Leading Edge
Review

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# Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior

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**The dentate gyrus of the mammalian hippocampus continuously generates new neurons during adulthood. These adult-born neurons become functionally active and are thought to contribute to learning and memory, especially during their maturation phase, when they have extraordinary plasticity. In this Review, we discuss the molecular machinery involved in the generation of new neurons from a pool of adult neural stem cells and their integration into functional hippocampal circuits. We also summarize the potential functions of these newborn neurons in the adult brain, their contribution to behavior, and their relevance to disease.**

Over 50 years have passed since the first report of neurogenesis in the hippocampal dentate gyrus (DG) of the adult rodent brain (Altman and Das, 1965). Although the scientific consensus of the time was that the adult brain did not generate new neurons, this discovery was confirmed by numerous subsequent studies. It is now widely accepted that adult neurogenesis occurs in the DG of humans (Eriksson et al., 1998; Spalding et al., 2013), as well as most mammals and several other vertebrates. Adult neurogenesis is the most robust form of plasticity in the adult brain and likely contributes to memory formation. In addition, adult-born neurons have been used to study neuronal development, and defects in neurogenesis have been associated with several human neurological and psychiatric diseases. In this review, we summarize the current knowledge about DG neurogenesis, its origins, regulation, and relevance to disease. We also focus on recent findings on the differentiation, network integration, and function of adult-born dentate granule cells (DGCs).

## The Subgranular Zone: Adult Neural Stem Cells and Their Niche

The sub-granular zone (SGZ) of the hippocampal DG is one of the stem-cell-containing niches in the adult mammalian brain (Figure 1A). This thin band between the granule cell layer and the hilus provides a unique microenvironment for an adult neural stem cell (NSC) population. The permissive milieu of the SGZ allows NSC proliferation while promoting the specification and differentiation of dentate granule neurons. Adult-born dentate granule neurons pass through several consecutive developmental stages before they become functionally integrated into the hippocampal circuitry. Type 1 radial glia-like cells (RGLs) are thought to represent the NSC population and can generate proliferating intermediate progenitor cells (IPCs, type 2 cells) with transient amplifying characteristics. These type 2 cells can give rise to neuroblasts (type 3) that subsequently differentiate into mature dentate granule neurons (Figure 1B). Apart from the neural progenitor population, this area contains several other cell types that are thought to support neurogenesis, as well as a dense vascular network that is tightly associated with NSCs.

## Progenitors: Is This a Homogeneous Population?

Two of the defining characteristics of stem cells are the capacity for self-renewal through cell division and the ability to generate specialized cell types through differentiation. However, stem cell populations are often heterogeneous within a tissue, and distinct stem cells may coexist for the same lineage. Different models of the identity and activities of NSCs in the adult mammalian brain have been proposed. GFAP-, Nestin-, and Sox2-expressing radial RGL cells (type 1 cells) exhibit NSC properties. Clonal analysis of individual RGLs has revealed self-renewal and multipotent capacities in this population (Bonaguidi et al., 2011). Alternative RGL properties have also been reported (Encinas et al., 2011; Sierra et al., 2015), suggesting that heterogeneity among RGLs may exist (Gebara et al., 2016). Whether and how such NSC heterogeneity contributes to varying levels of self-renewal and differentiation capacity among RGLs needs to be addressed. Furthermore, non-radial Sox2-expressing precursors have also been proposed to exhibit multipotent characteristics, and additional proliferating cell populations may act as NSCs under certain conditions. A recent study used single-cell gene expression analysis to elucidate the heterogeneity of NSCs and found that only a few genes were specific to quiescent NSCs (Shin et al., 2015). These results point to a more complex scenario for the developmental sequence in the adult hippocampal lineage than our prevailing simplified model may suggest. In addition, two recent studies demonstrated that single NSCs are not long-term self-renewing (Barbosa et al., 2015; Calzolari et al., 2015), supporting the emerging concept that NSCs may only persist at a population level. The advancement of new in vivo imaging approaches will undoubtedly further help shed light on this question.

The origin of adult NSCs is still only partly understood. According to a prevailing model, adult NSCs originate from the whole

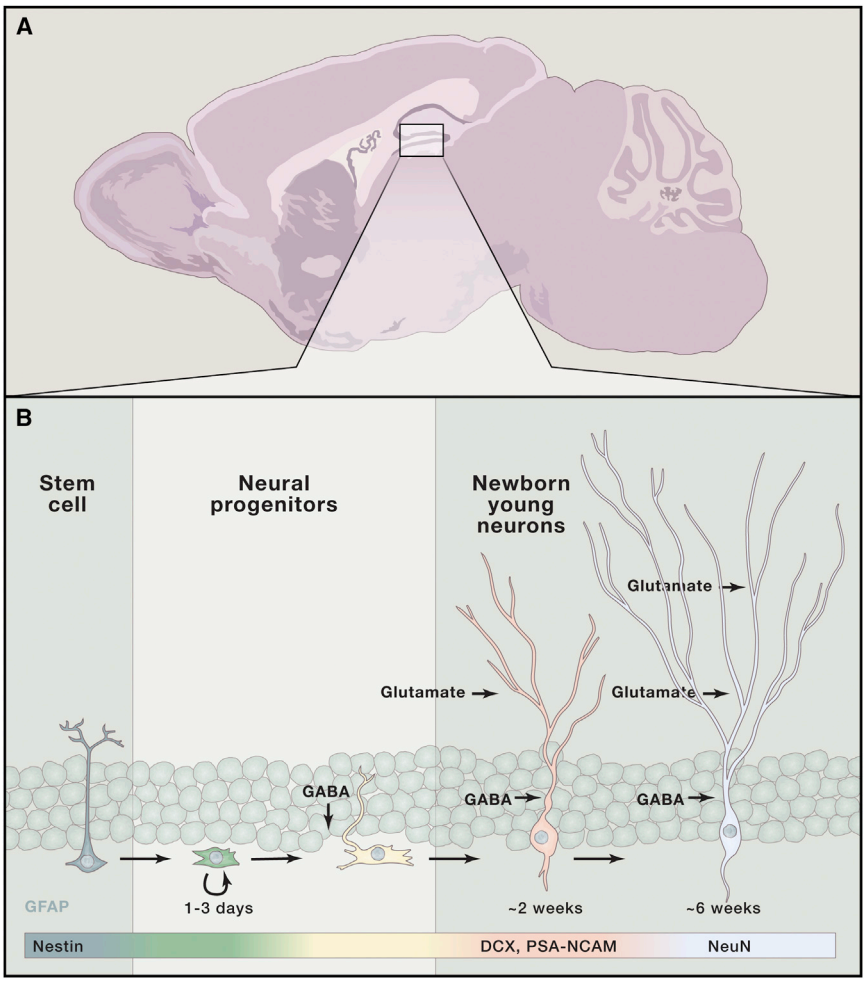



**Figure 1. The Adult Hippocampal Niche**

(A) Scheme showing the hippocampal formation in the adult rodent brain. The box highlights the SGZ of the dentate gyrus as one of the germinal zones in the adult mammalian brain.

(B) Newborn neurons in the subgranular zone of the dentate gyrus pass through several consecutive developmental stages. Type 1 RGLs can generate proliferating IPCs (type 2 cells) with transient amplifying characteristics. These type 2 cells can give rise to neuroblasts (type 3) to subsequently differentiate into dentate granule neurons. During their maturation, a transition occurs from GABA excitatory to GABA inhibitory and glutamate excitatory inputs around 2–3 weeks after birth. The developmental trajectory is accompanied by subsequent expression of stage-specific molecular markers.

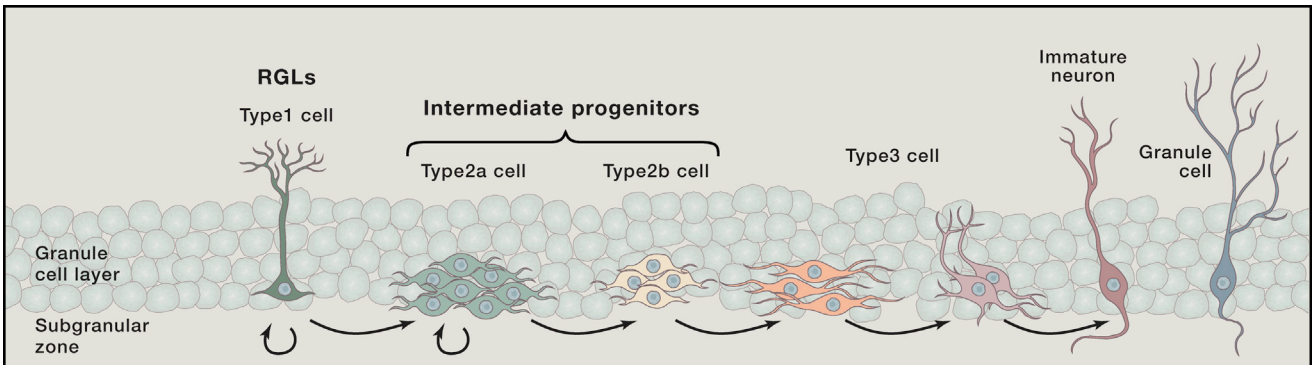
length of the dentate neuroepithelium, which produces both embryonically generated granule neurons and adult NSCs. However, a recent study also proposed that adult NSCs originate during late gestation from a population of sonic hedgehog (Shh)-responsive cells in the ventral hippocampus. The descendants of these cells then relocate into the dorsal hippocampus to become the source for adult NSCs in the SGZ (Li et al., 2013a). The lack of more sophisticated tracing tools still leaves some general questions about their origin unanswered. Do adult precursors arise from neural precursors that are also responsible for embryonic neurogenesis, or do they arise from a quiescent population that is set aside during early development as a reserved pool?

## Regulation within a Developmental Continuum: Where and When Do Signals Meet?

Numerous studies over the past decades have revealed several key factors and signaling mechanisms that regulate adult neurogenesis within a defined local microenvironment. As adult stem cells pass through genetically and morphologically identifiable stages, regulation can be targeted at several steps throughout their development. In this review, we discuss our current understanding of the intrinsic and extrinsic signaling mechanisms involved in regulating distinct stages of adult neurogenesis (Figure 2). We also attempt to draw a more unifying picture of how, when, and where canonical signaling pathways crosstalk to facilitate a dynamic modulation of neurogenesis. Signal convergence may occur at several levels within, and in close proximity to, the signal-receiving cell. The surrounding niche provides the environment for a first level of signal integration. Here, local or temporal morphogen gradients could have opposing or cumulative effects on the signaling outcome. A second and more complex level is the network of signaling components existing within a particular context of the signal-receiving cell itself (receptors and intermediate downstream targets). Their different expressions in space and time may set or alter the threshold for certain signals from the niche by integrating or differentiating incoming information. We will start by reviewing the current knowledge about the signaling components (morphogens, growth factors, cytokines, and neurotransmitters), transcription factors, and metabolic components that have been shown to be involved in adult neurogenesis. We will then give an outlook on how this plethora of incoming signals could possibly be integrated into the cellular program.

## Notch Signaling

Studies of invertebrates and vertebrates indicate that Notch signaling is highly pleiotropic, as it plays fundamental roles in a wide array of developmental processes. The specific context in which Notch signaling is activated dictates the particular downstream process that is triggered: cell proliferation, cell-fate determination, or apoptosis. The role of Notch signaling has previously been studied during development of the hippocampus, where it appears to be involved in maintaining the proliferative and undifferentiated stages of neural progenitor cells (NPCs) (Breunig et al., 2007). In addition to their developmental functions, Notch pathway components are expressed in the




| | Quiescence | Proliferation/ Activation | Fate specification | Morphogenesis and differentiation | Maturation |
|---|---|---|---|---|---|
| **Signals** | BMP, Notch, GABA | Canonical Wnt, IGF, Shh, FGF-2, EGF, Dopamine | Canonical Wnt, NT-3 | Wnt/PCP, Notch, BDNF, Reelin, GABA | Glutamate, NRG2, Neuronal activity |
| **Transcription factors** | Hes5, FoxO, REST | Sox2, Tlx | Tbr2, Ascl1/Mash1, NeuroD1, Prox1 | NeuroD1,Prox1, CREB, AP-1? | CREB |
| **Epigenetic regulation** | | Gadd45b, LSD1 | Fmrp, Mbd1 | Gadd45b, MeCP2 | MeCP2, HDAC2 |

**Figure 2. Signals, Transcription Factors, and Epigenetic Regulators during Adult Hippocampal Neurogenesis**
Stage- and cell-specific effects of different signaling pathways, transcription factors, and epigenetic regulators during lineage progression.

adult nervous system. Various studies have shown that the effects of Notch on adult neurogenesis are context dependent. Notch1 was found to be required for self-renewal and the expansion of nestin-expressing NSCs in the adult hippocampus. In line with these findings, inactivation of the Notch pathway component RBPj resulted in an initial increase in hippocampal neurogenesis by causing premature differentiation of Sox2-positive progenitors, which in turn resulted in depletion of the progenitor cell pool and suppression of adult hippocampal neurogenesis (Ehm et al., 2010). Furthermore, a study focusing on the Notch intracellular domain (NICD) showed that overexpression of this downstream effector induced proliferation and expansion of the NSC pool. The same study demonstrated that Notch signaling also modulated dendritic morphogenesis: conditional knockout of Notch1 resulted in significantly less complex arborization, whereas overexpression increased dendritic complexity (Breunig et al., 2007). The effect on dendritic development seems, however, to be restricted to immature cells, since manipulation of Notch signaling in adult neurons was shown to have no effect on dendritic arborization (Dahlhaus et al., 2008).

Due to its pleiotropic nature, the activity of Notch signaling can have diametrically opposed effects within distinct developmental contexts. Divergent functions of the Notch receptors, as well as differences in the intensity of Notch signaling, are thought to contribute to the heterogeneity in adult NSC behavior. The way in which Notch signaling is integrated with the signals from other pathways could be one possible explanation for its context-dependent roles.

## Hedgehog Signaling

Sonic hedgehog (Shh) is the major activating ligand to initiate Hedgehog signaling in the brain and has been shown to play important roles in the formation and patterning of adult germinal niches in the brain. Adult NSCs in the DG appear to originate from Shh-responsive progenitors in the ventral hippocampus (Ahn and Joyner, 2005; Li et al., 2013a). The receptor Patched (Ptc) and the transmembrane protein Smoothened (Smo) are expressed in the adult hippocampus and in progenitors derived from this region (Lai et al., 2003). The sources of Shh have not yet been clearly identified; however, tracing studies using *Gli1-nLacZ* reporter mice have revealed Shh signaling activity in NSCs (Ahn and Joyner, 2005). Exogenous Shh has been shown to directly promote progenitor proliferation in vitro. Overexpression of Shh within the DG using an adeno-associated viral system resulted in a marked increase in hippocampal progenitor cell proliferation in vivo. Pharmacological inhibition of Shh signaling through cyclopamine reduced hippocampal progenitor proliferation when directly delivered into the adult hippocampus (Lai et al., 2003). Postnatal progenitors failed to develop after embryonic ablation of Smo in GFAP+ and Nestin+ neural precursor cells (Han et al., 2008). In contrast, expression of a constitutively active Smo resulted in a marked expansion of the DG, indicating an important role for Shh signaling in the expansion and establishment of postnatal hippocampal progenitors. Interestingly, decreased Shh target gene expression and a similar devastating effect on postnatal neurogenesis were observed in animals lacking primary cilia (Breunig et al., 2008; Han et al., 2008). The selective targeting of the Shh-signaling

machinery to cilia is thought to enable RGL precursors to differentially respond to mitogenic Shh signals, thereby functioning as cellular ''antennae'' (Breunig et al., 2008).

### Bone Morphogenetic Protein Signaling

Bone morphogenetic proteins (BMPs) comprise a group of more than 20 ligands that constitute the largest subgroup of the transforming growth factor-beta (TGF-beta) superfamily of cytokines. They are highly expressed in the embryonic and adult nervous system and exert a plethora of effects, including cell survival, proliferation, and fate specification. Negative regulation of BMP activity can be achieved through Chordin, Noggin, and Neurogenesin-1, which bind and antagonize BMPs directly in the extracellular space. In adult neurogenic niches, BMPs can act as short-range morphogens due to a limited spread and their ability to bind to extracellular matrix components. As with many morphogens, the precise action of BMPs depends on the context in which the signaling occurs (context in the niche, as well as within the signal-receiving cell). In the postnatal hippocampus, BMPs are chronically secreted by granule neurons, NSCs, and other niche cells and are essential for regulating the equilibrium between proliferation and quiescence (Bonaguidi et al., 2008; Bond et al., 2014; Mira et al., 2010; Yousef et al., 2015). Not only are BMPs necessary for maintaining quiescence, but they also play crucial roles in controlling the rate at which DGCs mature (Bond et al., 2014). Such a dual role may be explained by a differential expression of the BMP receptors. While NSCs express BMPR-Ia, which is downregulated in IPCs, neurons and neuroblasts express BMPR-Ib (Mira et al., 2010), suggesting a receptor-context-specific signal integration.

Several BMP inhibitors, such as Chordin, Noggin, and Neurogenesin-1, are present in the hippocampal niche and are thought to locally adjust the levels of BMP signaling (Bonaguidi et al., 2008). By adulthood, a strong Noggin signal is concentrated in the DG, which has been shown to be controlled by the RNA binding protein FXR2 (Guo et al., 2011b). BMP signaling, in addition to other pathways, has also been shown to be involved in linking the mechanism of voluntary exercise with changes in neurogenesis. Finally, an age-associated increase in BMP signaling has recently been reported and may partly contribute to the decline of neurogenesis in old animals, suggesting that inhibition of this pathway could potentially allow rescue of this age-related drop (Yousef et al., 2015).

### Wnt Signaling

Canonical Wnt signaling is fundamental for the proper development of cortex and hippocampus during development. In addition to promoting self-renewal and maintaining neural progenitors during early neurogenesis, it induces the differentiation of intermediate progenitors during mid and late neurogenesis. Recent work suggests an important function for the Wnt pathway not only during development, but also in the adult brain. Wnt3, which is produced by local hippocampal astrocytes, was shown to stimulate Wnt/β-Catenin signaling in isolated AHPs and induce their differentiation toward the neuronal lineage (Kuwabara et al., 2009; Lie et al., 2005). In vivo experiments further demonstrated the regulative properties of Wnt signaling during adult hippocampal neurogenesis. While activation of Wnt signaling in the SGZ increased neurogenesis, its inhibition caused a reduction in proliferation and neuronal differentiation (Lie et al., 2005). *Prox1* and *Neurod1* were shown to be among the major direct transcriptional targets of Wnt/β-Catenin-TCF/LEF signaling, and they are known to control genes specifically involved in neuronal differentiation (Gao et al., 2009; Kuwabara et al., 2009; Lavado et al., 2010). Gao et al. (2009) demonstrated that *NeuroD1* is required for hippocampal neurogenesis by facilitating survival and maturation. An intriguing link between Wnt/β-Catenin signaling, neuronal differentiation, and the expression of *NeuroD1* was proposed in a study by Kuwabara et al. (2009). Here, the presence of dual regulatory elements within the *NeuroD1* promoter was shown to enable a molecular configuration in which *NeuroD1* transcription was either repressed by *Sox2* in undifferentiated cells or activated by Wnt signaling through TCF/LEF in dividing neuronal progenitors. Despite its pivotal role in promoting neuronal differentiation, activation of the Wnt/β-Catenin signaling pathway was shown to promote proliferation rather than differentiation. However, modulation of the pathway was achieved by injecting lentivirus-expressing shRNAs to suppress expression of Disrupted in Schizophrenia 1 (DISC1), which directly interacts with GSK3β, resulting in its inhibition and subsequent stabilization of β-Catenin (Mao et al., 2009).

While complicating our view of Wnt signaling, the involvement of Wnt signaling in both aspects—progenitor pool maintenance and neuronal cell fate—does not appear to be contradictory. In fact, manipulation of Wnt signaling is a formidable challenge, since the commonly used components (GSK3β, β-Catenin, etc.) are likely to cause pleiotropic effects, as they themselves interact with other signaling pathways. Several studies of Wnt antagonists have shown how aging and neuronal activity dynamically control adult hippocampal neurogenesis through modulation of this central pathway. The expression of Dickkopf-related protein 1 (Dkk1), a secreted Wnt antagonist, was shown to increase with age in the adult hippocampus, and Dkk1 deletion from granule neurons was sufficient to restore neurogenesis in old mice (Seib et al., 2013). In line with these observations, dorsal hippocampal infusion of Dkk1 resulted in impaired object recognition memory consolidation (Fortress et al., 2013). Moreover, secreted frizzled-related protein 3 (Sfrp3) was shown to be secreted by DGCs, and loss of Sfrp3 resulted in the activation of quiescent radial NSCs and a subsequent increase in dendritic complexity (Jang et al., 2013). Neuronal activity, mimicked by electroconvulsive stimulations and optogenetics, was shown to decrease the expression of Sfrp3 (Jang et al., 2013), demonstrating for the first time a link between neuronal activity and Wnt-mediated adult neurogenesis. However, whether these two Wnt antagonists act on the same or different downstream mechanisms remains unknown.

As Wnt signaling alone provides the basis for a wide range of possible interactions, how do these signals converge in space and time to allow a stage-specific regulation? A recent study focusing on the temporal signaling properties revealed a remarkable transition of Wnt signaling responsiveness from the canonical branch (β-Catenin-dependent) to the non-canonical branch (PCP pathway) in the course of neuronal differentiation. While canonical Wnt signaling progressively faded, the emerging

non-canonical branch was required for late stages of maturation, such as dendrite initiation, radial migration, and dendritic patterning (Schafer et al., 2015). These results demonstrated that Wnt signals in the hippocampal niche are highly stage dependent and that integration occurs in a context-specific manner within the signal-receiving cell. Careful analysis of Wnt pathway interactors in space and time will undoubtedly help us understand the various interactions and mechanisms involved.

## Growth Factors, Neurotrophic Factors, Cytokines, and Neurotransmitters

Numerous growth factors, neurotrophic factors, and neurotransmitters have also been reported to be part of the regulatory signaling macrocosm within the hippocampal niche. For brevity, we will discuss those factors that were studied in the context of adult hippocampal neurogenesis and will refer to more specific literature for further details.

Neurotrophic factors are extracellular signaling proteins that bind to receptor tyrosine kinases known as Trk receptors and their co-receptor p75NTR. Among the four identified neurotrophic factors, the role of brain-derived neurotrophic factor (BDNF) has been studied most extensively. Neuronal maturation—particularly, the dendritic growth of adult-born neurons—is accelerated by behavioral experience, such as exercise and exposure to enriched environments, and the neuronal activity associated with it. This activity-dependent increase in dendrite length and complexity appears to be mediated by the cell-autonomous, autocrine action of BDNF (Wang et al., 2015). Other secreted molecules may also be involved in stimulating the growth and maturation of DGCs in response to neuronal activity; for example, Wnt ligand release is elevated by activity (Wayman et al., 2006). A recent study followed the dendrite growth of adult-born DGCs using longitudinal in vivo imaging over a period of several weeks, thereby capturing the growth, addition, and pruning of dendrite branches in individual neurons (Gonçalves et al., 2016). Exposing the mice to an enriched environment resulted in faster growth and increased branching; however, these changes were countered by earlier and more extensive pruning, so that by the end of the first month post-mitosis, dendritic morphology in enriched environment mice was similar to that of mice reared under standard conditions. Stunting dendritic growth by disrupting Wnt signaling also resulted in dendrites with similar branching structure, albeit with smaller length. Interestingly, newborn neurons that extended more branches underwent more pruning, resulting in a similar dendritic structure for all DGCs. These findings suggest a homeostatic control of dendritic morphology that reverses the activity-dependent changes of dendrite morphology.

Growth factors are a large group of extracellular proteins controlling cell growth and maintenance. Several growth factors have been shown to be involved in regulating adult hippocampal neurogenesis, including fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1). FGF-2 (Kang and Hébert, 2015) and IGF-1 have both been reported to promote NPC proliferation and production of new neurons. In addition, IGF-1 was found to control subventricular zone (SVZ) neuroblast migration and to instruct adult NPCs in the hippocampus to become oligodendrocytes by inhibiting BMP signaling (Hsieh et al., 2004).

Adult neurogenesis is also strongly modulated by microglia and inflammation. Inflammation is known to sharply inhibit neurogenesis in the adult brain (Ekdahl et al., 2003) through the microglial release of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$). However, activated microglia do not necessarily inhibit neurogenesis and may even promote neurogenesis if the balance of secreted molecules in the neurogenic niche is anti-inflammatory (Battista et al., 2006). In fact, microglia are thought to be able to promote neurogenesis, for example, in response to exercise (Vukovic et al., 2012), primarily through the fractalkine (CX3CL1) signaling pathway.

## Metabolic States in the Adult Hippocampal Lineage

Metabolic control has been identified as an important regulator of stem cell activity in a variety of tissues. Stem cells seem to be in a metabolic state that is different from their progeny (Folmes et al., 2011; Varum et al., 2011). A recent study showed that de novo lipogenesis is crucial for adult stem cell behavior and that proliferation is significantly reduced upon genetic deletion or pharmacological inhibition of the key enzyme fatty acid synthase (Knobloch et al., 2013). Furthermore, physical activity has been shown to improve adult hippocampal neurogenesis, and endurance-related factors reflecting the metabolic state of the muscle are thought to mediate the effects exercise has on adult neurogenesis (Guerrieri and van Praag, 2015; Kobilo et al., 2014). Additionally, recent transcriptomic data suggest that the switch from a glycolytic metabolism to a largely mitochondrial-driven metabolism occurs at a very early stage in the lineage. Stem cells upregulate genes for oxidative phosphorylation at the time they become activated and enter proliferation (Shin et al., 2015).

Many molecular pathways and transcription factors involved in regulating adult neurogenesis have been shown to influence cell metabolism outside the brain. However, it remains unclear whether metabolic changes occur secondary to fate switches or are instructive for adult stem cell behavior. Further studies are needed to shed light on how metabolic states are interconnected with other signaling mechanisms that converge on controlling the balance of stem cell quiescence, activation, and differentiation.

## Transcription Factors and Epigenetics

Transcription factors are essential for regulation of gene expression and play a central role in coordinating lineage progression during development. Over the past decades, numerous studies have identified proteins expressed at specific stages of adult hippocampal neurogenesis, which have since been used as markers. Most of these proteins appear to be transcription factors with key functions in controlling the transcriptional program during lineage progression. Here, we discuss some of these major transcription factors with respect to the stage at which they exert a predominant function.

The SRY-related high-mobility group (HMG) box (Sox) family member Sox2 is among the most extensively studied transcription factors in NSC behavior and function. Sox2 is highly

expressed in type 1 and type 2a cells and controls the multipotency and proliferative capacities of NSCs (Favaro et al., 2009; Steiner et al., 2006). The transcription factor itself can be regulated by several signaling pathways that are particularly active in type 1 cells. Notch/RBPJk signaling, for example, directly controls the expression of Sox2, and overexpression of Sox2 was shown to be sufficient to rescue the self-renewal defect in RBPJk-deficient stem cells. Thus, Notch/RBPJk-dependent pathways act as essential regulators of adult NSC maintenance through the transcriptional regulation of Sox2 expression (Ehm et al., 2010).

Sox2, on the other hand, controls the expression of several target genes. The nuclear orphan receptor Tlx, which in turn promotes proliferation and self-renewal of adult NSCs through the canonical Wnt pathway, was shown to be positively regulated by Sox2 (Shimozaki et al., 2011). Tlx may also control NSC proliferation by suppressing pathways that promote quiescence, including the p53 pathway, cell-cycle inhibitor p21, and other pathways (Niu et al., 2011). An interaction of Tlx with the histone deacetylases HDAC3 and HDAC5, as well as with the lysine-specific demethylase 1 (LSD1), has been reported to be essential for Tlx-dependent regulation of stem cell proliferation (Sun et al., 2007). Finally, Sox2 was found to modulate Shh signaling by controlling the expression of Shh (Favaro et al., 2009), as well as to inhibit the Wnt signaling-induced transcriptional activation of NeuroD, thereby preventing neuronal differentiation (Kuwabara et al., 2009).

Other transcription factors that are predominantly active in NSCs are those of the Hes family, the Forkhead O-box (FoxO) family, the nuclear factor 1 (NF1) family, the transcriptional regulator Hmga2, and the transcriptional repressor Bmi-1. Common to all is the ability to functionally regulate the expression of differentiation inhibitors, cell-cycle inhibitors, and signaling pathways involved in controlling NSC behavior. The repressor element 1-silencing transcription (REST) factor is a particular case insofar as it is not only expressed in NSCs but also in mature granule neurons (Gao et al., 2011). REST is required to maintain NSCs in a quiescent and undifferentiated state, at least in part by preventing precocious expression of the neuronal differentiation program (Gao et al., 2011; Kim et al., 2015).

Achaete-scute homolog 1 (Ascl1/Mash1) is a member of the basic helix-loop-helix (bHLH) family of transcription factors and is expressed by dividing type 2a cells (Uda et al., 2007). As a proneuronal transcription factor, Ascl1/Mash1 has been shown to play two opposing roles during embryonic neurogenesis: promoting proliferation and driving cell-cycle exit and differentiation. Ascl1/Mash1 operates downstream of Tlx in the control of stem cell proliferation in vitro and closely interacts with Notch signaling in neural precursor cells (Andersen et al., 2014). Interestingly, Hes proteins that are induced by Notch activity act as potent repressors of gene expression, and proneuronal bHLH transcription factors are among their main targets. Due to an auto-regulatory repression and short half-lives, the cellular expression levels of Hes proteins oscillate. This oscillation in turn drives in opposite phase the oscillation of their targets, including Neurogenin 2 (Neurog2) and Ascl1/Mash1. It is noteworthy that the oscillating expression of Ascl1/Mash1 promotes proliferation of neural progenitors, whereas its stable expression drives differentiation (Imayoshi et al., 2013). In the adult hippocampus, Ascl1/Mash1 is indeed increased upon loss of RBKJk, and its expression is confined to about one-third of the activated NSCs, suggesting a dynamic regulation (Andersen et al., 2014).

The T-box transcription factor Tbr2 is another principal regulator of embryonic neurogenesis, controlling the formation of glutamatergic neurons in the developing cerebral cortex (Arnold et al., 2008). In the adult hippocampus, elimination of Tbr2 augmented stem cell proliferation and blocked the generation of late IPCs and dentate granule neurons (Hodge et al., 2008). Tbr2 seems to be crucial for the progression of neuronal fate decisions and was shown to counteract Sox2, the key determinant of NSC identity (Hodge et al., 2012).

A specific feature of the early dentate granule neuron lineage is the simultaneous expression of the bHLH transcription factor NeuroD1 and the homeobox factor Prox1. Both transcription factors are direct targets of canonical Wnt signaling (Gao et al., 2009; Kuwabara et al., 2009). Overexpression of either NeuroD1 or Prox1 promoted neuronal differentiation of NSCs in vivo, and conditional ablation resulted in decreased generation of DCX-positive immature neurons (Gao et al., 2009; Lavado et al., 2010). While Prox1 ablation increased cell death of late-stage precursors (Lavado et al., 2010), NeuroD1 appeared to be crucial for the survival of maturing dentate granule neurons (Gao et al., 2009). Given their simultaneous expression patterns, as well as their mutual operation downstream of Wnt signaling, Prox1 and NeuroD1 appear to be key players in a terminal network specifying the dentate granule neuron subtype. The majority of transcription factors involved in adult neurogenesis exert transient expression patterns. Prox1 seems to deviate from this principle in that its expression is maintained in mature dentate granule neurons after initiation at the stage of type 2b cells. A recent study reported that Prox1 was necessary to maintain the identity of mature dentate granule neurons. Conditional ablation of Prox1 from newly generated mature neurons resulted in reduced levels of Calbindin and aberrant expression of CA3-specific genes (Iwano et al., 2012). These pleiotropic actions of Prox1 could be plausibly explained by the existence of a multitude of Prox1 targets, which may place Prox1 as a central crosstalk anchor between different signaling pathways. Studies from the embryonic brain suggest that Prox1 may be acting at such a crosstalk point between key cell-fate regulators and diverse signaling pathways. However, cell-type- and context-specific interaction studies are needed to reveal the basis of the Prox1-associated transcription network during dentate granule neurogenesis.

Further regulatory properties arise through processes that control gene expression, such as miRNAs (Han et al., 2016) and epigenetic mechanisms, which determine the DNA and histone accessibility of critical genes to shape the cellular transcriptome landscape. Only recently has epigenetic regulation become the focus of attention with regard to adult neurogenesis, especially concerning maintenance and exit from quiescence in adult NSCs. Interestingly, DNA demethylation appears to be induced by neuronal activity in the DG, resulting in the proliferation of neural progenitors and growth of newborn DGCs (Guo

et al., 2011a; Ma et al., 2009). For further studies elucidating the importance of epigenetic mechanisms contributing to adult NSC maintenance and lineage progression, we refer the reader to more detailed reviews on this topic.

## Molecular Networks: Signal Convergence on a Systems Level

To understand how genes and signals contribute to a complex biological process like neurogenesis, we are faced with the task of assessing phenotypes within the CNS, a complex and highly organized system. Most laboratory experiments currently rely on models that can only account for a few features at a time. To better understand the basis for signal convergence within such a complex biological process, it is necessary to adopt data-driven approaches that permit the measurement of large-scale cellular and molecular phenotypes.

Recent advances in whole-transcriptome single-cell sequencing have laid the groundwork for identifying genome-wide molecular transitions of stem cell behavior. A recent study used a nestin reporter mouse to isolate putative NSCs from the SGZ and developed a single-cell omics analysis for reconstructing the molecular events along a calculated continuous developmental trajectory (Shin et al., 2015). Together with two other studies (Hanchate et al., 2015; Llorens-Bobadilla et al., 2015), these approaches demonstrate that single-cell analysis enables the reconstruction of temporal dynamics and molecular events during lineage progression. A combination of advanced experimental methods and computational tools can help elucidate more precisely the developmental lineages and identify defined or intermediate stages within a developmental continuum. More recently, this approach was used to demonstrate how a continuum of activation stages can be identified even within a defined population of DG neurons following exposure to an enriched environment: by combining cell sorting with single-nuclei RNA sequencing (RNA-seq), the authors were able to capture the transcriptional patterns associated with neuronal activation, including immediate early genes (IEGs) (Lacar et al., 2016). This first set of transcriptomics studies has very recently been complemented by the development of Div-Seq, a method that combines single nucleus RNA-seq with EdU pulse labeling to profile individual dividing cells (Habib et al., 2016).

It is beyond question that the further advancement of high-throughput quantification methods will permit the implementation of a systems biology approach on various levels. The single-cell omics studies are a first step toward such integrative network approaches.

On a molecular level, signaling components, transcription factors, and other molecules may all together be part of a multi-dimensional, partially self-regulatory network program. Cumulative evidence from the last decades of stem cell research suggests that transcription factors are particularly interconnected and form networks with cross-regulatory properties. Such forms of interconnection may provide the basis for a system that is self-sustaining and stable but also prone to unwind through dysregulation of a single interconnected factor (Figures 3A and 3B). As fate decisions, growth rate, and the tempo of maturation are subject to dynamic modulation through extrinsic signals, molecular networks appear to provide an ideal platform for integrating diverging and converging developmental signals into cellular programs (Figure 3B). How these signals are integrated remains largely unknown. However, different extrinsic signals may compete for the rate and tempo of network destabilization, as well as for the recruitment of alternative networks during lineage progression. As cells follow a developmental sequence, with each stage being determined by specific molecular networks, various transient network instability points may exist within intermediate stages (Figure 3C, right). The internal stability of a network at a given time may in turn determine the impact the signal has within that particular stage. Certain signals could benefit from such intermediate instability and gain momentum to dynamically modulate lineage progression. Alternating expression patterns of interconnected transcription factors, for example, as described in the case of the oscillating Hes transcription factor family (Imayoshi et al., 2013; Shimojo et al., 2008), are likely to cause alternating states of network stability and instability (Figure 3C, left). We still do not understand enough to predict such network states, and further studies are needed to combine biological data with computational network theories. Features such as network components (which molecules/genes participate in the network program?), network topology (how is the network organized? Figure 3A), and network logic (what are the internal network rules and what molecules/genes determine the internal stability? Figure 3B) should be implemented to understand network dynamics (Figure 3C). Such a holistic approach will be essential to test and develop hypotheses that look beyond the borders of a still simple developmental model.

## Functional Implications of Neurogenesis

As detailed above, molecular networks are involved in controlling the developmental process of adult-born DGCs and are particularly responsive to neuronal activity and environmental factors. This molecular machinery propels adult-born neurons through a maturation period that resembles that of neurons generated around birth, thus providing newborn DGCs with transient functional properties that are unique in the adult brain. In the next sections, we describe the steps in the functional maturation of newborn neurons and how their immature properties may be essential to their role in the adult brain.

## Electrophysiology of Adult-Born DGCs

Adult-born DGCs have been shown to differentiate into functional neurons with functional passive membrane properties, synaptic inputs, and action potentials. Their electrophysiological characteristics are initially distinct from those of mature DG neurons: they have higher input resistance, lower threshold voltage, and a slower membrane time constant and they are more prone to long-term potentiation (LTP). As adult-born neurons mature, they are thought to recapitulate embryonic and post-natal developmental steps and eventually become indistinguishable from DGCs born during early development (Espósito et al., 2005; Laplagne et al., 2006). They start by having high input resistance, more depolarized resting membrane potentials, and low-amplitude action potentials. Gamma-aminobutyric acid (GABA) currents are initially excitatory and tonic (Ge et al., 2006). Phasic

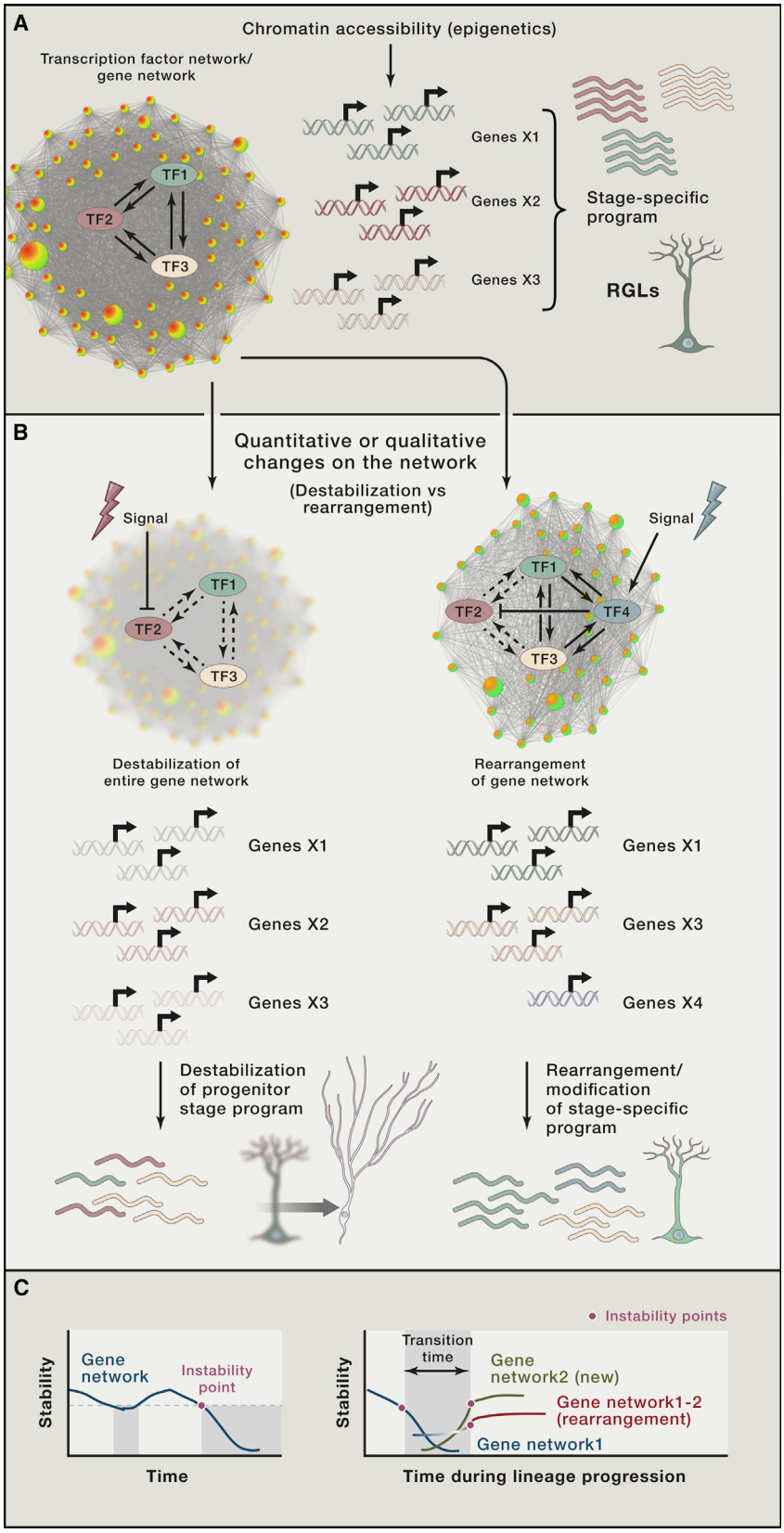



**Figure 3. Gene Networks as Platforms for Signal Convergence**

(A) Transcription factors are interconnected and form gene networks in which cross-regulation among its network components ensures that the system is self-sustaining and stable. These transcription factor networks result in a stage-specific transcriptional signature (gene-expression program). Different network programs may exist for different stages during lineage progression. Further regulatory properties are given through epigenetic modifications, which determine chromatin accessibility and thereby the ultimate cellular signature.

(B) Transcription factor networks can be modulated via extrinsic signals. Dysregulation of single interconnected transcription factors (in scheme TF1, 2, or 3, left) can result in destabilization of the entire network, which in turn dismantles the cellular program or stage. On the other hand, signals may also induce qualitative changes by rearranging or modifying the gene network (right).

(C) Different extrinsic signals may synergize or compete for the rate and tempo of network destabilization or rearrangement. The term "network dynamics" describes the internal stability of a network at a given time as a function of its topology and logic. Variable states of internal stability (such as those caused by oscillating expression levels of single transcription factors) may determine the impact a signal has on the network. Network states with lower stability (gray) could allow a faster or easier destabilization through certain signals (left). As cells follow a developmental sequence, various transient network instability points may exist between different network programs. Certain signals could benefit from such intermediate instability and gain momentum to dynamically modulate the recruitment of alternative or modified network programs during lineage progression (right).

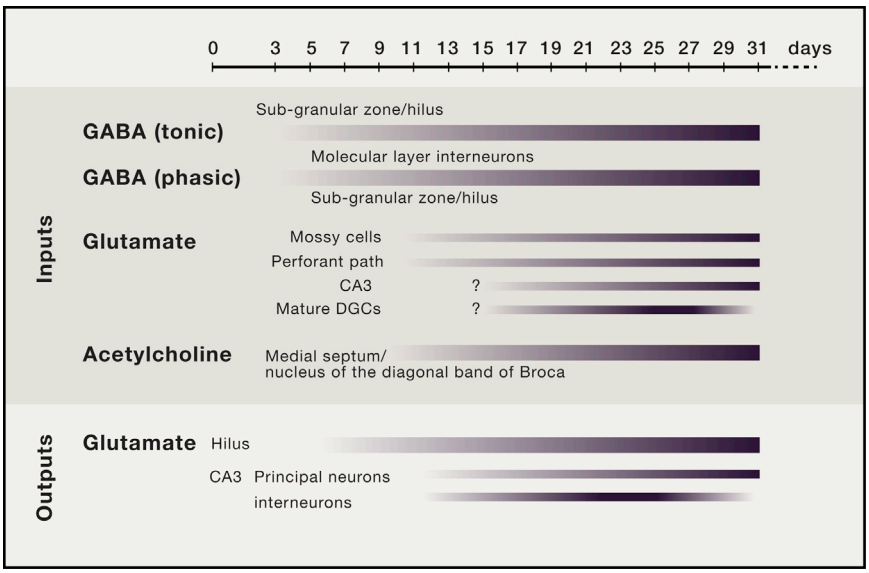



**Figure 4. Synaptic Inputs and Outputs of Adult-Born DGCs**

Time course of synaptic connectivity in developing adult-born DGCs (adapted from Deshpande et al., 2013, with data from Espósito et al., 2005; Ge et al., 2006; Zhao et al., 2006; Vivar et al., 2012; Chancey et al., 2014; Restivo et al., 2015).

GABA and glutamate post-synaptic currents are present after ~14 days. Similar to what happens during early postnatal development, the GABA reversal potential is initially higher than the resting membrane potential, resulting in excitatory GABA PSCs. This situation is gradually reversed as the $Cl^-$ transporter, NKCC1, is replaced by KCC2, and by the third week, GABA currents are inhibitory. Early synaptic input is essential for the correct development and synaptic integration of adult-born DGCs, starting with GABA-induced depolarization (Ge et al., 2006).

The first synaptic inputs into newborn cells are thought to be inhibitory interneurons in the SGZ and the hilus (Espósito et al., 2005; Ge et al., 2006), with a tonic GABA component that likely originates from transmitter spillover. Dendritic spines form at 16 days in a process that appears to be controlled by local astrocytes (Sultan et al., 2015; Zhao et al., 2006), but the first glutamatergic inputs into newborn neurons seem to originate as early as 10 days from hilar mossy cells (Deshpande et al., 2013). Inputs from cells in the molecular layer also appear during the second week, as do the first long-range connections from the medial septum and the nucleus of the diagonal band of Broca, providing cholinergic innervation. Inputs from the entorhinal cortex (EC) are present from the third week. Other synaptic inputs include a back-projection from CA3 and a seemingly transient input from mature DGCs that is present during the first month (Vivar et al., 2012), as well as inputs from the subiculum (Figure 4). Interestingly, the connectivity profile of newborn DGCs appears to depend on the behavioral experience of the animal (Bergami et al., 2015). Running and enriched environment exposure during weeks 2–6 were found to result in an increase in otherwise rare inputs from interneurons in CA3 and CA1 and from the mammillary bodies, as well as an increase in connectivity from the hippocampus, subiculum, and cortex. Although these changes were mostly transient, changes in cortical connectivity seemed to persist. Interestingly, voluntary exercise and enriched environment exposure appeared to have different effects on connectivity, with the former increasing connections only from the cortex, but not local and hippocampal connectivity, which could potentially account for the different behavioral consequences of exercise and enrichment. While both approaches resulted in an increase in the number of newborn neurons, mice exposed to an enriched environment outperformed those that engaged in voluntary exercise in challenging contextual fear-conditioning tasks that required discriminating between similar contexts (Clemenson et al., 2015). DGC axons (mossy fibers) make contact with granular cell layer (GCL) interneurons, as well as with interneurons and mossy cells in the hilus and CA3 cells. In newborn cells, DGC axons are found in the hilus as early as 7 days after GFP-expressing retroviral infection and reach CA3 at 10–11 days (Zhao et al., 2006), where they form functional glutamatergic connections as early as 17 days post-mitosis.

Therefore, by the end of the first month, adult-born DGCs are already integrated in the circuitry of the hippocampus, and their morphological growth is mostly complete. They receive incoming synaptic inputs, fire action potentials, and establish functional synapses onto hilus and CA3 cells. However, the electrophysiological features of DGCs at this age are different from those of mature cells, giving them unique properties that are thought to be important for their functional role. First, adult-born neurons are more excitable than mature DGCs between the fourth and sixth week post-mitosis due to a different balance in excitation/inhibition (Table 1). In addition, synaptic plasticity is enhanced: adult-born neurons have reduced LTP induction thresholds and increased LTP amplitude, which is at least partially attributable to a higher contribution of the NR2B-receptor subtype to NMDAR-mediated currents and less feed-forward inhibition at this developmental stage (Ge et al., 2007; Li et al., 2013b). The consequence of these differences in excitability and plasticity is a "critical period" during which immature adult-born neurons respond to a broad range of input stimuli and are quick to reinforce active connections. As newborn neurons mature further, they come under stronger inhibitory control, and the range of stimuli that elicit firing becomes narrower, resulting in sparser activity typical of mature DGCs (Danielson et al., 2016; Marín-Burgin et al., 2012). This period of unusual activity and plasticity is likely to be essential for the function of adult-born neurons, as obtaining these properties transiently in a specific subpopulation of cells is not thought to be easy using standard plasticity mechanisms present elsewhere in the mammalian brain.

**Table 1. Selected Electrophysiological Properties of Adult-Born DGCs**

| Property | Week 4 | Mature |
|---|---|---|
| Resting membrane potential (mV) | −76 ± 0.5 (Mongiat et al., 2009) | −81 ± 0.5 (Mongiat et al., 2009); −65 ± 4 (Ge et al., 2006); −68 ± 2 (Pernía-Andrade and Jonas, 2014)[a] |
| Input resistance (MΩ) | 519 ± 30 (Mongiat et al., 2009) | 224 ± 7 (Mongiat et al., 2009); 143 ± 10.8 (Pernía-Andrade and Jonas, 2014)[a] |
| GABA current reversal potential (mV) | −56 ± 1 (Marín-Burgin et al., 2012) | −75 ± 6 (Ge et al., 2006) |

[a]in vivo, awake recordings.

## Dentate Gyrus: From Structure to Function

The DG is an area of the brain characterized by a large, dense population of glutamatergic granule cells with very sparse activity (Chawla et al., 2005; Danielson et al., 2016; Jung and McNaughton, 1993). It is a major input region to the hippocampus and is therefore thought to play an essential role in learning, episodic memory, and spatial navigation tasks associated with that structure. DGCs receive their primary input from perforant path fibers originating in layer II of both lateral entorhinal cortex (LEC) and medial entorhinal cortex (MEC). In addition, they receive commissural inputs from the contralateral hippocampus, diverse neuromodulatory afferents, most notably cholinergic input from the septum, dopaminergic inputs from the midbrain (Du et al., 2016), feedback inputs from CA3 (Vivar et al., 2012), glutamatergic inputs from mossy cells, and inhibitory inputs from interneurons in the hilus, as well as granule and molecular layers. One striking anatomical feature of the DG is the fact that it contains significantly more principal neurons than its input or output regions. The rat DG is composed of around 1 million DGCs, whereas layer II of the EC has ~0.11 million and CA3 has ~0.25 million principal cells. Several theoretical studies have associated this disparity in dimensionality of coding and the low firing probability of DGCs with a putative function in discriminating between similar yet different experiences—a task equivalent to the computational concept of pattern separation (O'Reilly and McClelland, 1994; Treves and Rolls, 1994). This hypothesis is supported by studies of hippocampal lesions and manipulations of the electrophysiological properties of DGCs.

Since DGCs have very low firing probabilities, only a small population of DGCs is activated by these inputs at any given time, resulting in sparse representation of contexts and events. The sparseness of these DG representations—also known as "engrams"—is thought to be crucial for creating non-overlapping (or orthogonal) responses to different experiences, thereby keeping memories distinct. The sparseness of coding in the DG is largely due to strong inhibitory inputs from interneuron populations in the DG and hilus, including chandelier cells and the so-called MOPP (molecular layer perforant path-associated) cells (Li et al., 2013b) in the molecular layer, as well as basket cells in the subgranular layer, HIPP (hilar perforant path-associated) cells, and HICAP (hilar commissural-associational pathway-related) cells in the hilus. Remarkably, only ~2% of DGCs respond when exposed to any given context, as recorded by IEG expression (Chawla et al., 2005), and a recent study has found that the size of neuronal representations (cell ensembles) depends heavily on lateral inhibition from somatostatin-expressing interneurons in the hilus, which tend to be primarily HIPP cells (Stefanelli et al., 2016). Optogenetic stimulation of DGCs active during a context-dependent fear-conditioning task is sufficient to elicit a fear memory (Liu et al., 2012). Yet, it is unclear whether the re-activation of this neuronal population occurs during, and is necessary for, natural memory recall. A study using IEG expression as a proxy for neuronal activity found no preferential re-activation of DG neurons upon re-exposure to the conditioned context, whereas CA1 neurons preferentially reactivated during the same recall test (Deng et al., 2013); on the other hand, optogenetically silencing DG or CA3 cells activated during memory encoding prevented memory recall (Denny et al., 2014).

Other studies have found that the DG is involved in memory retrieval, but not in memory recall; however, it may be difficult to disentangle these two processes. Sparse representations in the DG are relayed to CA3, a hippocampal area characterized by recurrent connections that is hypothesized to function as an auto-association network; it has been shown to play a role in pattern completion, i.e., to recall a memory upon an incomplete cue or only a partial activation of its neuronal representation. However, CA3 is also thought to be able to perform pattern separation, and whether it performs one or the other task seems to depend on the input it receives directly from the EC and, particularly in the case of pattern separation, from the DG. In this manner, the hippocampal memory system is thought to have the flexibility to implement pattern separation and pattern completion, both essential functions for episodic memory formation and recall: the former permits generalization and recall from incomplete inputs, whereas the latter ensures that similar memories are kept distinct from each other. Disrupting DG function results in a decrease in the context specificity of CA3 activity, i.e., a shift from pattern separation to pattern completion (McHugh et al., 2007). Electrophysiological recordings of DG activity are difficult due to the high density of neurons in the granule layer and their low firing rates. Single-unit extracellular recordings have confirmed that DGCs have low firing rates (most cells have mean rates below 0.5 Hz and as low as 0.01 Hz) and exhibit stable place fields—that is, cells fired with high spatial selectivity with respect to the environment the animal moved in (Jung and McNaughton, 1993). Another study has found that small changes in the environment explored by rats resulted in large changes in the firing patterns and correlations of DGCs even when grid fields recorded from the perforant path did not change their firing rates (Leutgeb et al., 2007). These findings further confirmed the role of the DG in pattern separation. The involvement of DG and CA3 in pattern separation has been confirmed in humans by MRI studies done in conjunction with a visual memory task, where subjects were presented with images of

common objects. BOLD (blood-oxygen-level-dependent) fMRI responses in DG/CA3 were similar when an image was presented to test subjects for the first time and when highly similar images were presented. A different pattern of activity was seen in DG/CA3 upon presentation of a repeated image, but not in CA1 or EC, indicating that the latter areas do not have the same sensitivity to small differences (Bakker et al., 2008).

### Functional Role of Adult-Born Neurons

A better understanding of the contribution of adult neurogenesis to learning and memory has evolved side-by-side with our understanding of DG function. Adult-born neurons are unlikely to influence behavior before they integrate DG networks, fire action potentials, and establish synapses, but their presence is thought to be particularly impactful between the fourth and sixth weeks post-mitosis, as they undergo a period of increased excitability and plasticity (see above). New neurons eventually mature to the point where their properties are similar to those of other DGCs; they are unlikely to have a unique impact on behavior at this stage, as evidenced by the fact that optogenetically silencing newborn neurons at 4 weeks, but not at 2 or 8 weeks, could impair hippocampal memory retrieval (Gu et al., 2012). Early studies of the function of neurogenesis have some discrepancies and sometimes even contradictory findings that can, in hindsight, be attributed to these factors. Likewise, computational modeling of the effects of adult neurogenesis on hippocampal function has generated different theories for the role of newborn neurons. These include encoding of temporal information into memories (Aimone et al., 2006; Becker and Wojtowicz, 2007), avoidance of memory interference and cognitive flexibility during learning of new tasks (Chambers et al., 2004), and balancing pattern separation/integration (Aimone et al., 2009). While there is evidence for many of these functions, consensus on a unified theoretical framework for adult neurogenesis has not been reached and will likely require more experimental data. It might also be the case that adult-born neurons perform distinct functions in the DG depending on the environmental inputs and cognitive demands present during maturation. Experience during this early maturation period changes the timing of the integration of neurons into hippocampal networks and shapes their connectivity (Bergami et al., 2015; Gonçalves et al., 2016; Piatti et al., 2011; Zhao et al., 2006). It is therefore conceivable that distinct demands and distinct connectivity can result in distinct functions for adult-born neurons.

Reducing the number of newborn cells has been found to result in specific cognitive deficits. Spatial memory was affected in many instances, in particular long-term memory retention in the Morris water maze test. Context-dependent memory, and specifically performance in contextual fear conditioning tasks, was also found to depend on neurogenesis (Ko et al., 2009; Saxe et al., 2006; Tronel et al., 2012). There is also some evidence that newborn neurons may be involved in reducing interference between memories that occur at different times (Rangel et al., 2014). Yet another proposed distinct function for adult neurogenesis is cognitive flexibility, that is, the ability to adopt new strategies to successfully complete a previously learned task when contingencies change, such as when a familiar cue no longer indicates the position of the platform in the Morris water maze. However, it can also be argued that this function is a manifestation of improved spatial memory and contextualization.

Several studies have associated adult neurogenesis in the DG with improved performance in pattern-separation behavioral tasks. Pattern separation is defined at a computational level as a process that produces differentiated outputs from similar inputs—in the case of memories, by reducing the overlap in their representations. However, several brain areas may contribute to pattern separation, and in practice, it is impossible to fully characterize memory representations in the brain, thus rendering it impossible to fully characterize the inputs and outputs of the circuits involved in pattern separation. What is possible to record is the behavioral output of mice that attempt to discriminate similar contexts or stimuli. Therefore, these tasks are referred to as "behavioral" pattern-separation tasks, or more specifically as spatial, temporal, or odor pattern separation, depending on the nature of the task. Bussey and collaborators showed that mice with permanently reduced neurogenesis following focal X-ray irradiation or blocking Wnt activity had impaired performance in two tests of spatial pattern separation: a navigational radial arm maze task and an operant chamber, touch screen-based memory task (Clelland et al., 2009). In both cases, mice with ablated neurogenesis had difficulty performing the task at hand when the spatial separation between choices was low, although performance was not reduced when the choices were spatially further apart. Other studies of context discrimination and pattern separation in mice with ablated or silenced newborn neurons had concurring findings (Nakashiba et al., 2012). Similarly, mice with increased neurogenesis, either through behavioral interventions (exercise, enriched environment) or by genetically enhancing the survival of new neurons, performed better in contextual fear-conditioning tasks that required distinguishing between similar environments (Clemenson et al., 2015; Sahay et al., 2011).

Although it is difficult to quantify or manipulate the amount of neurogenesis in human subjects, human neurogenesis is known to decrease with age, as it does in rodents (Spalding et al., 2013). MRI studies in rodents and monkeys have found that cerebral blood volume (CBV) in the DG, a correlate of neurogenesis, is particularly sensitive to aging and is specifically increased by exercise in both rodents and humans (Small et al., 2004). Behavioral pattern separation in humans was shown to undergo an age-related decline in performance, reminiscent of the decay in neurogenesis in the DG (Stark et al., 2010). Interestingly, the ability to recall previously seen images did not vary with age, as older test subjects only had difficulty identifying pictures similar to those they had previously seen. Repeat presentations of the same pictures were easily recognized across age groups, indicating that aging affects pattern-separation tasks, but not recognition memory. Moreover, fMRI studies have shown that the CA3/DG requires a higher degree of dissimilarity in order to display the activity signature of exposure to a novel experience, reflecting impaired pattern separation. The field would greatly benefit from more direct in vivo measurements of neurogenesis in humans. One promising approach came from a report describing the use of magnetic resonance spectroscopy biomarkers for quantifying NSCs and NPCs in human subjects

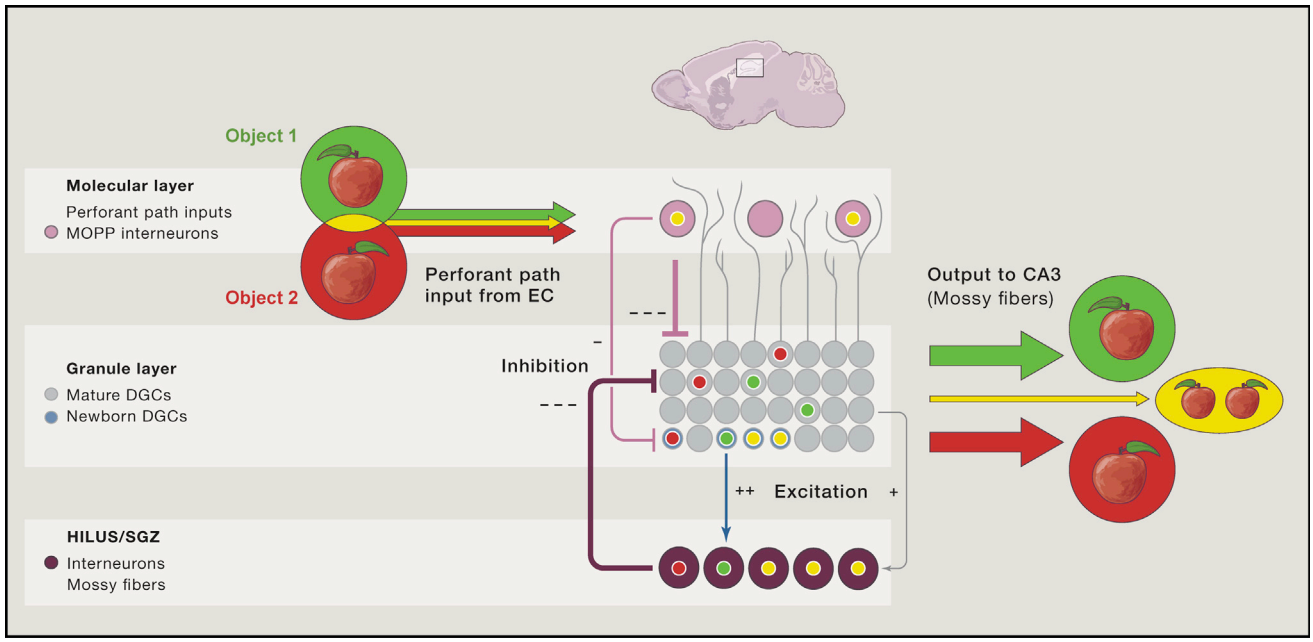



**Figure 5. Connectivity of Adult-Born DGCs Potentially Enhances Pattern Separation through Feedback Inhibition**

Memories of similar objects or events are thought to be encoded by separate but partially overlapping populations of activated DGCs (red and green, with overlap in yellow), here exemplified by a recall task where the subject is asked to identify which of two images is novel. In this example, the two apples differ only in their green leaves. The more similar the perforant path inputs from the EC, the greater the overlap of their representations in the DG. Mature DGCs (gray) receive strong inhibitory inputs from interneurons (purple) in the hilus, molecular, and sub-granular zones (denoted by – – –). Immature adult-born DGCs (blue) are more active than mature DGCs (gray) due to their intrinsic properties and reduced inhibitory inputs (denoted by –). However, the firing of immature neurons is also thought to strongly enhance feedback inhibition from hilar interneurons, resulting in overall sparser DG responses and, consequently, a decreased overlap of memory representations. Therefore, although the responses of newborn DGCs are less discriminating, with a large overlap between representations, they are thought to enhance pattern separation by minimizing the overlap between object representations of their mature counterparts. These representations are then relayed to CA3 through the mossy fiber outputs. Most mossy fibers respond to only one of the images (red and green arrows), although some, primarily those of newborn neurons, fire in response to both (yellow arrow).

(Manganas et al., 2007), although the results were controversial, indicating that this method may need further refinement before gaining widespread acceptance.

Despite the evidence for a function of adult neurogenesis in behavioral pattern separation, the exact mechanism through which newborn cells enhance DG function is still not known. Immature neurons are more excitable and hence will respond to a broader range of stimuli. It is therefore paradoxical that they would contribute to behavior pattern separation, a function that supposedly requires non-overlapping, finely tuned neuronal responses. However, some studies of DG activity have found that newborn cells contribute to a decrease in DG network activity (Ikrar et al., 2013; Lacefield et al., 2012). This reduction in activity makes DG responses more sparse, which would be advantageous for behavioral pattern-separation tasks. There is substantial evidence that reducing neurogenesis leads to a decrease in inhibition in the DG, whereas increasing neurogenesis leads to the activation of interneurons and sparsification of DG representations (Drew et al., 2016; Singer et al., 2011). Therefore, it appears that one way that immature neurons may contribute to behavioral pattern separation is by modulating feedback inhibitory circuits in the DG so that fewer DGCs, and in particular fewer mature DGCs, respond to incoming stimuli (Figure 5). Nevertheless, further work is needed to characterize the circuits involved in this process and quantify their net effect on DG excitability; experiments in hippocampal slices suggest that immature adult-born neurons are poorly coupled to inhibitory neurons in the DG and hilus (Temprana et al., 2015) while being subject to feedback and feedforward inhibitory inputs themselves (Dieni et al., 2016; Li et al., 2012, 2013b). Moreover, recent work has shown that newborn neurons transiently form strong connections to inhibitory circuits in CA3 (Restivo et al., 2015). Taken together, these data indicate that the inhibitory networks associated with developing adult-born DGCs are complex and dynamic. Ultimately, increases in neurogenesis have been predicted to result in the elimination of more distant memories, either through increased inhibition of mature DGCs, degradation and interference of very sparse representations, or simply competitive rewiring of DG outputs. Interestingly, there is experimental evidence that this elimination of memories may be true (Akers et al., 2014), but forgetting and increased pattern separation may be hard to differentiate experimentally: any small change to a conditioned stimulus will cause it to be perceived by the animal as a novel stimulus, instead of triggering a memory (recall).

Monitoring the activity of DGC populations during behavioral tasks will likely provide invaluable information for understanding

the effects of newborn neurons in hippocampal function. Electrophysiological recordings in the DG have not been able to distinguish between mature and immature DGCs, but a recent study has succeeded in recording the activity of genetically labeled newborn and mature neurons using in vivo calcium imaging (Danielson et al., 2016). As expected, immature adult-born DGCs were found to be more active than their mature counterparts. By allowing mice to run under head fixation on a long treadmill with multisensory spatial cues, the authors were able to determine that mature neurons had higher spatial selectivity than newborn cells and underwent remapping of their representations. Optogenetic inactivation of immature DGCs resulted in impaired contextual discrimination, consistent with previous behavioral studies where neurogenesis was ablated and consistent with a role for adult-born cells in behavioral pattern separation. One potential caveat with some methods for imaging the DG in vivo (Danielson et al., 2016; Gonçalves et al., 2016) is that they require the removal of a part of CA1, which could disrupt parts of the circuitry of the hippocampus, but developments in imaging technology seem to have circumvented this limitation and will hopefully soon enable the imaging of multiple hippocampal subfields with minimal damage to brain tissue (Pilz et al., 2016).

## Adult Neurogenesis and Diseases of the CNS

Studies aimed at detecting neurogenesis in humans have shifted from merely questioning the existence of neurogenesis to exploring the contribution that adult-born cells make to the function of the human brain in health and disease. However, most of these studies have been based on indirect methods due to the lack of tools to directly observe adult neurogenesis in living humans. In the past, this approach has limited the field to correlating impaired functions from disease states with alterations in adult neurogenesis. Nevertheless, understanding what role adult neurogenesis plays within a disease state, or which consequences arise from its involvement, may help to reveal some basic principles of its physiological functions. To date, there is no clinical evidence of an isolated impairment of adult hippocampal neurogenesis in the absence of other abnormalities, but numerous studies have reported alterations in adult neurogenesis that are associated with several neurological and psychiatric disorders, providing a link between adult neurogenesis and human disease. In some cases, these alterations in neurogenesis are thought to contribute to disease symptoms and even to accelerate disease progression. A possible reason for this is that newborn neurons are thought to account for a disproportionally large fraction of DG activity. In addition, they may regulate DGC firing through feedback inhibition and, since DG responses are so sparse, even small differences in the activity can have a significant impact. In this section, we will briefly discuss the effects of a few pathological conditions on adult neurogenesis in rodent models and human patients, and we refer the reader to further literature for more detail.

Alterations in adult neurogenesis and a reduced size of the hippocampus have been reported for most psychiatric disorders, including schizophrenia, major depression, addiction, and anxiety. A significant subpopulation of patients with major depression, for example, was shown to have a reduced hippocampal volume and cognitive defects. It has been proposed that depressive disorders might be caused by impaired adult hippocampal neurogenesis, partially because of the observation that antidepressants and depressive phenotypes affect levels of SGZ neurogenesis (Miller and Hen, 2015). Moreover, neurogenesis was found to be required for many of the behavioral effects of antidepressants (Santarelli et al., 2003). Evidence from human studies supported the observations made in rodent models; however, limitations in study design and the lack of comprehensive tools highlight the need for further validation to provide evidence for a neurogenic cause of depression.

Schizophrenia is a complex genetic disorder that has variable affective symptoms and cognitive deficits. Several studies have implicated an impairment in adult hippocampal neurogenesis as part of the pathology. Furthermore, several candidate genes have been suggested to play critical roles in adult neurogenesis. Ablation of DISC1, one of the best-characterized susceptibility genes, results in reduced levels of hippocampal neurogenesis, altered morphogenesis, and granule cell positioning, as well as impaired hippocampus-dependent behavior in rodents (Duan et al., 2007; Kvajo et al., 2008). However, DISC1 mutations are not unique to schizophrenia; they are also risk loci for major depression and bipolar disorder. Uncertainty remains about the actual disorder that is being modeled by the DISC1 mutant mouse.

Aberrant neurogenesis is also thought to contribute to mesial temporal lobe epilepsy (mTLE), the most common form of epilepsy in adults. There is evidence that mTLE may be triggered by an increase in neuronal excitability in the DG, in what is known in the field as the dentate gate hypothesis. Seizure activity increases adult neurogenesis but also results in aberrant migration, morphology, and connectivity of newborn cells (Parent et al., 1997). These cells frequently extend projections to the granule layer of the DG and are thereby thought to contribute to an increase in excitability, thus aggravating the disease.

Several neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD) and Huntington's disease (HD), have also been associated with alterations in adult neurogenesis (reviewed in Winner and Winkler [2015]). Mouse models of PD were found to have decreased neurogenesis, primarily due to an increase in cell death. While mouse models of AD were also found to have altered neurogenesis, these changes were not consistent and depended on the type of model, age of the animal, and other factors. Interestingly, knockin mice for the Apolipoprotein E4 (ApoE4) isoform had reduced neurogenesis due to a disruption of GABAergic inputs essential for newborn neuron maturation. These defects, as well as the associated deficits in learning and memory, could be rescued by the transplantation of hilar inhibitory interneurons (Tong et al., 2014). Neurogenesis was also reduced in models of HD due to decreased proliferation of neuronal progenitors, although no defects were found in neuronal differentiation (Lazic et al., 2004).

In vitro disease modeling using induced pluripotent stem cell (iPSC) technology has provided new possibilities for modeling human diseases in a dish. Recent advances in mimicking the region-specific sequence of developmental signaling pathways have led to an in vitro model for human DGC neurogenesis (Yu et al., 2014). This in vitro system has recently been used to model

mental disorders such as schizophrenia and bipolar disorder (Mertens et al., 2015; Yu et al., 2014).

## Conclusions and Future Directions

The last decade has seen tremendous progress in our understanding of the processes underlying adult neurogenesis and its function in the mammalian brain. Adult-born neurons have been found to contribute to learning and memory in rodents, and there are indications that they may fill a similar role in humans. There is still no consensus as to the exact functional contributions of adult-born DGCs, and it is possible that their role is highly adaptive to cognitive demands, especially since newborn neurons undergo a period of extraordinary plasticity as they mature. Nevertheless, it has become widely recognized that the DG is involved in behavioral pattern-separation tasks, and a growing body of research suggests a role for adult-born neurons in supporting this function. Perhaps the most remarkable feature of adult neurogenesis is that it produces a constant turnover of neurons with unique immature properties. These neurons respond to environmental cues through complex molecular regulatory networks and therefore bear the indelible mark of the environment they mature in. Due to their higher excitability, they are likely to have a significant impact on DG activity, despite their low numbers. Whether they play a role after full maturation remains unknown, but memories are hippocampus-dependent for a relatively short period, anyway, before being consolidated to other brain areas. It is therefore possible that the short-lived critical period of newborn neurons contributes to disambiguating or linking memories of events that occur during this time.

Recent technological developments will drive the next discovery wave of the mechanisms behind the proliferation, differentiation, and function of adult-born neurons. In vivo imaging techniques will likely provide invaluable information about adult NSC exit from quiescence and proliferation and will also enable activity recordings from large populations of identifiable DGCs during behavioral tasks. Optogenetics and engineered receptors now allow the silencing or activation of adult-born neurons in a specific and acute manner, with minimal effects on other cells and without triggering compensatory mechanisms that could otherwise mask the true contribution of newborn neurons to hippocampal function. Progress has also been made in the study of human adult neurogenesis. A recent report confirmed earlier findings of neurogenesis in humans and estimated rates of neuronal birth and death by measuring the $^{14}C$ content of genomic DNA in neurons from post-mortem tissue (Spalding et al., 2013). Additionally, MRI data and cognitive testing have advanced our understanding of human DG function while suggesting a possible correlation between neurogenesis and behavioral pattern separation in humans, but new approaches are needed for quantifying neurogenesis in human subjects in vivo, even if through indirect or correlative measurements. Recent efforts in this direction, for example, using PET imaging (Tamura et al., 2016), hold significant promise. Finally, advances in the analysis of gene expression have already provided a new insight into the molecular mechanisms and signaling cascades involved in the differentiation and functional activation of individual newborn neurons. These advances will foreseeably lead to new potential therapeutic targets for stimulating neurogenesis and modulating the activity of adult-born DGCs, and they may eventually contribute to future regenerative approaches for treating neurological disease.

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