vegetable or product, which makes it difficult to determine how much of which flavonoids had the greatest impact on cognition.

Human studies are complicated by nature and, while the majority of studies suggest flavonoids provide positive benefits to cognitive functions, it remains unclear which particular aspect of cognition is most improved with flavonoid consumption (208). Studies using rodents on specific flavonoid-rich diets report enhanced learning and memory. Changes in cognitive function are apparent in aged mice and their ability to complete hippocampus-dependent cognitive tasks efficiently. With age, hippocampal neurogenesis declines dramatically, and this decline is accompanied by a slower learning curve in the MWM task as the aged mice take longer to swim to the hidden platform compared with young mice (344). The effectiveness of supplementing the diet with flavonoid-rich extracts from berries or tea is evident in a number of motor and cognitive tasks in aged animals. For example, aged rats who received grape juice (10–50%) for 6–8 wk exhibited improved performance on behavioral tests, including the hippocampus-dependent MWM, rod walking, and accelerating rotarod, all tasks that are normally impaired in aged mice (309). Consistent with this finding, diets supplemented with blueberry (1.86%), spinach (0.91%), or strawberry (1.48%) extract reversed the cognitive and motor deficits associated with age, as assessed by a working memory water maze paradigm and rod walk in aged rats (146). Aged rats on a blueberry-supplemented (2%) diet for 8 wk made fewer errors on a radial arm water maze task than those on a control diet. This improved performance corresponded to an increase in the number of newborn cells in the hippocampus of blueberry-supplemented aged rats compared with control aged rats (62).

Enhanced cell proliferation may be mediated by the increase in IGF-I signaling, which is a known regulator of neurogenesis, as a result of the blueberry diet (62). While this study also used a blueberry supplement in lieu of a specific flavonoid, the findings were of particular interest because they linked the effects of blueberries on cognition to hippocampal neurogenesis, identifying possible mechanisms such as neurogenesis and growth factor signaling through which blueberries modulate cognitive function. IGF-I and other growth factors activate signaling cascades such as MAPK and CREB, which are involved fundamentally in learning and memory processes. Hippocampal levels of CREB phosphorylation and downstream target genes such as BDNF and Bcl-2 decreased with age but could be rescued with administration of green tea catechins in aged mice (192). Similar age-related declines in hippocampal levels of phospho-ERK1/2 and phosphorylated Akt were reversed in aged rats on a blueberry-supplemented diet (359). These studies reveal potential signaling pathways and mechanisms that may be crucial in enhancing cognitive function and synaptic plasticity by the flavonoids and/or other compounds found in the diets.

5. Learning

Interestingly, what was initially one of the more controversial relationships between behavior and neurogenesis is the effect of learning on new neurons (FIGURE 4). While there is considerable research into the effects of new neurons on hippocampus-dependent learning (see sect. V), studies of the effects of learning itself on the proliferation and survival of new neurons initially showed mixed results (122, 340). This initial confusion likely stems from two things: 1) not all learning paradigms and tasks are equivalent, and 2) the effect of learning appears to be temporally complex, with learning promoting the survival of some young neurons while suppressing the survival of earlier-born neurons (87). Notably, the training alone does not appear sufficient; in a study of aged rats, only the subset of animals that learned the task showed the aforementioned effects on survival (89).

Subsequent studies have illustrated that the effects of learning on neurogenesis are quite complex. Interestingly, the negative effects of learning on survival noted above appear to be necessary for performance on the task, suggesting the relationship between learning and survival is acutely important (90). Learning affects not only the survival of cells but also the maturation of newborn neurons, with training in MWM increasing the complexity of dendritic arborization and the spine density of adult-born GCs (187, 337). Indeed, this learning effect appears to influence what the neurons will eventually respond to (155, 319, 325).
**V. FUNCTION OF NEUROGENESIS**

Despite its extensive characterization, the functional importance of adult neurogenesis, particularly in humans, remains a big question for the broader community. Although the existence of adult neurogenesis was generally accepted by the mid 1990s, the appreciation that adult-born neurons had a meaningful role, as opposed to being simply a functionless evolutionary artifact (a “neural appendix” of sorts), has taken much longer. Presently, the question is not whether neurogenesis is relevant for cognition in rodents; rather, the debate has shifted towards the scope of its function (e.g., is neurogenesis function equivalent to DG function?) and its relevance in primates. This sharp transition from skepticism about its relevance to ascribing it a key role in memory formation has arisen due to several factors, including theoretical and computational examinations of how new neurons would relate to the well-characterized DG network, focused behavioral studies designed to test clear hypotheses on function, and sophisticated knockdown approaches that can specifically and sometimes reversibly impair neurogenesis.

Previous reviews have exhaustively described the numerous behavioral studies that have targeted neurogenesis (82). Here, we will take a different approach by explaining the major functional hypotheses that are currently being considered by the community, highlighting both the theoretical and computational justification for the hypotheses as well as the associated supporting behavioral evidence (5, 252) prior to describing the broader approaches to characterizing neurogenesis knockdown behavior. Notably, the following hypotheses are not exclusive of one another, because the unique time scales of neurogenesis maturation may allow new neurons to have different impacts on memory at different stages of their development.

**A. Hypothesis 1: Role for Adult Neurogenesis in Pattern Separation**

The primary function that has been most associated with adult neurogenesis is pattern separation (4, 285) (FIGURE 5). Broadly, pattern separation can be thought of as a network process whose outputs are less similar, or less overlapping to one another, than its inputs.

1. **What is pattern separation?**

The concept of “pattern separation” has been used somewhat differently in different domains (i.e., computational, behavioral, and physiology), so it helps to define the concept more directly as it relates to the DG. Broadly speaking, a computational pattern separation function can be attrib-

![FIGURE 5](http://physrev.physiology.org/)  
**FIGURE 5.** Pattern separation theory for the neurogenic dentate gyrus. *Left:* illustration of the cognitive phenomenon of pattern separation. Two events, consisting of highly similar objects and configurations, can be learned to be different if neurogenesis is present in the DG, whereas without neurogenesis the memories will be the same. *Right:* potential mechanisms for how neurogenesis may improve pattern separation. *Top right:* having new neurons available can permit the second event to utilize new neurons instead of the same old neurons to encode the differences in contexts. *Bottom right:* an alternative mechanism is that reducing neurogenesis increases the baseline activity of mature granule cells, leading to higher statistical overlap (and thus interference) between representations.
uted to almost any neural network, as individual neurons respond in different ways to different possible inputs. Behaviorally, pattern separation is more typically linked to discrimination tasks, which are straightforward to design metrics for, but are often difficult to attribute to specific regions. Electrophysiology studies of pattern separation are often in between these definitions; they can investigate the correlation of neuronal activity within a region but, because it is remains difficult to measure large populations simultaneously, one cannot fully investigate the computational mechanisms underlying the separation.

The computational pattern separation function that has been typically associated with the DG is more specifically related to the hippocampus' function in memory formation. Information provided by the cortex (which presumably is in the form of high level event and spatial representations) to the hippocampus is potentially highly correlated; two different contexts may be composed of many of the same objects and spatial features yet ideally should be stored as distinct memories. This decorrelation of cortical inputs to optimally form memories has been attributed to the DG for several reasons (223, 255, 335, 336). First, the large number of GCs relative to the principal cell numbers of the entorhinal cortex (EC) and CA3 is similar to the machine learning technique of projecting information into a higher dimensional space to better classify or separate it (e.g., support vector machines; Refs. 23, 82). Having more neurons available overall allows fewer neurons to be used to represent the same information, and sparse codes are ideally suited for reducing overlap (which can equate to interference) between DG outputs. Second, the high tonic inhibition arising from both feed-forward and feedback inhibitory populations and related low activity of GCs in vivo are well suited to achieve this sparse coding, supporting the idea that this form of separation is indeed used by the DG (147, 190). Finally, the sparsely projecting yet powerful mossy fiber output synapses of GCs are potentially well suited for utilizing a sparse, separated code during memory formation (135). The hypothesis that the DG as a whole has a pattern separation role has been further validated by behavioral studies in mice and rats, in which DG lesions impair spatial discrimination (116, 161, 222, 234), and in human imaging studies, in which the CA3/DG area appears to be particularly involved in the discrimination of similar visual objects (24).

Not surprisingly, given this long-held view of the DG, there are numerous computational models that have examined the role of new neurons in pattern separation (29, 355, 361). It is worth noting that these models have typically not examined the role of new neurons in the aforementioned rationale for the DG in pattern separation (i.e., why a highly divergent, sparsely active network needs new neurons). Rather, in these models, neurogenesis reduces interference by directing new learning towards new neurons (a common theme through almost all neurogenesis models; Ref. 5), thus ensuring that new memories and old memories are encoded distinctly. In some respect, while this function can be related to the pattern separation theory, the mechanism somewhat contrasts with the fairly passive, classic DG pattern separation motif. One possibility is that these two views of pattern separation can be unified if the activation of “classic” separating mature neurons and directed learning on immature neurons were distinct in the timing regime (i.e., when they fire is as important as what they fire to) (276).

2. Evidence for a neurogenesis pattern separation function

While the computational mechanisms explaining a neurogenesis/pattern separation link are not fully understood, behavioral data using different neurogenesis knockdown approaches have provided strong evidence of a connection between neurogenesis and tasks involving spatial discrimination. Several spatial discrimination tasks have been used, including the radial-arm maze (67, 291), fear context discrimination (244, 284), and the two-choice discrimination task (67, 73, 224). This latter task has become one of the more prominent tasks to examine spatial discrimination, as it uses a touchscreen operant chamber that permits a relatively large number of observations and typically shows that the discrimination of proximal (in space) choices is selectively impaired without neurogenesis.

B. Hypothesis 2: Role for Adult Neurogenesis in Encoding Temporal Context

While the pattern separation function above has a long history in the DG, a related potential function for new neurons represents a new perspective on DG and hippocampal memory formation. As mentioned previously, one observation of almost all computational models of adult neurogenesis is that new neurons are the primary recipients of learning (5). From one perspective, driving learning to young neurons can improve pattern separation by ensuring that new memories and old memories do not interfere with one another (361). However, this direct pattern separation role of new neurons is most evident when the process is considered from a temporal perspective. Specifically, although memories formed at different times would be separated because different populations of young neurons were involved, this separation would not apply for memories for novel events experienced at or around the same time (FIGURE 6).

Notably, unlike pattern separation, the role of new neurons in encoding time is an added function of neurogenesis that is distinct from prior hypotheses of DG function. This potential role was arrived at independently by two groups using slightly different theoretical rationales and has been subse-
quently observed in several computational studies (6, 7, 30). In both cases, young neurons have been shown to be better suited to encode novel information than mature neurons, which have already been configured to encode familiar information.

1. Evidence for temporal separation and neurogenesis

The big challenge for the temporal separation theory for neurogenesis is the lack of clear behavioral evidence for such a function. Perhaps due to its status as a novel type of memory, there are no direct animal behavior tests for this function similar to the spatial discrimination tasks that have been used to argue for a general pattern separation role. Nonetheless, there is some evidence that tasks that require integration of events that occur close in time require neurogenesis. For example, the hippocampus has long been known to be required for trace conditioning, a form of fear conditioning that uses a time delay between the conditioned stimulus (i.e., tone) and the unconditioned stimulus (i.e., eyepuff), and this task was one of the first behaviors for which a neurogenesis dependence was observed (307, 308).

However, although a temporal component can be attributed to this and other tasks, it is important to note that these studies were not designed to examine the relationship of neurogenesis to temporal coding but rather to hippocampal memory in general. Notably, Morris et al. (235) have recently reported a time-dependent task that was motivated by this neurogenesis hypothesis. While they did not ablate new neurons, they observed that the DG as a whole is necessary for temporal associations over longer time scales (235). It remains to be seen if other specific tasks that investigate the role of time in behavior directly can be developed and if these tasks depend on neurogenesis.

C. Hypothesis 3: Role for Adult Neurogenesis in Memory Resolution

A somewhat different perspective on neurogenesis function relates to what new neurons encode as opposed to how new neurons encode (FIGURE 7). In addition to its pattern separation function, the other primary function attributed to the DG is referred to as conjunctive encoding (161, 255). Basi-
cally, conjunctive encoding simply refers to the DG’s (and downstream hippocampus’s) integration of spatial and nonspatial information into memories. Rather than simply encoding place or objects, DG neurons likely encode a higher representation that combines the what and where (and potential when, see above) of experienced events for encoding into memory.

The challenge of linking the varied forms of pattern separation described above, combined with the appreciation that the DG may be involved in a more complex role in memory formation, has led us to propose that the DG, aided in large part through its neurogenesis process, has a substantial role in dictating the “resolution” of memories that are encoded by the hippocampus (4, 347). The DG memory resolution hypothesis is as follows. First, the sparse representation of the DG population and strong inhibition suggest that mature GCs are highly selective to what they respond to (i.e., “tightly tuned”). In addition to making them excellent pattern separators, this high selectivity makes mature GCs very informative about those features that they respond to. When a mature neuron is activated, there is only a relatively sparse set of features that could have been responsible. As a result, when an event is encoded, the set of mature neurons activated provides a very “high-resolution” encoding of those familiar features that drove their activity. There is a notable downside to this high-resolution coding: if each mature GC only encodes a finite number of features, it stands to reason that there will be features that are not encoded well by any mature GCs. While there are a substantial number of GCs (in rodents the GC layer is one of the largest forebrain regions by number), it is unlikely that they can guarantee that any event that may be encountered will have mature GCs capable of encoding it.

In contrast, young GCs are theoretically far less selective in response to the inputs. This lack of selectivity could be due...
to several features, namely, their altered physiological properties (2, 97, 114, 232, 269) and differential excitatory and inhibitory connectivity (193, 371). Individually, this lack of selectivity reduces the information content of young neurons relative to their mature counterparts and, in an absolute sense, makes networks with young neurons less effective at pattern separation (6). However, as a population, the broad tuning of young neurons can form a powerful distributed code that can uniquely represent features, and because this code consists of low-information units, it is likely capable of representing any cortical input that may be provided.

Alone, this combination of sparse (mature GCs) and distributed (immature GCs) coding schemes is a powerful approach to episodic memory encoding. However, improving the high-information sparse-coding representations over time is probably beneficial. Thus the memory resolution hypothesis predicts that low-information young neurons eventually become high-information mature neurons by virtue of their increased propensity for synaptic plasticity (115, 295). By focusing plasticity towards young neurons, the mature neurons can essentially fix their coding on familiar features indefinitely, with little interference over time. Notably, this directing of learning towards immature neurons to preserve the representations of mature neurons has been a key feature of numerous computational models of neurogenesis (5, 6, 13, 14, 361).

1. Evidence for a memory resolution function for neurogenesis

The principal prediction of the memory resolution hypothesis is that mature neurons will be highly selective to what they respond to and that young neurons will be somewhat less discriminating when young, but that they will mature to represent events from their development. This prediction is difficult to validate directly due to the challenges associated with in vivo physiology in the DG and because there is no clear method to determine which features are salient to an animal at a given time. However, there have been numerous studies using immediate early gene (IEG) assessments of DG activity during experiences in EEs and behavioral tasks (9, 83, 155, 275, 319, 325) and of CA3 following neurogenesis disruption (248). The results from these IEG studies have been somewhat mixed and at times difficult to interpret for a number of reasons (3). In summary, it appears that young neurons do learn to encode what they are exposed to when young; however, the overall activity of both the young and mature populations is quite low and it is unclear how representative the IEG-labeled population is of the active population.

From a behavioral level, the memory resolution hypothesis would suggest that animals without neurogenesis should show little effects on memory tasks that rely principally on familiar features; however, tasks that involve the flexible use of novel information would be predicted to suffer. There have not been many attempts to break down behavioral tasks in this manner while investigating neurogenesis; however, one recent study used diptheria toxin (DT) to ablate adult-born neurons that were thought of as previously specializing to the trained event, showing that the loss of already trained adult-born neurons did impair subsequent performance on those tasks (16).

2. Effect of neurogenesis reduction on behavior

Given that the neurogenesis occurs in the hippocampus, a critical brain region for declarative memory in humans, it has long been thought that adult-born neurons should contribute to hippocampus-dependent learning and memory. With the use of methylazoxymethanol acetate (MAM), a DNA methylating agent, to ablate neurogenesis, it was first shown that hippocampal neurogenesis was critical for the formation of hippocampus-dependent association memories using a trace version of a fear conditioning protocol and was dispensable for hippocampus-independent memory using a delayed version of a fear conditioning protocol (307). However, later studies of the consequences of adult neurogenesis ablation using irradiation or other nonspecific methods showed rather inconsistent data, possibly due to the nonspecific nature of neurogenesis ablating approaches, the behavioral assays used, and variations in experimental conditions from study to study (82). The latest studies using more specific methods for neurogenesis manipulation and behavioral tasks aimed at DG-related functions have not only established a role for adult-born neurons in hippocampus-dependent learning and memory but also revealed several key functional features of adult neurogenesis (82).

First, as described above, adult neurogenesis appears to be required for pattern separation, which is currently described as a key function of the DG. This critical role for adult neurogenesis was revealed by using tasks that tested animals’ ability to discriminate between closely located spatial positions or similar contexts (67, 73, 284). Recent molecular and physiological studies demonstrated that the DG can achieve pattern separation by using distinct population codes and different firing patterns to represent different environmental inputs (83, 190). It will be interesting to test whether such an event-specific activity pattern is affected by altering the rate of adult neurogenesis. In addition, tasks related to pattern separation have been noted to improve in response to genetic and behavioral manipulations that increase neurogenesis (73, 284).

Second, adult neurogenesis is more critically involved in memory discrimination at the time of memory recall. Mice with adult-born neurons ablated after the acquisition of contextual or spatial memories showed an impaired behavioral performance upon recall of those memories (16, 129). Using a contextual fear conditioning paradigm, Tonegawa and colleagues (199, 274) recently reported that optoge-
netic activation of GCs responding to a contextual exposure episode was sufficient to create an artificial fear memory to the exposed context and that artificially activating those GCs subsequent to the fear memory formation was sufficient to induce a fear response in a neutral environment. Further studies are needed to determine whether adult-born GCs are involved in forming false memories.

Third, adult neurogenesis is also required for the long-term consolidation of contextual memory. After the formation of the contextual fear memory, the recall of fear memory is initially dependent on the hippocampus but becomes independent of the hippocampus after several weeks (11). Kitamura et al. (164) showed that ablation of neurogenesis by irradiation prolonged the hippocampus dependency of contextual fear memory in mice, suggesting a role for neurogenesis in promoting system memory consolidation. Given that the hippocampus is considered to be a temporary storage site for memory, neurogenesis may serve as a mechanism for forgetting in the hippocampus through general retroactive interference, regardless of memory content (107).

Fourth, adult-born neurons at different maturation stages may make different contributions to learning and memory, given the differences in their physiological characteristics. By reducing the number of adult-born DGCs at different maturation stages in Nestin-tk transgenic mice, it was found that immature adult-born DGCs but not their mature counterparts were required for learning and memory (85). Furthermore, specifically silencing the adult-born DGCs at 4 wk of age but not their 2- or 8-wk-old counterparts by optogenetic techniques after task acquisition resulted in impaired memory recall (129). Finally, blocking the output of both developmentally born and adult-born mature DGCs did not impair contextual discrimination in mice but caused a deficit in the recall of previously formed spatial or contextual memories when presented with an incomplete set of cues (244). These results led Tonegawa and colleagues (244) to propose that immature GCs and mature GCs were responsible for pattern separation and pattern completion, respectively. However, this hypothesis is challenged by the observations that ablation or inhibition of young adult-born GCs impaired spatial and contextual memory recall and by the finding that optogenetically inhibiting GC activity did not affect the recall of contextual fear memory (16, 129, 162). Future studies are needed to resolve this disparity.

In addition to its role in learning and memory, the hippocampus has been implicated in emotional regulation. Instead of a unitary structure, the functional role of the hippocampus varies along its dorsoventral axis, with the dorsal and ventral parts involved in cognitive and emotional functions, respectively (26, 101). This hypothesis was supported by both differential connections between the hippocampus and other brain regions and distinct lesion effects, as well as a recent optogenetic characterization along the dorsoventral axis (162, 236, 322). Early studies of hippocampal neurogenesis also revealed a correlation between the rate of neurogenesis and emotional status, with increased neurogenesis being observed in antidepressant-treated animals and decreased neurogenesis in stressed animals, as discussed above. It has therefore been proposed that adult hippocampal neurogenesis could also be an important affective regulator (142).

Although initial correlative studies provided experimental evidence for this hypothesis, subsequent studies reported data inconsistent with this hypothesis, suggesting that there is a rather complicated relationship between hippocampal neurogenesis and affective states in animal models. For example, the increase in NPC proliferation observed with antidepressants is dependent on the strain of mice studied: antidepressants only enhance NPC proliferation in 129svEv and DBA mice but not in C56BL/6, balb/c, or A/J mice (78, 138, 227, 289). Furthermore, stress does not always cause a reduction in neurogenesis. It was recently reported that, instead of inhibiting neurogenesis, acute stress actually increased proliferation of NPCs in the dorsal hippocampus, leading to enhanced activity in newborn GCs and an improvement in fear extinction (163).

Studies examining the causal relationship between hippocampal neurogenesis and affective regulation also suggest that hippocampal neurogenesis is not a master regulator of emotional status in rodents; instead, neurogenesis may influence the emotional circuitry indirectly. In the first studies addressing the causality of neurogenesis and affective regulation, Santarelli et al. (289) reported that hippocampal neurogenesis was required for the effectiveness of fluoxetine (an antidepressant of the SSRI class) in certain tests for anxiety- and depression-like behaviors, such as the novelty suppressed feeding task and the splash test. However, subsequent studies showed that the dependence of antidepressant efficacy on hippocampal neurogenesis was not found in other tests for anxiety- and depression-like behaviors, such as the open field test or forced swimming test (78). In addition, hippocampal neurogenesis was only involved in the efficacy of limited classes of antidepressants (267), probably because anxiety and depression have complex etiologies and different classes of antidepressants may have different therapeutic targets. A recent study showed that hippocampal neurogenesis also played a role in stress adaptation, which in turn could influence anxiety- and depression-like behavior in a distinctive way in different, commonly used behavioral tasks (312).

In summary, the relationship between hippocampal neurogenesis and emotional regulation is rather complicated. Because of the heterogeneous nature of depression and poorly understood neural networks underlying the disease, it is difficult to pinpoint the exact mechanism for adult-born
GCs in affective regulation. It is possible that adult-born GCs are part of brain networks that underlie certain aspects of emotional disorders and are affected by certain antidepressants, making hippocampal neurogenesis indirectly involved in emotional regulation. For example, as discussed above, hippocampal neurogenesis is thought to be an important factor in pattern separation, so impaired neurogenesis may result in overgeneralization to emotional stimuli, which are found in certain types of anxiety disorders. Indeed, a recent study showed that there was a negative correlation between performance in a pattern separation task and the score received on the Depression Anxiety Stress Scales (302). Hence, it could be the hippocampal neurogenesis-mediated cognitive function that plays a role in affective regulation.

3. Relationship of neurogenesis and cognitive deficits in humans

Given the challenges associated with directly measuring neurogenesis in humans, the role of new neurons in human cognitive disorders remains unknown. Nonetheless, the lower neurogenesis levels typically seen in aged or diseased mice suggest that human neurogenesis may be potentially lower in psychiatric and neurological conditions and, in turn, linked to impaired cognition. For instance, both stress and aging, which have dramatic effects on neurogenesis levels in mice, are well known to be associated with memory impairments in clinical human populations (40, 205). Likewise, patients who have undergone radiation therapy for brain tumors are known to experience cognitive and memory deficits. While radiation clearly has broad effects on neural tissue, it is notable that radiation remains one of the most potent techniques for experimentally ablating neurogenesis and that the observed cognitive deficits are consistent with functions linked to neurogenesis (270, 279).

Despite the lack of direct evidence, there are several studies that have sought to link cognitive functions related to neurogenesis in rodents to human clinical conditions. In particular, the pattern separation function described above has been measured in humans and observed to be impaired in aged individuals. Furthermore, this age-dependent decline in performance is correlated with a regional fMRI effect specific to the DG/CA3 area (317, 366). In all, the observations that human cognition correlates with neurogenesis-related behaviors support the contention that activities and interventions that improve neurogenesis may be useful strategies to alleviate cognitive deficits in humans.

VI. TECHNOLOGY FOR STUDYING THE FUNCTION OF ADULT NEUROGENESIS

As adult neurogenesis has become an increasingly well-accepted concept over the past couple of decades, focus has been directed toward developing new technologies to investigate both the NSC development processes and the function of adult neurogenesis. There is also increasing interest in developing techniques to manipulate the levels of neurogenesis for both scientific and clinical purposes. Below we discuss the development of these new techniques designed to study adult neurogenesis.

A. Regulating the Number of Adult-Born Neurons

Adult hippocampal neurogenesis can be influenced by many factors, such as the genetic background of the animal, the age of the animal (185), mood (283), and housing environment (341). The number of adult-born neurons can be regulated at the levels of cell proliferation, differentiation, and survival.

Recently, various strategies have been developed to disrupt or augment neurogenesis specifically in the hippocampus. These methods provide spatial and cellular specificity to manipulate neurogenesis in the adult brain. The Herpes simplex virus type 1 thymidine kinase (HSV-1 TK) is a phosphotransferase that phosphorylates a broad range of pyrimidine nucleosides and purine nucleosides, including thymidine, deoxycytidine, and ganciclovir (GCV), which is a synthetic analog of 2'-deoxy-guanosine (65). Phosphorylated GCV is further metabolized to its triphosphate, GCV-TP, and incorporates into the nascent DNA, which induces chromosome breaks and sister chromatid exchanges (329) and thus causes cell apoptosis (280, 329). Combining GCV with a genetically expressed HSV-1 TK in adult-born neurons by use of a specific promoter such as GFAP and nestin (226, 363) has been used to specifically ablate adult-born neurons (85, 290).

DT is another toxin that is widely used for lineage-specific ablation (265). DT is synthesized by Corynebacterium diphtheriae (220). It has been adapted for expression in mammalian cells (220), and a single molecule might be sufficient to kill a cell. DT is a secretory precursor polypeptide and can be cleaved into two fragments, A (DTA) and B (DTB) chains, joined by a disulfide bridge. DTB binds to the receptor on the cell surface and is absorbed into the cells via endocytosis. Once DTB is transported into the cytoplasm, it catalyzes the transfer of ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD+) to a modified histidine residue on elongation factor 2, resulting in termination of protein synthesis (68) and subsequent cell death. Ideally, the delivery of DT into NPCs by retrovirus could specifically ablate newborn neurons in the adult brain. Since mice and rats are naturally resistant to DT, the genetic expression of an identified monkey DT receptor (a membrane-anchored form of the heparin-binding EGF-like growth factor) (241) in NPCs in these species provides another approach that ablates newborn neurons in the adult brain induced by DT (47, 287).
Only a fraction of the cells generated in the adult brain survives more than several months because the majority (~60–80% in standard housing) undergoes apoptosis (54, 79). Therefore, antiapoptotic techniques can potentially be used to increase neurogenesis. A recent example of this approach is the use of a mouse line with a genetic knockout of the proapoptotic gene Bax in NPCs to selectively increase adult neurogenesis (284).

B. Mapping the Circuit of Adult-Born Neurons

To date, most of the anatomical characterization of how new neurons physically connect to the existing circuitry has relied on circumstantial evidence from electrophysiology and the gross morphology of the neurons (97, 179, 214, 371). Developing more advanced techniques for dissecting the circuit of adult-born neurons is potentially very critical for fully understanding their integration and ultimately their function.

A genetically modified rabies virus was developed recently to trace the monosynaptic input of particular cell types (357). It has been successfully used to map the circuits of the piriform cortical neurons (132, 230), amygdala (132), retinal ganglion cells (318), and motor neurons (368). Unlike traditional tracers, this genetically modified rabies virus is able to target specific cell types and is subsequently retrogradely transported to presynaptic neurons only across one synapse, thereby labeling direct connections onto the initially infected cells (51).

Two modifications are made to generate this genetically modified rabies virus. 1) Rabies glycoprotein is necessary for a virus to retrogradely transport from target neurons to their presynaptic partners. Replacing rabies glycoprotein with fluorescent protein in a virus genome allows visualization of the presynaptic neurons and prevents the rabies virus from jumping across multiple synapses. 2) Pseudotyping rabies virus with EnvA then allows the rabies virus to target a specific cell type. EnvA is an avian sarcoma and leukemia viral envelope protein and can be used to guide virus infection specifically into cells that express the TVA viral receptor. Because TVA is only found in birds and not in mammals, introducing TVA into host cells in mice restricts EnvA pseudotyped rabies virus infection to only the particular cell type.

The use of this technique to map the circuit of newborn neurons in adult hippocampus requires both the genetically modified rabies virus and help from the aforementioned techniques to target young neurons. To get rabies glycoprotein and TVA to be specifically expressed in newborn neurons, the Moloney murine leukemia retrovirus can be used to specifically target genes to nonquiescent NSCs, NPCs, and their progeny. Therefore, combining a retrovirus and a genetically modified rabies virus is an ideal tool to study the monosynaptic circuit of adult-born neurons (86, 195, 349).

C. Imaging the Activity of Adult-Born Neurons

Ultimately, fully understanding the function of immature neurons will likely require an ability to observe the activity of young neurons in the context of the functioning DG circuit during behavior. A recent technique gaining widespread use in rodents is large-scale calcium imaging of the neural circuits. Combining optical microscopy with a fluorescent [Ca\(^{2+}\)] indicator can be used to record activity of population of neurons. Because action potentials trigger Ca\(^{2+}\) influx through voltage-gated calcium channels, changes of free [Ca\(^{2+}\)] can be used as a reliable readout for neural activity. The most popular techniques to qualitatively measure [Ca\(^{2+}\)] have been the use of either synthetic dyes or proteins that change their intensity of fluorescence upon calcium binding (212).

Chemical indicators are often a derivative of BAPTA, an EGTA homolog (338). They have high selectivity for calcium, and their fluorescence significantly increases in response to binding calcium (339). Membrane-permeable indicators (369) or dextran-conjugated indicators (240) such as Oregon Green BAPTA-1 (OGB-1) and fluo 4 have been widely used in functional imaging in vivo. This technique has been used in slice studies of the DG (213). In one study, the DG was bathed with a calcium indicator and retrovirus-labeled, adult-born, and embryonically born neurons could then be measured in the same experimental condition in vitro (213).

Genetically encoded calcium indicators (GECIs) overcome several of the primary limitations of chemical indicators, such as cell type specificity. GECIs can be targeted to specific neuronal populations and are able to be stably expressed in cells over months. GECIs are based on fusions of fluorescent proteins and calcium buffers, such as calmodulin (CaM) (242) or troponin C (TnC) (211). When calcium binds to CaM/TnC, conformational changes induce changes in fluorescence intensity (242). Long-term expression of GECIs in adult-born neurons by a retrovirus or progenitor-specific promoter would allow the imaging of the activity of newborn neurons in behaving animals in vivo over long periods of time and would greatly facilitate the demonstration of what adult-born neurons encode.

D. Controlling the Activity of Adult-Born Neurons

Manipulating the activity of newborn neurons in the adult brain is required to further understand the function of adult neurogenesis. Inactivating or activating newborn neurons
by genetics or viral vector using optogenetic strategies (103) has the potential to directly investigate the impact of adult-born neurons on the local circuit. The most common optogenetic tools are Clamydymonas reinhardtii channelrhodopsin-2 (ChR2) and Natronomonas pharaonis halorhodopsin (NpHR), which are microbial light-sensitive proteins. ChR2 is a cation channel that mainly allows Na+ to enter the cell and activate cells following exposure to blue light (479 nm). NpHR is a chloride pump that activates upon illumination with 580 nm yellow light and causes inhibition of neurons. The millisecond temporal scale of optogenetics allows control of neuronal activity in real time. The introduction of ChR2 to adult-born neurons by retrovirus has shown that newborn neurons generated in the adult brain are able to form functional synapses with their postsynaptic target CA3 pyramidal neurons (330). Direct activation using ChR2 of a group of neurons in the DG that are activated during fear conditioning has been shown to be sufficient to mediate the fear memory recall (200). It will be interesting to directly activate or inhibit newborn neurons by optogenetics during learning.

In addition to optogenetics, other methods can be used to control neuronal activity. Tetanus toxin (TeTx) is a neurotoxin produced by the bacteria Clostridium tetani (165). TeTx suppresses neurotransmitter release by cleaving the synaptic vesicle-associated membrane protein VAMP2/synaptobrevin2 (294). Therefore, expressing TeTx in neurons will inhibit their output and inactivate their function. A similar method involves the use of the Drosophila allatostatin (AL) receptor (AlstR), which is a G protein-coupled inward rectifier K+ channel (39). Applying AL to AlstR-expressing neurons blocks generation of action potentials (181). Inducible and rapidly reversible AL-dependent silencing of a cell has been demonstrated in cortex and spinal cord in the anesthetized rat, mouse, and monkey (119, 323, 324). Finally, an engineered muscarinic receptor, hM3Dq, is specifically activated by a synthetic ligand, clozapine-N-oxide (CNO), but not by its endogenous ligand, acetylcholine (15). CNO activates Gq signaling pathway in hippocampal neurons and induces burst firing (8, 113).

E. Future of Neurogenesis Technology and Research

While current technology has provided us many insights into how these new neurons arise and what they do, there is still much that we need to learn about these new cells. Ultimately, understanding the cellular nature of the neurogenesis process from NSC to GC as well as how the functions of adult-born neurons is going to require a combination of many of these approaches. For instance, externally regulatable genetic approaches manipulating how progenitors respond to their local environment and external cues will reveal much about the proliferation and differentiation processes. In short, while the technological advances of the last couple of decades have revealed most of what we know about adult neurogenesis, we may yet learn that we still know very little.

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Current address of G. D. Clemmons: Dept. of Neurobiology and Behavior, Univ. of California Irvine, Irvine, CA 92697.

Address for reprint requests and other correspondence: F. H. Gage, Laboratory of Genetics, Salk Institute for Biological Studies, La Jolla, CA 92037 (e-mail: gage@salk.edu).

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DISCLOSURES

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