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Watching Synaptogenesis in the Adult Brain

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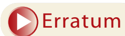

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Key Words

synapse development, synaptic plasticity, activity-dependent synaptic formation, olfactory bulb, dentate gyrus

Abstract

Although the lifelong addition of new neurons to the olfactory bulb and dentate gyrus of mammalian brains is by now an accepted fact, the function of adult-generated neurons still largely remains a mystery. The ability of new neurons to form synapses with preexisting neurons without disrupting circuit function is central to the hypothesized role of adult neurogenesis as a substrate for learning and memory. With the development of several new genetic labeling and imaging techniques, the study of synapse development and integration of these new neurons into mature circuits both in vitro and in vivo is rapidly advancing our insight into their structural plasticity. Investigators' observation of synaptogenesis occurring in the adult brain is beginning to shed light on the flexibility that adult neurogenesis offers to mature circuits and the potential contribution of the transient plasticity that new neurons provide toward circuit refinement and adaptation to changing environmental demands.

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OB: olfactory bulb
DG: dentate gyrus

INTRODUCTION

Adult neurogenesis in mammals was first described in the early 1960s (Altman & Das 1965), but it was not until much later that investigators broadly accepted that the addition of new neurons occurs throughout life in both the olfactory bulb (OB) (Lois & Alvarez-Buylla 1993, Luskin 1993) and the dentate gyrus (DG) (Bayer 1980, Bayer et al. 1982, Gage et al. 1995). Although most neurons in the brain are added to immature circuits during assembly, neurons generated in adulthood face an additional challenge as they integrate into mature, fully functional circuits. Relatively little is known about the mechanisms that regulate the synaptic development of adult-born neurons and their connectivity within mature circuits. This review aims to present key aspects of the emerging understanding of synaptogenesis in adult-born neurons, as well as how activity in the brain modulates this process.

Synapse formation during adult neurogenesis raises several intriguing questions. Does synapse formation in adult-born neurons simply recapitulate the steps that occur during embryonic and neonatal development, or is it regulated by specific mechanisms specialized for integration into functioning circuits? How do new neurons make synapses with mature circuits without disrupting existing connectivity? An understanding of synaptogenesis in the adult brain will not only shed light on the putative function of adult neurogenesis in information processing and storage, but also provide new insights to develop strategies for successful neuronal replacement therapies to treat brain injury and neurodegenerative conditions.

We begin this review by discussing some of the techniques currently being used to study synaptogenesis in adult-born neurons. Next, we proceed to critically examine current literature on how the various types of adult-born neurons develop synaptic connections with their respective circuits and how this process is modulated by activity. We also discuss the functional properties of new neurons and their potential contribution toward refining the existing circuit. Finally, we conclude by reflecting on recent trends and discoveries in this dynamic field and exploring future directions toward understanding the integration of new neurons into adult

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circuits and the role of adult-born neurons in brain function.

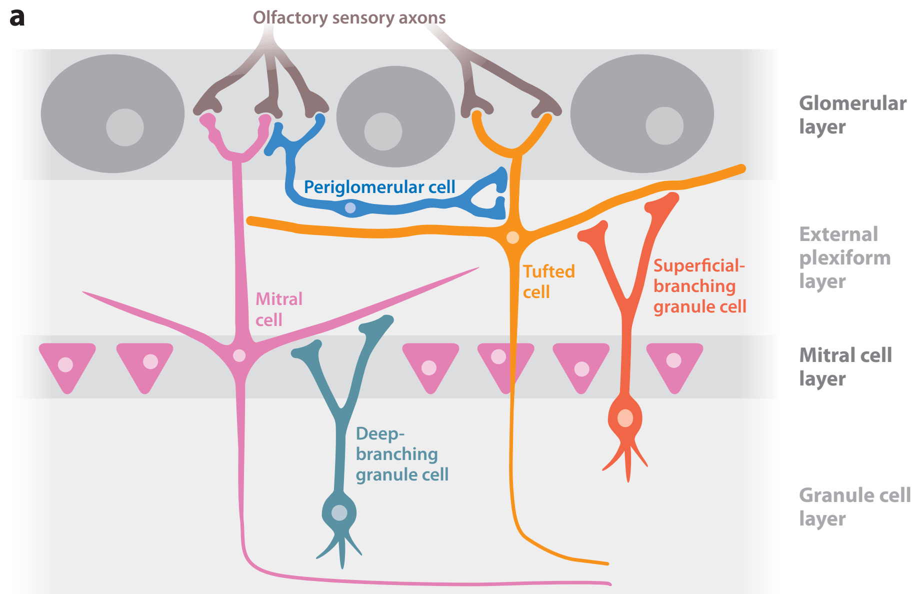
TECHNIQUES FOR OBSERVING SYNAPTOGENESIS

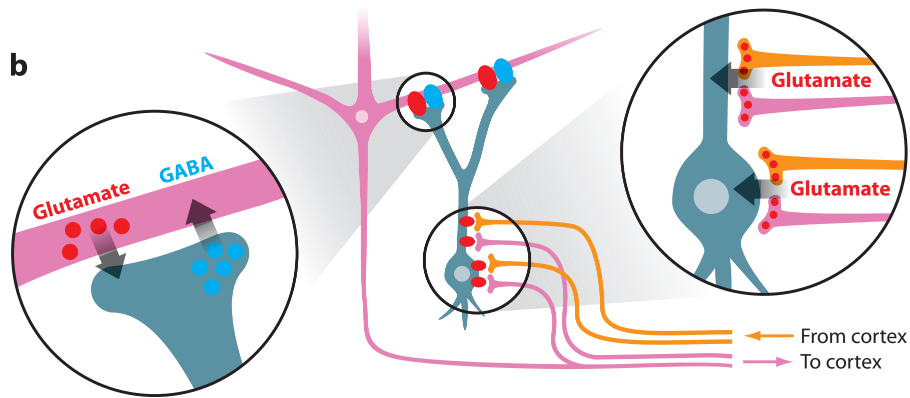
Recent technical advances have accelerated the study of synapse formation in adult neurogenesis. In particular, two genetic methods that facilitate the selective labeling of new neurons with fluorescent proteins have been especially useful. First, oncoretroviral vectors can be used to label new neurons genetically (Jessberger et al. 2007, Kelsch et al. 2007), as they exclusively infect actively dividing cells, such as neuronal progenitors, but are unable to infect postmitotic cells, such as neurons (Roe et al. 1993). Second, investigators have developed several transgenic mouse lines that enable selective labeling of adult-born neurons. In two of these transgenic lines, expression of a fluorescent protein, either green fluorescent protein (GFP) or red fluorescent protein (dsRed), is driven by promoters that are active only in immature neurons, namely doublecortin (Wang et al. 2007) and nestin (Mignone et al. 2004). This process results in specific labeling of immature neurons in both the OB and the DG (Brown et al. 2003, Yamaguchi et al. 2000). In another line, the proopiomelanocortin (POMC) promoter drives GFP expression, which, unexpectedly, labels new neurons in the DG (Overstreet et al. 2004).

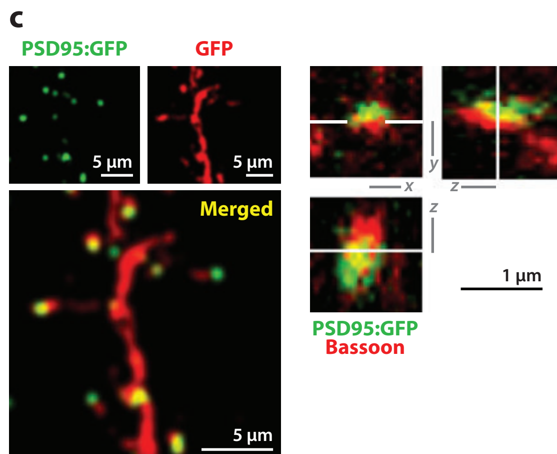
Genetic Labeling of Synapses of Adult-Born Neurons

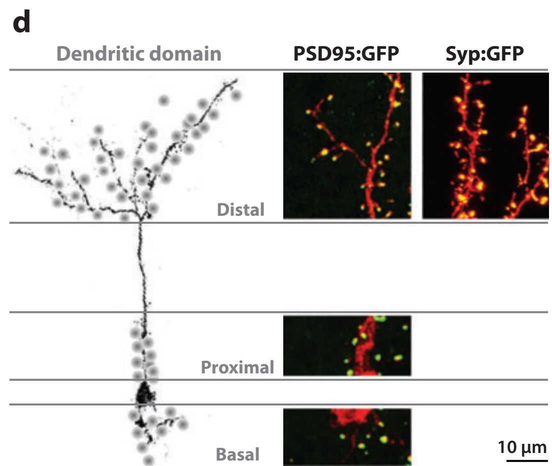
Synapse formation has traditionally been studied in three main ways. First, in some cases, synapses can be easily identified on the basis of their association with neuronal structural specializations. For instance, many excitatory input synapses are located in dendritic spines, in which case spines may be used as a morphological substitute for synapses. One limitation of this method is that a large proportion of synapses, such as excitatory input synapses on cell somata and inhibitory synapses, are simply not associated with spines (Price & Powell 1970, Woolf et al. 1991). In addition, it is not possible to accurately quantify the density and measure the size of output synapses by simple morphological analyses. Second, antibody labeling against synaptic markers is a powerful method to label synapses in cultured neurons. However, this method is suboptimal in brain sections because the large number of synapses present severely complicates the attribution of synapses to individual new neurons. Emerging imaging techniques, however, may be able to overcome some of these problems soon (Micheva & Smith 2007). Third, synapses can be unambiguously identified by electron microscopy, but this technique is labor intensive and has yet to be sufficiently developed for high-throughput analysis.

Labeling synapses with genetically encoded markers addresses some of the limitations of the above-mentioned techniques and significantly simplifies the quantification of synaptic development in new neurons (Kelsch et al. 2008, Livneh et al. 2009, Meyer & Smith 2006, Niell et al. 2004). The visualization of pre- and postsynaptic terminals can be achieved via expression of fluorescent proteins fused to proteins specifically located in synapses. For instance, synaptophysin is a protein located in neurotransmitter vesicles that is selectively localized at presynaptic terminals (Sudhof & Jahn 1991) and can be used to identify release sites on axon terminals (**Figures 1*d*, 2*b***). To identify postsynaptic terminals, PSD95, a scaffolding protein restricted to clusters in the postsynaptic density of most glutamatergic synapses (Ebihara et al. 2003, Gray et al. 2006, Niell et al. 2004, Sassoe-Pognetto et al. 2003, Sheng 2001, Washbourne et al. 2002), can be used (**Figures 1*c*,*d* and 2*c***). When introducing these genetically encoded markers, it is critical to ensure that only modest levels of overexpression are achieved because excessive levels of these proteins may interfere with synaptic development or function (El-Husseini et al. 2000). Fortunately,









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retroviral vectors, which deliver single copies of transgenes into their target cells, produce sufficiently low levels of expression that leave synapse number and strength unperturbed (Kelsch et al. 2008). With genetically encoded markers, one can, in principle, analyze the complete set of a neuron's excitatory input synapses and output synapses, including those not associated with spines or axon terminals, respectively. This method also allows for software-aided quantification of synapses (Kelsch et al. 2008). Furthermore, viral vectors can be engineered such that in addition to synaptic markers other genes of interest, such as those coding for ion channels, growth factors, or cell adhesion molecules, can be introduced in the same vector to assess the effects of various manipulations on synapse formation and dynamics.

The labeling methods mentioned above allow investigators to observe synapse development in combination with a variety of imaging and recording techniques. Here we briefly discuss the strengths and limitations of four major techniques currently used in the field.

Two-photon laser scanning fluorescence microscopy in vivo. Two-photon microscope technology has taken off considerably in recent years and is still the only technique that allows for synapse imaging in vivo. This technique is extremely useful for observing real-time changes to experimental manipulations and allows investigators to visualize synapse dynamics. Owing to detection limits, only neurons up to ~800 μm below the brain's surface can be imaged (Helmchen & Denk 2005), largely restricting this technology to the study of adult-born periglomerular neurons (PGNs) in the glomerular layer of the OB and distal dendrites of OB granule neurons, which are located in the external plexiform layer (**Figure 1*a***) (Mizrahi 2007). By removing part of the neocortex and white matter above the hippocampus, superficial dendrites of neurons in CA1 of the hippocampus can be imaged with two-photon technology (Mizrahi et al. 2004). However, adult-born granule cells in the DG cannot be imaged in the same manner without damaging a substantial part of the hippocampus. There

PGN: periglomerular neuron

Figure 1

Adult-born olfactory bulb granule cells and their synaptic wiring with the surrounding circuit. (*a*) Synaptic organization in the olfactory bulb. In the glomeruli of the olfactory bulb, olfactory sensory axons synapse on the apical dendrites of principal neurons, the mitral and tufted cells, as well as on periglomerular neurons (PGNs), which line these glomeruli. PGNs also form additional synaptic connections with the apical dendrites of principal neurons, whereas the lateral dendrites of principal neurons form synapses with granule cells (GC_{OB}). Two independent microcircuits may exist in the olfactory bulb, with GC_{OB} with either deep or superficial dendritic branching connecting exclusively to mitral or tufted cells, respectively. (*b*) Synaptic connectivity of olfactory bulb granule neurons. GC_{OB} form dendro-dendritic synapses with lateral dendrites of principal neurons in the bulb. These atypical synapses consist of a glutamatergic input synapse from the principal neuron onto the GC_{OB} and a GABAergic output synapse onto the same lateral dendrite of the principal neuron, both located in a single spine. In addition, GC_{OB} receive glutamatergic inputs onto their basal and proximal apical dendrites from centrifugal cortical axons and possibly also from axon collaterals of principal neurons. GC_{OB} are also contacted by GABAergic input synapses from local interneurons in the olfactory bulb as well as cholinergic and monoaminergic inputs. (*c*) Genetic labeling of synapses. *Left*: Progenitors of GC_{OB} were infected in the subventricular zone (SVZ) with retroviral vectors carrying genetic constructs encoding PSD95:GFP, a marker for postsynaptic glutamatergic sites, to generate PSD95:GFP-expressing GC_{OB}. PSD95:GFP-positive clusters can be detected by direct visualization of GFP (*shown as green puncta*). The dendritic morphology of the GC_{OB} was revealed by amplifying the low levels of PSD95:GFP in the cytoplasm (that could not be detected by intrinsic fluorescence) by immunostaining against GFP with a red fluorescent secondary antibody. The merged images of PSD95:GFP positive clusters (*green*) and dendritic morphology (*red*) allow investigators to attribute clusters to specific dendritic domains of individual GC_{OB} (scale bar, 5 μm). *Right*: Confocal three-dimensional image showing a PSD95:GFP positive cluster in a new GC_{OB} that is contacted by the presynaptic marker, bassoon (scale bar, 1 μm). (*d*) Synaptic distribution in the dendritic domains of granule cells. GC_{OB} have a basal dendrite and an apical dendrite, which consist of proximal and distal synaptic domains. The proximal domain is a specialized sector of the unbranched apical dendrite that emerges directly from the soma of GC_{OB}, which contains a high density of glutamatergic input synapses. The branched dendritic segment of the apical dendrite is known as the distal domain. Examples of genetic labeling of synapses in the dendritic domains of granule cells are given for postsynaptic glutamatergic synapses, PSD95:GFP, and for the presynaptic genetic marker, Synaptophysin:GFP (Syp:GFP).

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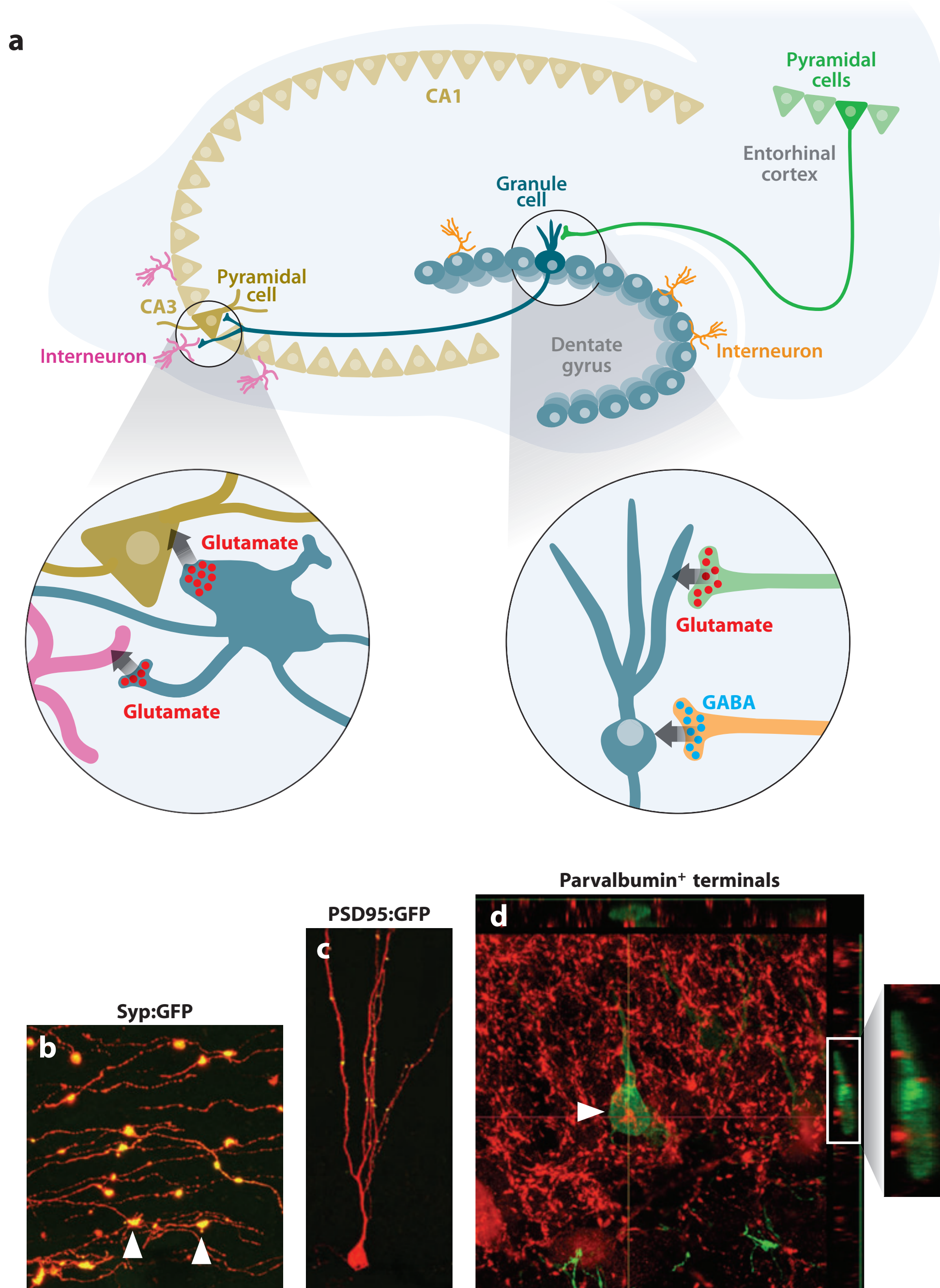
has been great interest in using two-photon microscopy associated with endoscope lenses to image deep within the brain, but several technical obstacles need to be solved before this method can be routinely used (Barretto et al. 2009).



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Confocal laser scanning microscopy. Because deep structures of the brain, such as the DG where adult-born granule cells are added, are beyond the depth limitation of two-photon microscopy in vivo, in vitro time-lapse confocal imaging of brain slices is sometimes carried out to study the dynamics of synapse formation (Galimberti et al. 2006, Toni et al. 2007). This technique is not widely used because of concerns about the integrity of cultured adult brain slices over long time periods with currently available culture techniques (Berdichevsky et al. 2009), as well as the possibility of abnormal synapse rearrangement due to fluctuations in culture conditions (Kirov et al. 2004). Confocal imaging of fixed slices is much more commonly used to study synaptogenesis, especially because this method of observation is technically straightforward and enables investigators to analyze many neurons at one time. Time course experiments can be performed to observe spine formation over days and months, but because only an instantaneous snapshot of the synapses can be obtained in fixed slices, this technique cannot be used to analyze the short-term dynamics of synapse formation in real time.

Electron microscopy. Electron microscopy is more technically challenging than confocal imaging but allows for simultaneous analysis of pre- and postsynaptic sites by visualizing synaptic vesicles and postsynaptic sites, respectively. Electron microscopy can also be used for three-dimensional high-resolution analysis of individual synapses on adult-born neurons and their synaptic partners (Toni et al. 2007). Recent developments within the past few years hint at the possibility of semiautomated sectioning and imaging of large neuropil volumes (Briggman & Denk 2006), thus facilitating high-throughput ultrastructural analyses of synapses.

Electrophysiology. Electrophysiological recording provides a functional confirmation of structural observations in studies of adult-born neurons. The frequency and amplitude of excitatory and inhibitory synaptic inputs help scientists understand changes in connectivity of new neurons during their maturation and the effects of diverse manipulations. Carleton et al. (2003) and van Praag et al. (2002) have used electrophysiology to describe synaptic properties of new neurons as they mature and integrate into their circuits. One of the most significant contributions of electrophysiology to the study of adult neurogenesis is the demonstration that new neurons display enhanced synaptic plasticity compared with fully mature neurons in both the OB and the DG (Nissant et al. 2009, Schmidt-Hieber et al. 2004). Scharfman et al. (2000) also used electrophysiological methods

Figure 2

Adult-born granule cells in the dentate gyrus and their synaptic wiring with the hippocampal and other circuits. (*a*) Synaptic organization of dentate granule cells. Adult-born granule cells in the dentate gyrus (GC_{DG}) receive excitatory glutamatergic input onto their apical dendrites from projection neurons in the entorhinal cortex and mossy cells in the hippocampus, as well as inhibitory GABAergic input from local interneurons. GC_{DG} project axons solely to the CA3 region of the hippocampus. At the CA3 region, these axons constitute two forms of specialized contact sites: large mossy terminals and en passant boutons. Large mossy terminals are compartmentalized release sites: The central portion of these terminals forms complex interdigitating synapses with proximal dendrites of CA3 pyramidal cells while the emanating filopodia of the terminals synapse on GABAergic interneurons in CA3. En passant boutons are smaller synaptic swellings along the axon collaterals that exclusively contact CA3 interneurons. (*b*) Genetic labeling of output synapses along axon collaterals of dentate granule cells. The release sites from GC_{DG} onto CA3 neurons, at large mossy terminals (*arrowheads*) and en passant boutons on the axons of adult-born GC_{DG}, can be visualized by a genetic presynaptic marker, synaptophysin:GFP (Syp:GFP, *yellow*). (*c*) Genetic labeling of input synapses in the apical dendrites of granule cells. Adult-born GC_{DG} develop glutamatergic input sites as visualized by PSD95:GFP (*yellow*) in their apical dendrite during their differentiation. Note the absence of PSD95:GFP-positive sites on the soma. (*d*) Identification of inhibitory innervation on the soma of adult-born dentate granule cells. Parvalbumin-positive inhibitory terminals (*red*) contacting the soma of an adult-born GC_{DG} (*arrowhead*) can be visualized by immunohistochemistry against parvalbumin. *Left*: Confocal image of a GFP-positive GC_{DG} labeled by retroviral infection of progenitors in the subgranular zone of the dentate gyrus. *Right*: Magnified z-stack cross-section of image on left.

GC_{OB}: olfactory bulb granule neurons

GC_{DG}: dentate gyrus granule neurons

to study how different manipulations affect neuronal integration into the adult brain, such as the effects of seizures on synaptic properties of adult-born dentate granule neurons.

SYNAPTOGENESIS IN NEURONS ADDED TO THE ADULT MAMMALIAN BRAIN

The three main types of neurons added to the adult brain are the granule cells (GC_{OB}) and PGNs in the OB and the granule cells in the DG (GC_{DG}). GC_{OB} constitute the largest population of adult-born neurons. They are GABAergic interneurons that connect to the lateral dendrites of the OB's principal neurons (mitral and tufted cells; **Figure 1*a***). PGNs are GABAergic and/or dopaminergic interneurons that modulate incoming information from olfactory sensory axons that connect to the apical dendrites of the olfactory bulb's principal neurons (**Figure 1*a***). Granule neurons in the DG are excitatory neurons that receive input from the entorhinal cortex and project to the CA3 region of the hippocampal formation (**Figure 2*a***).

SYNAPTOGENESIS IN ADULT-BORN OLFACTORY BULB GRANULE NEURONS

Stages of Synaptic Development

Adult-born GC_{OB} arise from neural progenitors in the subventricular zone (SVZ), which lines the walls of the lateral ventricles (Lois & Alvarez-Buylla 1994). Neuroblasts travel long distances via the rostral migratory stream (RMS) into the OB where they migrate radially into the granule cell layer (Lois & Alvarez-Buylla 1994). Of the ~30,000 new neurons produced daily in an adult mouse, more than 97% differentiate into GC_{OB} while the remaining develop into PGNs (Lois & Alvarez-Buylla 1994, Winner et al. 2002).

GC_{OB} are axonless neurons that have a basal and an apical dendrite (**Figure 1*d***). Their apical dendrite is composed of an unbranched segment emerging from the soma followed by a branched segment and can be divided into proximal and distal synaptic domains. The proximal synaptic domain is a region on the unbranched dendrite segment with a high concentration of glutamatergic input synapses. The distal domain consists of the branched dendritic segment and possesses spines containing bidirectional dendro-dendritic synapses, where input and output synapses are colocalized and functionally coupled. These bidirectional synapses receive glutamatergic input synapses from the lateral dendrites of principal neurons and release GABA back onto the same neurons (**Figure 1*b***) (Mori 1987). Dendro-dendritic synapses are the only output of GC_{OB} and are responsible for local inhibition of principal neurons in the OB (Chen et al. 2000, Halabisky & Strowbridge 2003, Mori 1987). The basal dendrite, or basal domain, and unbranched apical dendrite receive glutamatergic input from axon collaterals of the OB's principal neurons and olfactory cortex (Balu et al. 2007, Davis & Macrides 1981, Luskin & Price 1983, Mori et al. 1983).

The developmental stages of adult-born GC_{OB} have been defined according to morphological criteria (Petreanu & Alvarez-Buylla 2002). Stage one neurons are those in the process of migration in the RMS. At stage two, new GC_{OB} reach the granule cell layer and begin to extend their first neurites. At stage three, about ten days after the birth of GC_{OB} in the SVZ, the main dendritic arbor of new GC_{OB} continues to grow and cells start to receive inhibitory synaptic input (Carleton et al. 2003). At stage four, or about two weeks after their birth, new GC_{OB} start receiving excitatory synaptic input (Carleton et al. 2003). In adult-born GC_{OB}, excitatory synapses appear first on the proximal segment of the apical dendrite at this stage (Kelsch et al. 2008). At this time there are few spines and synaptic sites on the distal branches of the apical dendrite (Petreanu & Alvarez-Buylla 2002). Finally, at stage five, between three and four weeks after birth of GC_{OB}, the distal branches of their apical dendrites develop dense spines, achieving full spine

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density in these dendrites by four weeks of development (Petreanu & Alvarez-Buylla 2002). During this final stage of maturation, new GC_{OB} acquire the ability to fire fast action potentials (Carleton et al. 2003) and form most of the input and output synapses on their distal branches (Kelsch et al. 2008). Although synaptic development is mostly complete by four weeks after the generation of adult-born GC_{OB} (Carleton et al. 2003, Kelsch et al. 2008, Mizrahi 2007, Petreanu & Alvarez-Buylla 2002, Whitman & Greer 2007a), Mizrahi (2007) has observed rearrangement of spines after this time, which suggests that GC_{OB} may maintain some capacity for synaptic modification even when they are mature.

Adult-born granule neurons first develop input synapses in their proximal dendritic domain, which lacks output synapses, before developing most of their dendro-dendritic output synapses and prior to acquiring the ability to fire action potentials (Kelsch et al. 2008), i.e., they "listen" before they can "speak." This sequential pattern of synaptic development of adult-born GC_{OB} sharply contrasts with the maturation of cells born during neonatal development, which is when most GC_{OB} are generated (Lemasson et al. 2005). First, neonatal-born GC_{OB} develop the ability to fire action potentials early in their development, during stage three, around the same time they start receiving synaptic inputs (Carleton et al. 2003). Second, neurons added to the neonatal brain also develop input and output synapses on the distal and proximal regions of apical dendrites simultaneously (Kelsch et al. 2008).

The different modes of synaptic development between adult and neonatal neurons could be due to intrinsic properties of new GC_{OB} already determined in their respective precursors. Alternatively, local cues in the neonatal and adult OB environment may be responsible for these differences. Heterochronic transplantation of postmitotic precursors could be helpful to clarify which aspects of synaptic development are governed by cell-autonomous versus external cues.

Synapse Connectivity Within Olfactory Circuits

The lateral dendrites of mitral and tufted cells, which form dendro-dendritic synapses with GC_{OB}, are located in the deep and superficial external plexiform layers, respectively (**Figure 1*a***). Most GC_{OB} ramify distal dendritic branches only in one location within the external plexiform layer, not in both (Kelsch et al. 2007, Mori et al. 1983), and this phenomenon is genetically predetermined in neuronal progenitors as demonstrated by fate-mapping and transplantation studies (Kelsch et al. 2007, Merkle et al. 2007). The OB may have "independent microcircuits" such that specific populations of GC_{OB} target only one class of principal neurons (Mori 1987); GC_{OB} with superficial or deep dendrites may exclusively form synapses with tufted or mitral cells, respectively. However, the existence of these independent microcircuits has yet to be proven functionally. The concept of microcircuit-specific targeting of new neurons in the adult brain is consistent with the protomap model of circuit assembly (Rakic et al. 2009) and raises the possibility of genetically engineering stem cells to generate specific neuronal types to replace those lost to disease or injury.

Neuronal Addition and Turnover

The functional differences between deep and superficial GC_{OB} also extend to neuronal survival. Whereas neonatal-born GC_{OB} often reside in the superficial granule cell layers, adult-born neurons tend to localize within the deep layers (Imayoshi et al. 2008, Lemasson et al. 2005). Although most superficial and neonatal-born GC_{OB} survive for long periods approaching the animal's lifetime, deep and adult-born GC_{OB} tend to be short-lived (Imayoshi et al. 2008, Lemasson et al. 2005). A recent study using a transgenic labeling technique suggests that almost all deep, adult-born neurons are turned over and thus continuously replaced (Imayoshi et al. 2008), which supports Bayer's (1980) original

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observations. Two long-term studies suggest that cell death in adult-born GC_{OB} is limited to the first month after neuron birth (Lemasson et al. 2005, Winner et al. 2002), whereas another study suggests there is a further drop in cell survival after the first two months (Petreanu & Alvarez-Buylla 2002). However, the latter study has low temporal resolution after the two-month time point, and this result may also be caused by the high variability between samples. At least some of the adult-born GC_{OB} that persist throughout life maintain a synaptic density similar to the one they displayed a month after their birth (W. Kelsch & C. Lois, unpublished observations). In summary, the question of neuronal addition or turnover of adult-born GC_{OB} remains unresolved and warrants further clarification, especially in light of the implications on the potential role of adult neurogenesis for long-term memory storage.

Activity-Dependent Neuronal Survival

Only 50% of new GC_{OB} generated in the adult successfully integrate into the bulb's circuits and survive for extended time periods, and abundant evidence indicates that neuronal activity is important in determining their survival. Synaptic maturation in GC_{OB} occurs mostly in the third and fourth week of development (Carleton et al. 2003, Kelsch et al. 2008, Whitman & Greer 2007b). This period coincides with a time window during which the survival of GC_{OB} is most sensitive to sensory deprivation, when the proportion of surviving neurons is further reduced by half in a deprived bulb (Petreanu & Alvarez-Buylla 2002, Yamaguchi et al. 2000, Yamaguchi & Mori 2005). Silencing the circuit with pharmacologically enhanced inhibition also reduces survival of adult-born neurons during this critical period (Yamaguchi & Mori 2005). Rochefort et al. (2002) reported that exposure to an odor-enriched environment increases survival, particularly when the animal is rewarded for performing an odor-discrimination task (Alonso et al. 2006). However, the enhanced survival reported in these works has not been observed in other studies (Magavi et al. 2005). Although the source of this disparity is unclear, the enhanced survival reported may not be due solely to exposure to enriched odors, but also to the fact that the behavioral demand of the task may raise animals' attention levels (Alonso et al. 2006). These observations suggest that odor information processing via nascent synapses plays a critical role in the stable integration of new neurons in the adult olfactory system.

Activity-Dependent Synaptogenesis

Activity in the OB not only influences the survival of adult-born GC_{OB}, but also regulates their synaptic connectivity. When postnatal-born GC_{OB} are subject to sensory deprivation during the critical period, they display fewer synaptic spines (Saghatelyan et al. 2005). Kelsch et al. (2009) recently confirmed this finding using genetically encoded markers for excitatory synapses in adult-born GC_{OB}. The loss of input and output synapses triggered by sensory deprivation occurs only during early synaptic development and is not seen when sensory deprivation is performed after synaptic development is completed. This observation suggests that the critical period during which the survival of new neurons is dependent on sensory input coincides with a stage in which neurons have a high degree of plasticity, which facilitates the shaping of their synaptic organization. Similarly, a recent study by Nissant et al. (2009) has demonstrated that long-term potentiation can be induced in adult-born neurons during early stages of their maturation, but not after this period. It will be interesting to examine whether this critical period also applies to other forms of plasticity in adult-born neurons.

The effects of olfactory deprivation on synaptic development are complex: Adult-born GC_{OB} that survive after sensory deprivation display an increased density of proximal input synapses in the unbranched apical dendrite (Kelsch et al. 2009). This observation suggests that neurons may compensate for the absence of sensory input by receiving additional

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excitatory drive, which elevates their activity level above the threshold required for survival.

The relationship between cell-intrinsic excitability and synapse formation is not well understood. Recent experiments indicate that increasing the intrinsic excitability of adult-born GC_{OB} by expressing a voltage-gated bacterial sodium channel does not affect synapse formation or maintenance (Kelsch et al. 2009) and promotes stable integration of adult-born GC_{OB} (Lin et al. 2010). However, genetically increased excitability blocks sensory deprivation–triggered synaptic changes. GC_{OB} expressing this sodium channel that are born in a bulb deprived of sensory input develop a normal organization of glutamatergic input synapses, as measured by the density of their PSD95:GFP-positive clusters (Kelsch et al. 2009). Similarly, dampening the excitability of new GC_{OB} by overexpressing the potassium channel Kir2.1 does not affect the synapse numbers of the surviving neurons (Lin et al. 2010). Taken together with the finding that increased inhibition in the circuit decreases the survival of adult-born GCs (Yamaguchi & Mori 2005), these observations demonstrate that synaptogenesis in adult-born GC_{OB} is sensitive to changes in synaptic input and suggest that both survival selection and synaptic development are driven by a minimum threshold excitation level, to which several factors can contribute: first, local glutamatergic excitatory inputs from mitral or tufted cells, whose activity is regulated primarily by sensory experience; second, centrifugal glutamatergic inputs originating in other regions of the brain, such as the olfactory cortex, which act on the olfactory bulb; third, centrifugal inputs of neuromodulators such as acetylcholine, noradrenaline, or neuropeptides, which modulate neuronal activity on a longer timescale. For instance, cholinergic stimulation, which causes sustained depolarizations in GC_{OB} (Pressler et al. 2007), enhances the survival of new neurons both in the dentate gyrus and in the OB (Kaneko et al. 2006) and may be responsible for the reported enhanced survival of adult-born neurons when olfactory tasks involved increased attention levels (Alonso et al. 2006). Hence, phasic excitation provided by synaptic input from mitral and tufted cells is only one of the many determinants of survival and integration of new GCs into the bulb's circuits.

We hope future studies will resolve the ambiguities surrounding the regulation of neuronal survival by centrifugal and sensory-driven inputs. Meanwhile, the transient synaptic plasticity displayed during synaptic development of new GC_{OB} may be an attractive model with which to study how neuronal connectivity during circuit assembly is regulated by activity.

SYNAPTOGENESIS IN ADULT-BORN OLFACTORY BULB PERIGLOMERULAR NEURONS

The second class of neurons generated throughout life in the OB is the PGN, which surrounds glomeruli where olfactory sensory axons connect to the apical dendrites of the OB's principal neurons (**Figure 1*a***). PGNs receive excitatory synaptic input both from olfactory sensory axons as well as from the apical dendrites of principal neurons via dendro-dendritic synapses. The outputs of PGNs occur both through dendro-dendritic synapses and axonal output synapses with principal neurons, although not all PGNs have axons (Pinching & Powell 1971). PGNs are a highly diverse group of neurons and can be broadly divided into two groups: those whose dendrites synapse on only one or two glomeruli and those that synapse on many (Whitman & Greer 2007a). These neurons can be dopaminergic and/or GABAergic, and different subsets of GABAergic neurons also express different calcium-binding proteins such as calbindin and calretinin (Whitman & Greer 2007a). Different subtypes of PGNs are generated in the OB during embryogenesis, neonatal development, and adulthood (Batista-Brito et al. 2008, De Marchis et al. 2007).

Much less is known about the synaptic development of adult-born PGNs as compared with GC_{OB}. Given the diversity of adult-born

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PGNs, one would expect heterogeneity in their synaptic development and organization as well. Indeed, the maturational sequence of spontaneous inputs is not stereotypical for PGNs: Some neurons develop GABAergic inputs first, whereas others develop glutamatergic inputs first (Grubb et al. 2008). Excitatory inputs to PGNs appear early during their development, and their frequency continues to increase until six weeks after their birth (Grubb et al. 2008).

During the first six weeks after their birth, adult-born PGNs develop a full dendritic arbor. In vivo two-photon imaging has shown that the spines of adult-born PGNs become more stable as they mature (Livneh et al. 2009). During the maturation of PGNs, strong functional changes occur in the synapses between sensory neurons and PGNs. These changes appear to be mostly restricted to the postsynaptic sites on the PGNs, whereas the characteristically high release probability at olfactory sensory neuron terminals (Murphy et al. 2004) is already present as soon as functional synapses are formed (Grubb et al. 2008). The highly dynamic rearrangement of input synapses in PGNs may be attributed to the continuous turnover of olfactory sensory axons (Zou et al. 2004), from which they receive their primary input, or could simply be an intrinsic property of these neurons.

Activity-Dependent Neuronal Survival and Synaptogenesis

Because PGNs are the first relay station of olfactory sensory input, it is hardly surprising that, akin to GC_{OB}, adult-born PGNs also display activity-dependent survival. Investigators have reported that sensory deprivation decreases (Mandairon et al. 2006), whereas olfactory enrichment (Rochefort et al. 2002) and olfactory discrimination tasks (Alonso et al. 2006) increase, adult-born PGN survival. Also, sensory enrichment accelerates the development of their glutamatergic input synapses as visualized by genetic synaptic markers (Livneh et al. 2009).

PGNs may be an attractive model with which to study the formation of synaptic connections and how they are affected by activity in real time, since their superficial location in the olfactory bulb makes them the only adult-born neurons amenable to in vivo two-photon imaging with sufficient spatial resolution to visualize these changes (Livneh et al. 2009).

SYNAPTOGENESIS IN ADULT-BORN DENTATE GYRUS GRANULE NEURONS

Stages of Synaptic Development

The third type of adult-born neurons in mammals is the DG granule cell (GC_{DG}), which arises from progenitors in the subgranular zone just beneath the granular layer where mature neurons eventually reside. About 9000 new granule cells are produced daily in the DG of young rats (Cameron & McKay 2001). These neurons receive their main excitatory input from the entorhinal cortex and provide glutamatergic input primarily to the excitatory pyramidal neurons and inhibitory interneurons in the CA3 region of the hippocampus (**Figure 2*a***). In this manner, the DG acts as the main entry point for entorhinal cortex input into the hippocampus, relaying the information to CA3 for further processing before it is returned to the entorhinal cortex via CA1.

The distinct stages of neuronal maturation of adult-born GC_{DG} largely recapitulate those that occur during perinatal development, but at a slower pace (Overstreet-Wadiche et al. 2006a, Zhao et al. 2006). This observation could be due to the upregulation of DISC1 protein in the adult DG, which slows the increase in spine density of GC_{DG} during their development (Duan et al. 2007). The new GC_{DG} first receive GABAergic input to their dendrites approximately one week after they are generated. This innervation is initially depolarizing until two to four weeks, when it becomes hyperpolarizing (Esposito et al. 2005), owing to the transient expression of the inward chloride transporter

NKCC1 in immature neurons, which results in an elevated intracellular chloride concentration as compared with mature neurons (Ge et al. 2006). Expression of this transporter is necessary for normal development because its ablation leads to severely delayed neuronal maturation (Ge et al. 2006). In the second week after their birth, dendrites of GC_{DG} start to form spines and to receive glutamatergic input, and their GABAergic input becomes predominantly perisomatic (**Figure 2*d***) (Esposito et al. 2005, Ge et al. 2006). Concurrently, axonal projections from new neurons reach the CA3 region and begin to form contacts that continue to mature for months (Toni et al. 2008). By two months of age, adult-born neurons have similar morphological and electrophysiological properties to those formed during perinatal development (Ge et al. 2007; Laplagne et al. 2006, 2007).

Activity-Dependent Neuronal Survival

Akin to GCs in the OB, 50% of new GC_{DG} born in the adult die by four weeks of age (Kempermann et al. 1997a), and their survival is most sensitive to environmental influences between the first and third weeks of development (Tashiro et al. 2006).

Levels of neurogenesis and subsequent survival of GC_{DG} are strongly influenced by neuronal activity. Increased levels of adult neurogenesis in the DG accompany changes in experiences through exercise or enriched environments (Kempermann et al. 1997b, van Praag et al. 1999). New neurons that are activated during learning are preferentially selected for incorporation into active DG circuits (Kee et al. 2007). Conversely, new GC_{DG} whose *N*-methyl-D-aspartate (NMDA) receptor-mediated input is eliminated experience a drastic reduction in survival rates (Tashiro et al. 2006). These observations illustrate that, as described in the OB, neuronal activity plays a role in selecting new neurons that eventually survive and integrate into the DG circuits.

Activity-Dependent Synaptogenesis and Pathology: Excitability-Induced Rewiring of Adult-Born Neurons in the Dentate Gyrus

The functional maturation of adult-born GC_{DG} is highly sensitive to changes in activity, and the strongest perturbations of synapse formation in new GC_{DG} are caused by seizures (Parent et al. 1997). Experimentally induced seizures accelerate synaptic development of new neurons such that new GC_{DG} added to an epileptic brain start receiving GABAergic input to their dendrites prior to two weeks after their birth, significantly earlier than in the unperturbed DG (Overstreet-Wadiche et al. 2006b).

In experimental seizure models, the DG exhibits network changes that resemble those observed in human pathology of temporal lobe epilepsy. This reorganization of connectivity may be attributed to anomalous integration of new neurons, in addition to other changes in preexisting neurons. Differentiating neurons are most susceptible to develop aberrant connectivity, and some morphological changes are seen only in new neurons generated within days of the onset of seizure, but not in neurons born a week before (Jessberger et al. 2007). Seizure-induced synaptic alterations to GC_{DG} in animal models include increased numbers of mushroom spines (spines with characteristically large heads) and spiny, branched basal dendrites that extend into the polymorphic cell layer (Jessberger et al. 2007). Seizures also perturb the migration of new GC_{DG}. The cell bodies of new GC_{DG} born during seizures aberrantly localize within the hilus and these neurons fire in synchrony with CA3 pyramidal neurons, which suggest that they contribute to increased excitability within the hippocampus (Scharfman et al. 2000). Seizures can also result in mossy fiber sprouting, axonal projection by GC_{DG} to the supragranular molecular layer. The consequences of sprouting are controversial; electrophysiological studies have proposed that this aberrant connectivity produces either recurrent excitatory circuits and subsequent hippocampal hyperexcitability (Okazaki et al. 1995), or

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conversely, recurrent inhibition by preferentially targeting inhibitory neurons in the molecular layer (Sloviter 1992).

Preliminary analyses of the addition of individual new neurons with genetically enhanced excitability into the adult dentate gyrus in vivo suggest that these neurons experience increased perisomatic inhibition as well as a reduction in the frequency of excitatory inputs and density of glutamatergic input synapses (S. Sim, C.W. Lin, and C. Lois, unpublished observations). Notably, these neurons display many of the seizure-induced alterations such as larger dendritic spines and basal dendrites, but they lack mossy fiber sprouting. Investigators have also observed synaptic rearrangements in these neurons' axon terminals. Normal GC_{DG} have axonal specializations known as large mossy terminals, where they form complex synapses with pyramidal neurons in the CA3 region of the hippocampus. Neurons with genetically enhanced excitability lose many of their large mossy terminals, suggesting a reduction in inputs to excitatory CA3 neurons. These results support previous findings in hippocampal cultures showing that these axonal connections are fairly dynamic, since synaptic rearrangements as well as changes in the size of large mossy terminals have been documented in response to changes in spiking activity (Galimberti et al. 2006).

Synaptic Plasticity During a Critical Period

Similar to the situation described for GC_{OB}, there is a critical period within which new neurons in the adult DG display increased synaptic plasticity compared with mature neurons as demonstrated by an enhanced propensity for long-term potentiation (Schmidt-Hieber et al. 2004). This enhanced synaptic plasticity lasts until the second month after new neurons are generated and then decreases to levels comparable to those of the surrounding mature neurons (Ge et al. 2007). Long-term potentiation during this critical period possesses several defining characteristics: It is dependent on the presence of the NR2B subunit of the NMDA receptor and can be induced in the presence of intact inhibition (Ge et al. 2007). Similarly, a low-threshold calcium spike can boost fast sodium action potentials and contribute to long-term potentiation during this critical period (Schmidt-Hieber et al. 2004). Investigating how the flow of information through the hippocampus can shape the synaptic organization of new neurons will help investigators elucidate the role of adult neurogenesis in learning and memory.

PERSPECTIVES ON THE FUTURE

We conclude this review by raising several open questions that, we hope, may inspire future research.

First, adult neurogenesis is a widespread phenomenon in most vertebrates. It is interesting to note that mammals appear to be an exception among vertebrates: Their brains are composed mostly of long-lived, nonrenewable neurons born during the embryonic development. Why has a phenomenon that is common in so many classes of animals become less prevalent in mammals? Why is the human cerebellum or neocortex capable of processing, acquiring, and storing information for decades using a single set of neurons, whereas the dentate gyrus and olfactory bulb require the continuous addition of neurons into their circuits throughout life to perform their functions?

Second, in contrast with circuit assembly during embryonic development, which involves integration of new neurons in a mostly constant environment in utero, adult-born neurons integrate into mature, functioning circuits. This observation poses additional challenges because adult brain activity is constantly modulated by the ever-changing conditions of the outside world. Furthermore, because adult-generated neurons integrate into the brains of behaving animals, these neurons must form new synapses with minimal disruption to existing connectivity so that behavior is unperturbed. Do specialized mechanisms exist for synaptic integration in adult-born neurons, which differ from those used during embryonic brain development?

Third, synaptic plasticity of adult-born neurons is likely restricted to a specific time window early during their maturation. This phenomenon supports the idea that new neurons provide the mature circuit with a transient form of heightened plasticity, acting as a substrate for circuit refinement and adaptation. After this critical window, the neurons mature, become stably integrated into the brain, and partially lose their activity-dependent plasticity. The addition of cells endowed with such an initial short-lived flexibility and long-term stability enables information processing in the brain to be both versatile and reliable while faced with changing behavioral demands. The transient plasticity of new cells generated during adult neurogenesis may explain the requirement for additional new neurons to facilitate lifelong plasticity and reshaping of memory circuits. Which molecular mechanisms are responsible for this transient plasticity? Most mammalian brain regions, such as the thalamus, striatum, and neocortex, do not receive any new neurons after birth. Do neurons in these brain areas maintain their plasticity for longer periods of time compared with adult-born neurons added into the OB and DG?

Investigating these unanswered questions will shed some light on the mystery of why mammalian brain circuits are composed of two classes of neurons: those that live as long as the individual harboring them and those that are continuously added throughout life.

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