
Control of Early Events in Olfactory Processing by Adult Neurogenesis

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Abstract

The mature brain needs to have flexible control over behavior in the face of ever-changing needs. It achieves this control through morphological and physiological changes at the level of molecules, spines, dendrites, and axons and through processes of adult neurogenesis, entire cells. The functional maturation of newly generated cells in the adult forebrain involves the expression of neurotransmitter receptors before synaptic activity and excitatory γ -aminobutyric acid (GABAergic) influences prior to glutamatergic input. The production of new cells for incorporation into neural circuits that are already up and running gives rise to a unique situation that may require epigenetic regulation. However, once mature, new neurons must carve out a niche among more established cells to be useful. How do they survive and what are they used for? Recent studies have revealed that adult neurogenesis alters the olfactory bulb at all levels, from single cells to the network and system levels. It has also been suggested that cell turnover may be particularly beneficial for the processing of new information in dynamic networks. However, elucidating the functional meaning of adult neurogenesis must wait for the development of new paradigms to eliminate the pool of newly generated neurons but sparing the preexisting ones. Nevertheless, there is already considerable correlative evidence to indicate that adult neurogenesis is a plastic mechanism by which the performance of the brain can be optimized in a given environment.

Key words: experience, interneurons, plasticity, sensory map, survival

Introduction

Brain plasticity refers to the brain's ability to change structure and/or function during maturation, learning, environmental challenges, or disease. Multiple and dissociable plastic changes in the adult brain involve many different levels of organization, ranging from molecules to systems, with changes in neural elements occurring hand-in-hand with changes in supportive tissue elements, such as glia and blood vessels. There is now substantial evidence to show that new functional neurons are constitutively generated from endogenous pools of neural stem cells in restricted areas of the mammalian brain, throughout life. So, in addition to all the other known structural changes, entire new neurons can be added to the existing network circuitry. This addition of new neurons provides the brain with another tool for tinkering with the morphology of its own functional circuitry.

Although the ongoing neurogenesis and migration have been extensively documented, their functional consequences are still not clear. Is adult neurogenesis an atavism, an empty-running leftover from evolution? What is adult neurogenesis good for? Is there anything special about the new neurons besides their later birth? How are they integrated into the existing networks? How could they possibly contribute to brain

function? How does the brain know that more neurons are needed, and how is this demand translated into signals a precursor cell can "understand"? In fact, adult neurogenesis may represent an adaptive response to challenges imposed by an environment and/or internal state of the animal. The continuous production of new neurons raises a series of important questions concerning the possible role of neurogenesis in mature neuronal circuits. We attempt to address these questions here, using the olfactory bulb (OB) as a model system. Due to space constraints, we will concentrate on the OB of vertebrates and primarily those of mammals.

Production and integration of new neurons into the OB circuit

Postdevelopmental neurogenesis has been conserved throughout evolution, from crustaceans (Beltz and Sandeman 2003; Harrison et al. 2003; see Schmidt 2007) to insects (Scotto-Lomassese et al. 2003; see Cayre et al. 2007) and to higher vertebrates, including birds (Goldman and Nottebohm 1983; Alvarez-Buylla 1990), rodents (Altman and Das 1965; Hinds 1968; Lois and Alvarez-Buylla 1994), primates

(Gould, Reeves, et al. 1999; Kornack and Rakic 1999), and humans (Eriksson et al. 1998). If adult neurogenesis is well preserved throughout evolution, yet there are some fundamental changes between species. Why can some animal such as lizards regenerate entire brain parts, whereas “higher animals” cannot? Is adult neurogenesis advantageous or disadvantageous? At first glance, apparent evolutionary history seems to show that the extent of postnatal neurogenesis decreases with increasing brain complexity, with this process providing an “additional supply” of neurons capable of regenerating entire brain parts in lower vertebrates but restricted to neuronal “replacement” limited to a few regions of the brain in mammals. The extent of postdevelopment neurogenesis in a given species may well depend on a trade-off between the benefits conferred by newly generated neurons and the problems they generate for the network circuitry into which they integrate. These problems seem to have dominated in animals with more complex brains as in mammals in which adult neurogenesis under normal conditions is probably confined to just 2 regions (Figure 1): the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ), which contributes interneurons to the OB (for reviews, see Alvarez-Buylla and Garcia-Verdugo 2002; Rakic 2002) (Figure 2). There is some evidence to suggest that constitutive neurogenesis occurs in many other regions of the adult brain, but methodological issues (e.g., Kornack and Rakic 2001; Lie et al. 2002; Koketsu et al. 2003; Frielingsdorf et al. 2004) make it impossible to add these regions to the list of zones in which adult neurogenesis occurs, for the time being.

Producing new neurons

It is currently thought that astrocytes in the adult SVZ, which lines the border between the striatum and the lateral ventricle, act as slowly dividing neural stem cells capable of generating a progeny of neuroblast precursors (Doetsch et al. 1999; Laywell et al. 2000; Tramontin et al. 2003). Once generated in the germinal niche (see the glossary in Appendix 1), these neuroblasts proceed toward the OB along an intricate migration path, up to 5 mm long in rats, called the rostral migratory stream (RMS) (Figure 1). More than 30 000 neuroblasts exit the rodent SVZ for the RMS each day (reviewed in Alvarez-Buylla et al. 2001). Rather than being guided by radial glia, they migrate tangentially in chains through tubular structures formed by specialized astrocytes (Lois et al. 1996). Time-lapse video imaging can be used to analyze this migration directly and has shown that nuclear translocation always occurs in the direction of the leading process extension, often leaving a thin trailing process behind the cell body. Remarkably, cells move bidirectionally, with occasional migration back toward the SVZ. Several studies have reported migration to be rapid in explants (100 $\mu\text{m}/\text{h}$) or ex vivo (50 $\mu\text{m}/\text{h}$). However, the way in which neuroblasts migrate in vivo has remained unclear, due to the inability to monitor migration appropriately

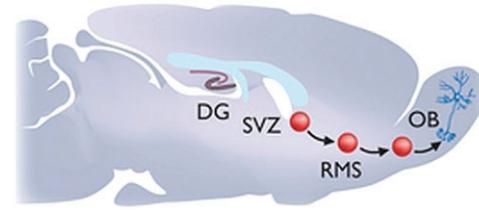


Figure 1 Neurogenesis and neural stem cells (NSC) in the adult mammal CNS. NSC from adult brains produce all cell types from the neuronal lineage: astrocytes, oligodendrocytes, and neurons. The 2 neurogenic zones in the adult brain that host NSC are the dentate gyrus (DG) of the hippocampus and the SVZ. In the hippocampus, stem cells located in the SGZ of the DG produce D cells, which function as transient precursors, and give rise to new granule neurons (type A cells or neuroblasts). The proliferation of newborn cells in this niche has been studied extensively, revealing multiple external factors—including neurotransmitters, hormones, growth factors, and environmental stimuli—involved in regulating the process. From the SVZ, neuroblasts migrate to the OB via the RMS, where they differentiate into bulbar interneurons. The migration along the RMS is regulated by interactions between neuroblasts and the local RMS microenvironment, involving different receptors and proteins involved in contact-mediated repulsion or attraction. After reaching the core of the OB, chain detachment and radial migration of neuroblasts are regulated by the external factors reelin, tenascin-R, and prokineticin-2. Newborn cell survival then depends upon sensory input.

from the adult neurogenic zone, through the migratory pathway or at the integration site. These areas are too deep for conventional fluorescence microscopy. A method based on integrated fiber confocal fluorescence microscopy has therefore been developed, for minimally invasive, long-term recordings several millimeters into anesthetized animals (see Appendix 2). The use of this novel technique has made it possible, for the first time, to visualize migrating newly generated neurons in living animals (Davenne et al. 2005). Once SVZ progenitor cells have been labeled, their migration could be monitored during several hours. It was found that the speed of migration in vivo is not constant. Instead, there seem to be spurts of migration interspersed with periods of inactivity.

After reaching the OB and detaching from the chains, the new cells migrate radially and mature into olfactory inhibitory interneurons of 2 main types—granule cells and juxtglomerular cells—that are found in different layers (Figure 2). The 2 cell types make only local contacts in the bulb, directly or indirectly modulating the processing of sensory information by the projection neurons of the OB, the mitral and tufted cells.

Maturation and integration of newly generated neurons

By transducing newly generated cells in the adult SVZ with replication-incompetent retroviruses and labeling them with enhanced green fluorescent protein (Appendix 3), researchers have been able to characterize the morphological and electrophysiological properties of the newly generated neurons during their migration and differentiation (Petreanu and Alvarez-Buylla 2002; Belluzzi et al. 2003; Carleton et al.

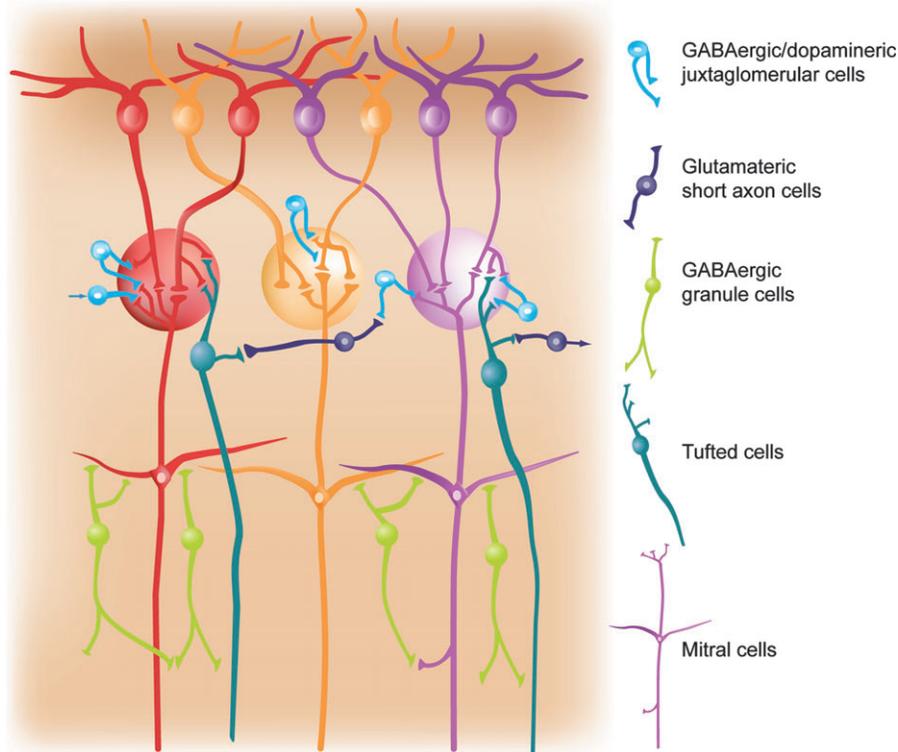


Figure 2 Basic circuitry of the main OB. Olfactory sensory neurons that express the same odorant receptor gene project their axons to either of 2 glomeruli in the OB. Three populations of sensory neurons, each expressing a different odorant receptor gene, are depicted in different colors. Their axons converge on specific glomeruli, where they synapse with the dendrite of local interneurons (periglomerular neurons) and second-order neurons (mitral cells). The lateral dendrites of mitral cells contact the apical dendrites of granule cells. Short axon cells are bulbar interneurons that contact both apical and lateral dendrites of mitral cells.

2003). The morphological complexity of the newly generated cells increases within a few weeks of their generation. Granule cells form more elaborate dendrites that extend into the external plexiform layer of the bulb, becoming fully morphologically mature within 2 weeks of their birth (Petreanu and Alvarez-Buylla 2002). In contrast, periglomerular cells take around 4 weeks to develop their full dendritic and axonal morphology (Belluzzi et al. 2003). During this maturation period, the immature bulbar interneurons express transient marker proteins, including TUJ1 and TUC4 (Winner et al. 2002). The first synapses made with the new cells are γ -aminobutyric acid (GABAergic); these synapses are followed a few days later by glutamatergic inputs (Belluzzi et al. 2003; Carleton et al. 2003). The main difference between new periglomerular and granule cells lies in the sequence in which voltage-dependent currents and synaptic connections are developed. In periglomerular cells, the voltage-dependent sodium current and consequently the capacity of the newly generated cells to fire action potentials seem to develop before the formation of synaptic contacts. In granule cells, full development of the sodium current is observed once a newly generated neuron has established synaptic connections (Belluzzi et al. 2003; Carleton et al. 2003). This difference suggests that the pattern of functional

integration of new neurons may be cell-type specific. The delayed maturation of granule cell excitability may help to prevent disruption of the existing circuitry during the integration of the new cells. However, as even mature granule cells have no axon, it may also be due to morphological differences between granule and periglomerular neurons. Further studies are required to determine whether the newly generated neurons form functional synapses with their downstream target neurons and release appropriate neurotransmitters, to provide an unequivocal demonstration of the integration of these cells into adult networks.

Adding new neurons to old circuits

A myriad of factors regulate the production, migration, and development of newly generated neurons in adults and determine whether these neurons die or survive. However, once a newly generated cell has reached its target, matured into a neuron, and survived, what does it do then? Why is it there? What is its function?

A role in information processing

If we consider this issue in a rather abstract and fundamental way, the new neurons may contribute to adult brain function

at the cellular, network, and systems levels. At the cellular level, the function of these new neurons seems quite obvious: they are just neurons. However, they could be a special type of neuron. They may differ from the existing cells in terms of target structure. Studies of the maturation of these newly generated cells have suggested that their cellular function may differ from that of the preexisting cells, at least transiently. Indeed, the new cells tend to be more excitable and more adaptable than the older, established neurons. In many ways, they act like the neurons of the embryonic brain, readily making new synapses with other neurons and changing the strength of these connections. Young granule cells in the adult hippocampus, for example, display much greater synaptic plasticity than older granule cells (Wang et al. 2000; Schmidt-Hieber et al. 2004), whereas newly generated granule and periglomerular cells in the OB have active membrane properties markedly different from those of the established neurons surrounding them (Carleton et al. 2003; Belluzzi et al. 2003), and display greater plasticity in response to sensory deprivation (Saghatelyan et al. 2005).

There is now clear evidence that olfactory interneurons produced postnatally in the OB are physiologically integrated (Carlen et al. 2002; Belluzzi et al. 2003, Carleton et al. 2003). However, the functional significance of the newly generated interneurons in the OB remains still unclear. Nevertheless, growing interest in adult neurogenesis has led to advances in our understanding of the molecular, cellular, and physiological properties and location of newly generated neurons. Evidence is accumulating that newborn neurons have properties not present in their established counterparts. These unique properties provide a fundamental framework for elucidating the functional role of the neurons generated in adult animals. Newly generated neurons in the OB have different characteristics at early and late postnatal stages, suggesting that postnatal neurogenesis does not give rise to a uniform population of bulbar interneurons. The newly generated interneurons are positioned deeper in the granule cell layer in late postnatal than in early postnatal periods (Lemasson et al. 2005). As no difference in apoptosis across the granule cell layer is observed between stages, this gradient may reflect differences in the recruitment of newly generated interneurons. This suggests that the cells generated in the early postnatal period and in adulthood respond differently to common signals and/or respond to different molecules during the life of the animal. The neurons generated later in the postnatal period also migrate to the OB more rapidly than those generated early in the postnatal period (Lemasson et al. 2005). The glial tube surrounding the migrating neuroblasts is difficult to detect before the third postnatal week (Peretto et al. 1997, 1999). This difference in migration speed may therefore be due to molecules being released from the glial processes of astrocytes along the RMS of the adult, facilitating the migration of neuroblasts (Mason et al. 2001; Murase and Horwitz 2002). The newly generated interneurons of the mature OB also differ from those estab-

lished during the development of the OB in having a much lower survival rate. It has been suggested that interneurons generated early in life, with a high rate of survival, play a critical role in determining the control of precocious olfactory processes over lifelong behavior (Lemasson et al. 2005), whereas interneurons generated during adulthood, which have a much lower rate of survival, fine-tune olfaction (Gheusi et al. 2000; Rochefort et al. 2002; Enwere et al. 2004). Investigations of the causes of these changes in the survival potential of neurons produced at different ages would help to broaden our understanding of these functions.

Although sensory deprivation limits the morphofunctional properties of granule cells generated early in the postnatal period and spares preexisting bulbar interneurons, compensatory mechanisms increase the intrinsic excitability of newly formed granule cells to keep overall levels of inhibitory input to mitral cells constant (Saghatelyan et al. 2005). Direct, across-age comparisons of this form of adaptive response are required to increase our understanding of the functional properties of the interneurons generated in adults. Newly generated bulbar neurons have pharmacological and physiological characteristics different from those of mature granule cells (Belluzzi et al. 2003; Carleton et al. 2003). The neurons generated in adult animals initially express extrasynaptic GABA_A receptors but lack *N*-methyl *D*-aspartate receptors. No spiking activity occurs as these neurons enter the OB after tangential migration, and the first synaptic events occur after the completion of radial migration. Finally, although most of the newly generated interneurons express Na⁺ channels, as they form reciprocal synapses with mitral cells, spiking activity does not occur until the neurons are fully mature. A recent study on the functional response of olfactory granule neurons generated in adult animals showed that, unlike preexisting neurons, newly generated granule cells preferentially respond to new odors after their synaptic integration, consistent with the view that these neurons have unique properties and make a specific contribution to the olfactory circuitry (Magavi et al. 2005). The greater sensitivity of newly generated cells to new odors seems to emerge after a period of familiarization with these odors, showing that the greater responsiveness of the newly formed neurons is stimulus specific and experience dependent. Thus, the bulbar interneurons generated in adult animals seem to be components with singular properties making a unique contribution to the olfactory system. It has been suggested that bulbar, and particularly hippocampal, neurogenesis in adults is involved in learning and memory, but we need to consider this phenomenon also in other contexts. In addition to contributing to endogenous repair, newly generated bulbar interneurons may be involved in inducing, facilitating, and/or maintaining olfactory-mediated behavior, such as food selection, mate selection, or caring for young (Shingo et al. 2003). Studies of the potential role of newly formed neurons in these processes may provide new insight into the functional significance of adult neurogenesis.

Although active neurogenesis clearly occurs in the adult OB, only a small percentage of the newly formed neurons survive. Indeed, only a subset of the newly generated interneurons integrates into bulbar circuits, the rest being destroyed. Quantitative studies have demonstrated that almost half of all newly generated interneurons are eliminated 1 to 2 months after their creation (Petreanu and Alvarez-Buylla 2002; Winner et al. 2002). The remaining 50%, which survive this initial period of cell elimination, generally survive for at least 19 more months (Winner et al. 2002). It is therefore of interest to determine the regulatory mechanisms controlling the survival of newly generated neurons. Several studies have suggested that long-lasting, odorant-dependent activity in the OB may modulate the survival of the new neurons and improve olfactory learning (Najbauer and Leon 1995; Petreanu and Alvarez-Buylla 2002; Rochefort et al. 2002; Mandairon et al. 2003). Mitogen-activated protein kinase activation may contribute to the odorant-induced increase in the survival of newly formed bulbar interneurons (Miwa and Storm 2005).

At the network level, the newly generated neurons may contribute to the properties driving the concerted activity of groups of cells. In the OB, oscillations and synchrony of neuronal activity are major network phenomena serving as potential targets for adult neurogenesis. Indeed, the OB receives and processes signals from olfactory sensory neurons and transmits them to higher processing centers. The mapping of sensory inputs has revealed that each odorant generates a reproducible spatial pattern of restricted activation in the glomerular layer of the OB. However, within the bulb, these confined patterns are transformed into widely distributed patterns by lateral interactions between relay neurons and local interneurons. Thus, odor information processing requires the spatial patterning of both sensory inputs and synaptic interactions mediated by local interneurons. Odor representation is thus highly dynamic and temporally orchestrated by lateral inhibition (reviewed in Lledo and Lagier 2005). We suggest that adult neurogenesis may lead to the persistent or transient enhancement of lateral inhibition in the OB network, facilitating odorant discrimination and increasing the chances of survival of newly generated granule cells. The continuous recruitment of local interneurons throughout life is therefore a key means of continually adapting transposition from the local to the temporal dimension, to optimize olfactory information processing.

Finally, in terms of functional plasticity, neurogenesis at the network level should be considered from the viewpoint of permissiveness versus instruction. It is unknown whether the new cells have prespecified roles, enabling them to instruct change in the networks they join, or whether they simply permit changes to occur in these circuits. However, studies of the functions of adult neurogenesis have mostly focused on the systems level. We suggest that new neurons optimize the working of the OB, enabling it to process new and complex stimuli. The perceptual and mnemonic func-

tions of the OB have understandably led to investigations into the importance of adult neurogenesis in these processes. The gap between cell generation and cognitive processes is large, but we are beginning to understand how the plasticity afforded by adult neurogenesis might contribute to the behavior of the animal (reviewed in Lledo and Gheusi 2003).

Roles in information processing and storage

As a first step toward the identification of systems functions to which adult neurogenesis contributes, it would seem sensible to ask whether individual levels of performance for a given function are correlated with individual levels of new cell generation and/or survival. Olfactory enrichment increases the survival of new cells in the OB, leading to improvements in olfactory memory (Rochefort et al. 2002). Conversely, olfactory discrimination is decreased by genetic manipulations aimed in reducing the number of new cells integrated into the adult bulb (Gheusi et al. 2000; Enwere et al. 2004). These observations suggest that adult neurogenesis may contribute to the learning and memory functions of the hippocampus and the perceptual and memory functions of the bulb. However, there are situations in which manipulation-induced changes in neurogenesis are not matched by parallel changes in bulb-dependent cognitive function (Mechawar et al. 2004).

Rather than looking for correlations between neurogenesis and function, a more direct approach would involve investigating whether a particular function itself regulates neurogenesis. For example, in the olfactory system of female mice, the proliferation of cells generated during adulthood increases after mating, during gestation, and during lactation (Shingo et al. 2003). This suggests that adult neurogenesis plays an important role in the strong olfactory perceptual and memory demands associated with reproduction (reviewed in Lledo et al. 2005).

Thus, most of the correlational and intervention evidence suggests that neurogenesis in the adult OB is important for olfactory discrimination and memory. The development of approaches designed to inhibit adult neurogenesis specifically and completely, such as conditional knockout approaches, should make it possible to investigate the functions of adult neurogenesis at the systems level, provided that approaches to behavioral characterization are developed with care.

Of course, one way to manipulate adult neurogenesis specifically and completely is to use a simulated computational model of the brain. This approach is extremely useful for demonstrating what adult neurogenesis might be able to do. Introducing activity-dependent survival of newly generated neurons into a model of the OB circuitry improves the orthogonalization of sensory representations, indicating improvements in olfactory discrimination in changing environmental conditions (Cecchi et al. 2001). Interestingly, whereas the addition of new neurons to a background of

existing cells with restricted plasticity makes it possible to learn new information without significantly degrading old memories, the turnover of cells on a background of plastic units facilitates not only new learning but also the forgetting of old information (Chambers et al. 2004; Deisseroth et al. 2004). These models probably provide the best evidence currently available for a commonly proposed function of adult neurogenesis: endowing a network with the ability to cope with “future” changes in input rather than simply being involved in the storage of “current” new information. Adult neurogenesis may therefore be a form of metaplasticity: a change in the brain facilitating further changes in the brain. This suggestion is reflected in observations of synaptic plasticity, highlighting the computational advantages of structural changes in circuit wiring (Chambers et al. 2004; Deisseroth et al. 2004). However, this idea is difficult to test experimentally as, strictly, it would be necessary to demonstrate that the information leading to the survival of a new neuron was in no way represented by that neuron’s contribution to network activity. Nevertheless, it would be interesting to determine whether the increase in neurogenesis resulting from one form of learning is required for information retention in a very different future task.

Concluding remarks

We still do not know why new neurons are incorporated into some parts of the adult brain and not into others, but research into the regulation of adult neurogenesis, and an increasing number of studies investigating the function of the new cells, has provided 2 major pieces of information. First, adult neurogenesis is affected by relevant behavior, including behavior specifically related to bulbar or hippocampal functions. Second, changes in the rate or extent of neurogenesis have an effect on subsequent behavior. Thus, experience may change the entire structure of adult circuits, and changes in structure may modify, or at least affect, subsequent behavior.

In the olfactory system, the correlation between neuronal activity and adult neurogenesis suggests that new neurons are involved in general aspects of bulbar function, probably sustaining the ability of the adult network to adapt information processing to relevant ethological needs. Several studies have demonstrated that odor representation at the first central relay is dynamic and highly complex and may therefore require unique plasticity mechanisms. Neurogenesis, migration, and the replacement of interneurons in the OB are probably involved in this adaptive mechanism. However, the precise nature of the requisite function for implementing adaptation remains unknown. The original bulbar interneurons are known to play a crucial role in transposing the spatial dimension of olfactory information, into a temporal dimension. They also distribute this information throughout the entire bulbar circuit. However, we do not yet know whether the newcomers can accomplish both these tasks. Patch-clamp recordings have shown that young newly generated

neurons are not yet competent for action potential-dependent GABA release (Carleton et al. 2003). The contribution to the activity of adult networks of newly formed granule cells may be different from that of established cells, at least during the early stages of their maturation.

Furthermore, adult neurogenesis does not simply introduce fresh cells into old circuits. It is also a matter of selection. About half the new interneurons die within a month of maturation. Our results show that newly formed granule cells maintain synaptic contacts during this period of rapid cell death. The selective elimination of neurons may make it possible to sculpt the circuitry rapidly. As a result, most of the newly generated neurons never reach the fully mature state and may therefore never play a role similar to that of the preexisting neurons. The ability to mold the integration of new neurons and to eliminate other neurons continually, without depleting the neuronal population, may provide neuronal networks with an adaptive potential unequaled by synaptic plasticity alone and may provide a mechanism for learning.

Two seemingly contradictory challenges: stability versus flexibility

The adult brain must guide the organism through an ever-changing world. In this context, it faces 2 conflicting challenges: maintaining behavior and long-term traces, requiring preservation of the necessary synaptic organization of a given circuitry, and maintaining a capacity to adapt following brain injuries, in learning conditions, or when faced with environmental changes. At first glance, neurogenesis in the adult brain would seem to bias the system in favor of flexibility; after all, the introduction of a completely new cell into a functioning circuit, or the removal of an existing neuron from the existing network, is a major step. However, computational models have suggested that adult neurogenesis, rather than greatly increasing the flexibility of the network, may serve to stabilize an already plastic network confronted with a large volume of new information.

The discovery of adult neurogenesis has led to efforts to understand the relationship between the presence of new cells and learning, in various model systems. However, the results obtained have been conflicting rather than consensual. For instance, Gould, Beylin, et al. (1999) reported an increase in the survival rates of newly formed hippocampal neurons in rats subjected to spatial learning in the Morris water maze. In contrast, van Praag et al. (1999) observed no change in neurogenesis following water-maze training in mice. More recent studies have confirmed the complex nature of adult neurogenesis, as both decreases and increases in neurogenesis have been reported with water-maze learning (e.g., Ambrogini et al. 2004; Van der Borght et al. 2005; Ehninger and Kempermann 2006). These discrepancies may result from changes in the functions of newly formed neurons during their maturation. They may also reflect

the temporal relationship between the labeling of newborn neurons and exposure to the memory task (Dobrossy et al. 2003). Many more new neurons than are actually needed may be produced, requiring the active and selective removal of those that have not established appropriate synaptic contacts. The learning-induced mechanisms involved are probably similar to those mediating brain development and maturation, shaping relevant and functional neural networks in the developing brain. We suggest that there may be a balance between the addition and elimination of newly formed neurons during learning. According to this view, learning depends not only on the addition of new neurons and new synaptic connections but also on regressive events culminating in the removal of neurons from the cellular network of the adult central nervous system (CNS). We recently tested these hypotheses. We found that the learning of olfactory discrimination increases the survival of newly formed neurons in the adult OB. Indeed, this result is far from surprising and is consistent with the large series of studies that have already shown the key role played by neuronal activity in increasing the degree of neurogenesis. However, the newly generated neurons reaching the network were unevenly distributed. The newcomers were restricted to functionally relevant circuits for a given learning task (Alonso et al. 2006). However, they were located in bulbar circuits specifically activated by the unreinforced odorant (S⁻) only and not in those activated by the reinforced odorant (S⁺). If the animals had learned that the reward was consistently associated with the odorant S⁺, then these results suggest that the newly generated neurons are unlikely to survive in neuronal circuits engaged in learning. Their presence may have deleterious effects on ongoing network activity. Further experiments are required to confirm or exclude these hypotheses.

Outlook

Improvements in our understanding of the role of adult neurogenesis in brain plasticity are not solely dependent on the collection of larger amounts of higher quality data concerning the dynamics, spatial extent, and longevity of experience-dependent structural changes in adult circuits. They also depend on 1) a fuller description of the integrative properties of individual newly generated neurons, 2) the development of better models of the representational redundancies present in neurons within neuronal networks, and (3) a more complete description of the guidance and selection mechanisms acting on the new neurons. Identification of the physical changes encoding particular long-term information remains a central issue today in neuroscience. Interdisciplinary approaches combining molecular, anatomical, physiological, and theoretical methods are most likely to succeed in efforts to address this and other questions relating to the physical substrate for long-term adaptation in the adult brain.

Those, like us, trying to determine the functional significance of adult neurogenesis may be inspired by its extremely

limited spatial distribution. What is so special about the OB and the hippocampus that these 2 regions are the only ones into which new neurons integrate? Alternatively, we could turn the question around: given the relative abundance of neurogenesis in the adult brains of reptiles and birds, why do so many regions of the adult mammalian brain not receive new neurons? The answers to these questions require a deeper understanding of the operation of a given network. Functionally, it is intriguing that both the OB and hippocampal networks are vital in the segmentation of olfactory or spatial information and in the “temporary” storage of this information. It has also been suggested that both the OB and the hippocampus deal with particularly large amounts of information (Chambers et al. 2004) or employ particular coding strategies to deal with that information (Cecchi et al. 2001). However, for every similarity between the OB and the hippocampus, large differences can be found between the 2 structures, not least in adult neurogenesis. Moreover, for every proposed common function of these 2 regions, other, nonneurogenic regions of the adult brain can be shown to have the same attributes. More detailed investigations of the function of adult neurogenesis are required to determine whether this process plays a similar, specific role in the adult OB and hippocampus.

In this review, we have tackled 2 of the main problems relating to the functional significance of adult neurogenesis. Both are fundamental issues. First, we have discussed the role of adult neurogenesis in the context of OB function. Assuming that the bulb processes odor information before relaying it to the olfactory cortex, we hypothesize that adult neurogenesis enables the OB to adjust the degree of processing of sensory information appropriately. Second, the existence of a pool of juvenile neurons, enabling the system to adapt to future similar situations, raises the possibility that adult neurogenesis acts post hoc to provide a structural basis for brain plasticity and learning. Research on adult neurogenesis in the olfactory system is thus not only interesting in itself but also provides new avenues of exploration to increase our understanding of adult brain adaptation.

Appendix 1: Neurogenic stem cell niches of the adult mammalian brain

Neural stem/precursor cells

Adult CNS stem cells have several cardinal features, such as an unlimited capacity for self-renewal, the ability to proliferate indefinitely in response to mitogens, and multipotency for the various neuroectodermal lineages of the CNS. The multipotent progenitors of the adult brain are proliferative cells with a limited capacity for self-renewal that can differentiate into at least 2 different cell lineages (Weiss et al. 1996). Lineage-specific precursors or progenitors are cells restricted to one particularly lineage (e.g., neuronal, astroglial, or oligodendroglial). CNS stem cells and all types of

precursor/progenitor may be considered, in general terms, to be neural precursor cells (NPCs). NPC is used as a generic term to encompass both stem and early progenitor cells.

The germinal niche

NPC self-renewal and differentiation are regulated by a specialized microenvironment—conventionally referred to as the germinal niche—in which these cells reside. Both environmental cues and intrinsic genetic programs are required to maintain stem cell properties and to direct, or regulate, stem cell proliferation and differentiation within niches (Doetsch 2003).

Niche cytoarchitecture

The SVZ of the lateral ventricle wall and the SGZ of the dentate gyrus of the hippocampus are germinal regions of the brain in which neural stem cells reside and support neurogenesis and gliogenesis throughout adult life. Cells with the structural and molecular characteristics of astrocytes are responsible for neurogenesis (true stem cells or type B cells) in the SVZ and SGZ (Doetsch et al. 1999; Laywell et al. 2000; Garcia et al. 2004). The SVZ astrocytes, which express glial fibrillary acidic protein (GFAP), are in intimate contact with all other SVZ cell types, including the rapidly dividing transit-amplifying (type C) cells and the lineage-committed (postmitotic) migratory neuroblasts (type A cells). The differentiation of the cell lineage passes from type B, through type C to type A cells, with type B cells believed to be the self-renewing primary precursors (Doetsch et al. 1999) (Figure 3). In the SGZ, GFAP-expressing astrocytes function as stem (type B) cells, undergoing self-renewal, proliferation, and differentiation into transit-amplifying (type D) cells, which then differentiate into lineage-committed migratory granule neurons (type G cells) (Palmer et al. 2000; Seri et al. 2001). The maintenance and differentiation of neural stem cells in brain niches seem to depend on these cells being in physical contact with the basal lamina, which acts as a scaffold and sequesters and/or modulates cytokines and growth factors derived from local cells (such as fibroblasts, macrophages, and pericytes) (Mercier et al. 2002). Type B cells in the SVZ are in close contact (interdigitated) with both the basal lamina and the blood vessels. In the SGZ, bursts of endothelial cell division are spatially and temporally related to clusters of neurogenesis (Palmer et al. 2000).

Appendix 2: Deep in vivo imaging of adult neurogenesis

An understanding of the migration of neuroblasts in the adult brain is required for the design of brain repair strategies based on the rerouting of endogenous neural progenitors away from their natural migratory path. Our lack of knowledge in this area results at least partly from the current impossibility of obtaining clear in vivo brain images for the adult neurogenic zone, the migratory pathway, and the in-

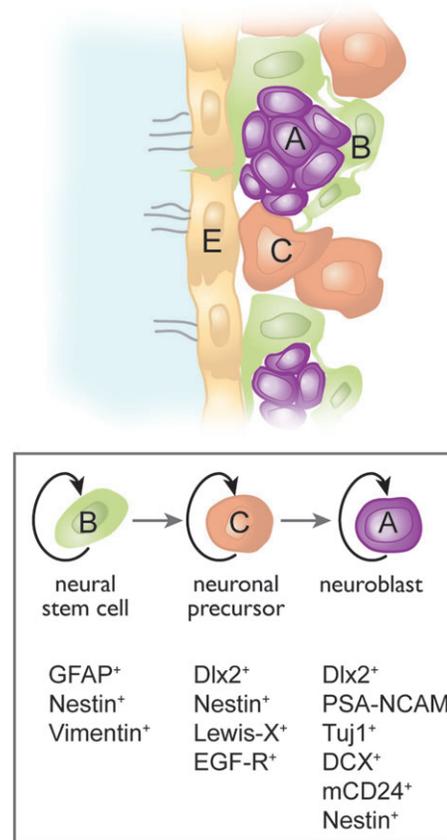


Figure 3 Neurogenic niche cytoarchitecture of the SVZ. A germinal niche is located near the lateral ventricle wall in which neural stem cells reside and support both neurogenesis and gliogenesis throughout life. The schema also indicates the intrinsic (i.e., genetic) factors that control adult neurogenesis in the SVZ. Note that adult neural stem cells are cells with the structural and molecular characteristics of astrocytes (called type B cells). The SVZ astrocytes, which express GFAP, divide to generate transit-amplifying cells (called type C cells), which in turn give rise to neuroblasts (type A cells) that migrate in the RMS to their final destination in the OB. Several intrinsic factors expressed by neural stem cells and progenitors are indicated. They control both proliferation rates and the fate of newborn cell. In addition, external factors (not shown here), such as neurotransmitters, hormones, and growth factors, may cooperate with intrinsic programs to modulate adult neurogenesis. (E) states for ependymal cells.

tegration site of new neurons. These brain regions are too deep for the use of conventional fluorescence microscopy, making such imaging a daunting task.

The noninvasive methods currently available for the imaging of deep brain structures in animals include ultrasounds, functional magnetic resonance imaging (fMRI), and positron emission tomography (PET). These techniques are much less efficient than microscopy, in terms of both acquisition time and resolution. In addition, although fMRI and PET allow functional imaging, the assessment of function with fMRI is indirect and based on measurements of blood flow, and far fewer functional contrast agents that can be used with PET are available than fluorescent probes. In contrast, optical imaging techniques, such as

conventional bench-top confocal and multiphoton fluorescence microscopy, combine micrometer-scale resolution with true neuronal functional imaging.

In recent years, improvements due to the use of new optical imaging techniques have greatly increased the range of problems in mammalian neurobiology that can be studied using visible light (Feng et al. 2000; Naldini and Verma 2000; Spergel et al. 2001; Zhang et al. 2002; Miyawaki et al. 2003; Bozza et al. 2004; Brecht et al. 2004; Griesbeck 2004; Hasan et al. 2004). Unfortunately, the use of novel high-resolution fluorescence imaging techniques has remained largely confined to *in vitro* preparations. For *in vivo* studies, the use of these techniques has been limited to superficial, or easily accessible, brain areas (Yuste et al. 2000) because light scattering attenuates the intensity of light exponentially with the distance traveled through solid tissue. In the mammalian brain, for example, the characteristic scattering length limits fluorescence imaging to depths of 500 μm (Kleinfeld et al. 1998). Efforts have recently been made to increase this limit, using a Ti:sapphire regenerative amplifier as an excitation source for ultra-deep two-photon microscopy (Theer et al. 2003). This technique has made it possible to visualize fluorescent neuronal cell bodies up to 1 mm deep within the neocortex of young mice (Theer et al. 2003). Despite such improvements, the imaging of migrating neuroblasts, which lie more than 5 mm into the adult forebrain, is not yet possible.

The development of fluorescence microendoscopy based on fiber optic technology has made it possible to image deep areas of the brain not previously accessible with light microscopy (Mehta et al. 2004). Fluorescence microendoscopy has the advantage of expanding the applicability of optical methods well beyond the imaging range of conventional microscopy and of allowing *in vivo* high-resolution fluorescence imaging in living animals (Jung and Schnitzer 2003; Jung et al. 2004; Adelsberger et al. 2005). In addition, once inserted in the brain, a fiber optic probe can be used for long recording sessions, with minimal bleaching and phototoxicity, allowing prolonged imaging sessions of cellular pro-

cesses. Indeed, we recently used optical fiber-based confocal fluorescence microendoscopy to study adult neurogenesis, visualizing migrating newly generated cells in living animals with a lateral optical resolution of 2 μm (Davenne et al. 2005; Lledo and Saghatelian 2005) (Figure 4). Migratory velocities were found to be nonlinear, with periods of inactivity interspersed between migratory spurts. We also observed bidirectional movements of migrating cells, which were occasionally seen to move back toward the SVZ.

The next step will be to use this approach to study the regulation of newly generated cell migration by intrinsic determinants and environmental factors. Further improvements in resolution will be necessary to record detailed cell movement and to resolve the dynamic spines of more mature neuroblasts, but the functional imaging of cells generated *in vivo* in adults may prove possible with the advent of voltage-sensitive or calcium indicator dyes driven by viral vectors.

Appendix 3: Viral vectors for labeling subsets of cells generated in adults

Studies of the various processes of maturation, migration, and integration of newborn neurons in the adult brain is simplified if it is possible to label particular types of cell reliably and to visualize them. In recent years, the use of viral vectors coupled with the production of green fluorescent protein (GFP) has revolutionized the investigation of adult neurogenesis. Unlike many of the methods commonly used to label cells fluorescently (e.g., lipophilic carbocyanin vital dyes such as DiI or DiO), the GFP label remains within the cell and its progeny, as long as the transgene encoding GFP remains present and as long as there are appropriate transcriptional elements to drive GFP expression. Thus, internalization, cell division, and cell growth do not decrease GFP fluorescence, whereas they would decrease the intensity of chemical dyes. In addition, as GFP is a heavy protein, it

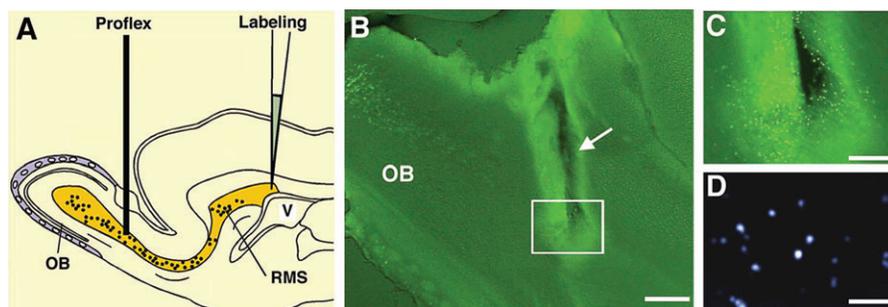


Figure 4 Deep brain imaging of neuronal precursors labeled with Cell Tracker Green (Molecular Probes, Poortgebouw, The Netherlands). **(A)** Cells in the SVZ were labeled by stereotaxic injection of the fluorophore. Two to 5 days later, migrating neuroblasts were detected with a 300- μm diameter optical probe (Proflex). **(B)** Following image acquisition in a living mouse, sagittal sections of the forebrain confirmed the location of the fiber optic (arrow) in the core of the OB. **(C)** Enlargement of boxed area shown in (B). **(D)** Example of image acquired at a penetration depth of 2.7 mm in the core of the OB. Thus, the fibered confocal fluorescence microscopy has proven unique capabilities to image cells *in vivo* in the brain of living animals with a micron-scale resolution and a limited invasiveness. Scale bars in (B): 500 μm ; (C): 100 μm , and (D): 50 μm . Modified from Davenne et al. (2005).

is not transferred from cell to cell—a critical property for the unequivocal tracing of cell lineages.

However, for imaging cells in intact tissues, GFP should ideally be expressed in a large subset of cells, but not in every cell. Many viral vectors are now available for gene transfer into neuronal cells (Washbourne and McAllister 2002; Kootstra and Verma 2003), offering a wide range of options in terms of infection specificity and efficiency and the delivery and regulation of particular transgenes (Davidson and Breakefield 2003).

Many viral vectors based on pseudorabies, herpes-, adeno-, and retrovirus backbones have been used to introduce reporter genes, such as GFP, into cultured cells and cells in intact animals. For example, Carlén et al. (2002) made use of a pseudorabies virus expressing GFP to cross the synapse between connected neurons. They injected this virus into the piriform cortex of adult mice exposed to bromodeoxyuridine (BrdU) in drinking water. BrdU and viral colabeling of periglomerular and granule cells show that new neurons reach the OB. Furthermore, the triple labeling of newly generated neurons shows that some BrdU-neuronal nuclei colabeled cells are also positive for *c-Fos* induction in adult mice exposed to odorants. Such results unequivocally support the notion that newly generated olfactory neurons are functionally integrated into the adult circuitry.

Unlike other viral systems, which preferentially infect quiescent cells, the Moloney murine leukemia virus (MoMuLV)-derived recombinant retroviral vectors selectively infect and permanently integrate into the genome of dividing cells, making it possible to visualize neuronal development from early progenitor cell stages. Thus, cycling neuroblasts can be labeled and their progeny followed for extended periods of time by injecting Moloney-based retroviral vectors encoding GFP into the adult SVZ. These vectors have made it possible to study the successive morphological maturation steps by which a neuroblast becomes a mature neuron (Petreanu and Alvarez-Buylla 2002). Following on from this work, the consecutive electrophysiological stages through which the neuroblast passes before becoming a fully mature neuron have been described, using similar GFP-encoding retroviral vectors (Beluzzi et al. 2003; Carleton et al. 2003). The proportion of different cell types generated from the transduced SVZ cells differs from that generated by transduced RMS cells (Hack et al. 2005). The construction of Moloney-based retroviral vectors carrying instructive genes (Hack et al. 2005; Kohwi et al. 2005; Marshall et al. 2005) makes it possible to alter the phenotypic fate of progenitor cells both predictably and efficiently—an intervention with potentially important clinical applications. As recombinant retroviral vectors target only those cells involved in postnatal neurogenesis, retroviral transduction could provide specific cell types for reconstruction of the brain after brain injury.

As for the Moloney-based retroviral vector approach, oncoretroviral vectors have been injected into the SVZ to

transduce dividing cells (Falk et al. 2002; Rogelius et al. 2005), but not neural stem cells, which are moderately quiescent (Morshead et al. 1994). Both types of retroviral vector transduce only rapidly dividing precursors and neuroblasts, the so-called A and C type cells. In contrast, lentiviral vectors, which transduce both dividing and nondividing cells, preferentially transduce neural stem cells (B type cells) when injected into the SVZ, due to the higher density of these cells in this forebrain region (Consiglio et al. 2004; Rogelius et al. 2005).

As MoMuLV-derived vectors are unable to deliver genes to nondividing cells, these retroviral particles have limited applications in the adult CNS. Lentiviruses are currently the most promising retroviruses for in vivo applications as their toxicity is low, and they can give long-term reporter gene expression. Lentiviruses containing specific promoters have been used in vivo for the specific labeling of neurons or glial cells (Jakobsson et al. 2003), and lentiviral systems have also been developed for RNAi (Rubinson et al. 2003; Tiscornia et al. 2003). Lentiviruses can also infect a wide range of species, and the lentiviral infection of embryos results in germline transmission of the integrated virus, providing a method for the stable expression of reporter genes in species other than mice (Lois et al. 2002; Rubinson et al. 2003; Tiscornia et al. 2003). These developments suggest that lentiviruses could be routinely used both to label neurons and to silence genes in vivo.

In summary, the use of viral vectors to deliver genes to the nervous system is very promising, both for basic research and for therapeutic applications. Several landmark applications of in vivo neuronal labeling have been reported in recent years. However, the continual development of new labeling strategies and advances in imaging techniques suggest that this is just the beginning. In the near future, the genetic manipulation of sparsely labeled neurons should become possible. In addition, improved GFP-based reporters for neuronal physiology studies should gradually find more in vivo applications. It may even become possible to use viral vectors for RNAi-based genetic screens for neuronal function in species in which traditional forward genetics has not previously proved feasible.

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References

- Adelsberger H, Garaschuk OJM, Milos J, Enache A, Konnerth A. 2005. In vivo monitoring of calcium waves in behaving mice. *Nat Neurosci.* 8:988–990.
- Alonso M, Viollet C, Gabellec M-M, Meas-Yadid V, Olivo-Marin J-C, Lledo P-M. 2006. Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb. *J Neurosci.* 26:10508–10513.
- Altman J, Das GD. 1965. Post-natal origin of microneurons in the rat brain. *Nature.* 207:953–956.
- Alvarez-Buylla A. 1990. Mechanism of neurogenesis in adult avian brain. *Experientia.* 46:948–955.
- Alvarez-Buylla A, Garcia-Verdugo JM. 2002. Neurogenesis in adult subventricular zone. *J Neurosci.* 22:629–634.
- Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD. 2001. A unified hypothesis on the lineage of neural stem cells. *Nat Rev Neurosci.* 2:287–293.
- Ambrogini P, Orsini L, Mancini C, Ferri P, Ciaroni S, Cuppini R. 2004. Learning may reduce neurogenesis in adult rat DG. *Neurosci Lett.* 359:13–16.
- Belluzzi O, Benedusi M, Ackman J, LoTurco JJ. 2003. Electrophysiological differentiation of new neurons in the olfactory bulb. *J Neurosci.* 23:10411–10418.
- Beltz BS, Sandeman DC. 2003. Regulation of life-long neurogenesis in the decapod brain. *Arthropod Struct Dev.* 32:39–60.
- Bozza T, McGann JP, Mombaerts P, Wachowiak M. 2004. In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron.* 42:9–21.
- Brecht M, Fee MS, Garaschuk O, Helmchen F, Margrie TW, Svovoda K, Osten P. 2004. Novel approaches to monitor and manipulate single neurons in vivo. *J Neurosci.* 24:9223–9227.
- Carlén M, Cassidy RM, Brismar H, Smith GA, Enquist LW, Frisen J. 2002. Functional integration of adult-born neurons. *Curr Biol.* 12:606–608.
- Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo P-M. 2003. Becoming a new neuron in the adult olfactory bulb. *Nat Neurosci.* 6:507–518.
- Cayre M, Scotto-Lomassese S, Malaterre J, Strambi C, Strambi A. Forthcoming 2007. Understanding the regulation and function of adult neurogenesis: contributions from an insect model, the house cricket. *Chem Senses.* 10.1093/chemse/bjm010.
- Cecchi GA, Petreanu LT, Alvarez-Buylla A, Magnasco MO. 2001. Unsupervised learning and adaptation in a model of adult neurogenesis. *J Comput Neurosci.* 11:175–182.
- Chambers RA, Potenza MN, Hoffman RE, Miranker W. 2004. Simulated apoptosis/neurogenesis regulates learning and memory capabilities of adaptive neural networks. *Neuropsychopharmacology.* 29:747–758.
- Consiglio A, Gritti A, Dolcetta D, Follenzi A, Bordignon C, Gage F, Vescovi AL, Naldini L. 2004. Robust in vivo gene transfer into adult mammalian neural stem cells by lentiviral vectors. *Proc Natl Acad Sci USA.* 101:14835–14840.
- Davenne M, Custody C, Charneau P, Lledo P-M. 2005. In vivo imaging of migrating neurons in the mammalian forebrain. *Chem Senses.* 30 (Suppl 1):i115–i116.
- Davidson BL, Breakefield XO. 2003. Viral vectors for gene delivery to the nervous system. *Nat Neurosci Rev.* 4:353–634.
- Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC. 2004. Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron.* 42:535–552.
- Derby CD. Forthcoming 2007. Why have neurogenesis in adult olfactory systems? The Presidential Symposium at the 2006 AChemS Conference. *Chem Senses.* 10.1093/chemse/bjm011.
- Dobrossy MD, Drapeau E, Arousseau C, Le Moal M, Piazza PV, Abrous DN. 2003. Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Mol Psychiatry.* 8:974–982.
- Doetsch F. 2003. A niche for adult neural stem cells. *Curr Opin Genet Dev.* 13:543–550.
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell.* 97:703–716.
- Ehninger D, Kempermann G. 2006. Paradoxical effects of learning the Morris water maze on adult hippocampal neurogenesis in mice may be explained by a combination of stress and physical activity. *Genes Brain Behav.* 5: 29–39.
- Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, Weiss S. 2004. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J Neurosci.* 24:8354–8365.
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. 1998. Neurogenesis in the adult human hippocampus. *Nat Med.* 4:1313–1317.
- Falk A, Holmstrom N, Carlen M, Cassidy R, Lundberg C, Frisen J. 2002. Gene delivery to adult neural stem cells. *Exp Cell Res.* 279:34–39.
- Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR. 2000. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron.* 28:41–51.
- Frielingdorf H, Schwarz K, Brundin P, Mohapel P. 2004. No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. *Proc Natl Acad Sci USA.* 101:10177–10182.
- Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV. 2004. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat Neurosci.* 7:1233–1241.
- Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo P-M. 2000. Importance of newly generated neurons in the adult OB for odor discrimination. *Proc Natl Acad Sci USA.* 97:1823–1828.
- Goldman SA, Nottebohm F. 1983. Neuronal production, migration and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci USA.* 80:2390–2394.
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. 1999. Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci.* 3:260–265.
- Gould E, Reeves AJ, Graziano MS, Gross CG. 1999. Neurogenesis in the neocortex of the adult primates. *Science.* 286:548–552.
- Griesbeck O. 2004. Fluorescent proteins as sensors for cellular functions. *Curr Opin Neurobiol.* 14:636–641.
- Hack MA, Saghatelian A, de Chevigny A, Pfeifer A, Ashery-Padan R, Lledo P-M, Gotz M. 2005. Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci.* 8:865–871.
- Harrison PJ, Cate HS, Steullet P, Derby CD. 2003. Amputation-induced activity of progenitor cells leads to rapid regeneration of olfactory tissue in lobsters. *J Neurobiol.* 55:97–114.

- Hasan MT, Friedrich RW, Larkum ME, Euler T, Both M, Duebel J, Waters J, Giese G, Bujard H, Griesbeck O, et al. 2004. Functional fluorescent Ca²⁺ indicator proteins in transgenic mice under TET control. *PLoS Biol.* 2:763–775.
- Hinds JW. 1968. Autoradiographic study of histogenesis in the mouse OB. II. Cell proliferation and migration. *J Comp Neurol.* 134:305–322.
- Jakobsson J, Ericson C, Jansson M, Bjork E, Lundberg C. 2003. Targeted transgene expression in rat brain using lentiviral vectors. *J Neurosci Res.* 73:876–885.
- Jung JC, Mehta AD, Aksay E, Stepnoski R, Schnitzer MJ. 2004. In vivo mammalian brain imaging using one- and two-photon fluorescence microendoscopy. *J Neurophysiol.* 92:3121–3133.
- Jung JC, Schnitzer MJ. 2003. Multiphoton endoscopy. *Opt Lett.* 28:902–904.
- Kleinfeld D, Mitra PP, Helmchen F, Denk W. 1998. Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layers 2 through 4 of rat neocortex. *Proc Natl Acad Sci USA.* 95:15741–15746.
- Kohwi M, Osumi N, Rubenstein JLR, Alvarez-Buylla A. 2005. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. *J Neurosci.* 25:6997–7003.
- Koketsu D, Mikami A, Miyamoto Y, Hisatsune T. 2003. Nonrenewal of neurons in the cerebral neocortex of adult macaque monkeys. *J Neurosci.* 23:937–942.
- Kootstra NA, Verma IM. 2003. Gene therapy with viral vectors. *Annu Rev Pharmacol Toxicol.* 43:413–439.
- Kornack DR, Rakic P. 1999. Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc Natl Acad Sci USA.* 96:5768–5773.
- Kornack DR, Rakic P. 2001. Cell proliferation without neurogenesis in adult primate neocortex. *Science.* 294:2127–2130.
- Laywell ED, Rakic P, Kukekov VG, Holland EC, Steindler DA. 2000. Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA.* 97:13883–13888.
- Lemasson M, Saghatelian A, Olivo-Marin JC, Lledo P-M. 2005. Neonatal and adult neurogenesis provide two distinct populations of granule cells in the mouse OB. *J Neurosci.* 25:6816–6825.
- Lie DC, Dziejczapolski G, Willhoite AR, Kaspar BK, Shults CW, Gage FH. 2002. The adult substantia nigra contains progenitor cells with neurogenic potential. *J Neurosci.* 22:6639–6649.
- Lledo P-M, Gheusi G. 2003. Olfactory processing in a changing brain. *Neuroreport.* 14:1655–1663.
- Lledo P-M, Gheusi G, Vincent JD. 2005. Information processing in the mammalian olfactory system. *Physiol Rev.* 85:281–317.
- Lledo P-M, Lagier S. 2006. Local interneurons transduce spatial coding into temporal patterning in the mammalian olfactory bulb. *Semin Cell Dev Biol.* 17:443–453.
- Lledo P-M, Saghatelian A. 2005. Integrating new neurons into the adult olfactory bulb: joining the network, life/death decisions, and the effects of sensory experience. *Trends Neurosci.* 28:248–254.
- Lois C, Alvarez-Buylla A. 1994. Long-distance neuronal migration in the adult mammalian brain. *Science.* 264:1145–1148.
- Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. 1996. Chain migration of neuronal precursors. *Science.* 271:978–981.
- Lois C, Hong EJ, Pease S, Brown EJ, Baltimore D. 2002. Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science.* 295:868–872.
- Magavi SSP, Mitchell BD, Szentirmai O, Macklis JD. 2005. Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo. *J Neurosci.* 25:10729–10739.
- Mandairon N, Jourdan F, Didier A. 2003. Deprivation of sensory inputs to the olfactory bulb up-regulates cell death and proliferation in the subventricular zone of adult mice. *Neuroscience.* 119:507–516.
- Marshall CAG, Novitsch BG, Goldman JE. 2005. Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. *J Neurosci.* 25:7289–7298.
- Mason HA, Ito S, Corfas G. 2001. Extracellular signals that regulate the tangential migration of OB neuronal precursors: inducers, inhibitors, and repellents. *J Neurosci.* 21:7654–7763.
- Mechawar N, Saghatelian A, Grailhe R, Lledo P-M, Changeux JP. 2004. Nicotinic receptors regulate the survival of newborn neurons in the adult OB. *Proc Natl Acad Sci USA.* 101:9822–9826.
- Mehta AD, Jung JC, Flusberg BA, Schnitzer MJ. 2004. Fiber optic in vivo imaging in the mammalian nervous system. *Curr Opin Neurobiol.* 14:617–628.
- Mercier F, Kitasako JT, Hatton GI. 2002. Anatomy of the brain neurogenic zones revisited: fractones and the fibroblast/macrophage network. *J Comp Neurol.* 451:170–188.
- Miwa N, Storm DR. 2005. Odorant-induced activation of extracellular signal-regulated kinase/mitogen-activated protein kinase in the OB promotes survival of newly formed granule cells. *J Neurosci.* 25:5404–5412.
- Miyawaki A, Sawano A, Kogure T. 2003. Lighting up cells: labelling proteins with fluorophores. *Nat Cell Biol.* 5(Suppl):S1–S7.
- Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D. 1994. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron.* 13:1071–1082.
- Murase S, Horwitz AF. 2002. Deleted in colorectal carcinoma and differentially expressed integrins mediate the directional migration of neural precursors in the rostral migratory stream. *J Neurosci.* 22:3568–3579.
- Najbauer J, Leon M. 1995. Olfactory experience modulated apoptosis in the developing olfactory bulb. *Brain Res.* 674:245–251.
- Naldini L, Verma IM. 2000. Lentiviral vectors. *Adv Virus Res.* 55:599–609.
- Palmer TD, Willhoite AR, Gage FH. 2000. Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol.* 425:479–494.
- Peretto P, Merighi A, Fasolo A, Bonfanti L. 1997. Glial tubes in the rostral migratory stream of the adult rats. *Brain Res Bull.* 42:9–21.
- Peretto P, Merighi A, Fasolo A, Bonfanti L. 1999. The subependymal layer in rodents: a site of structural plasticity and cell migration in the adult mammalian brain. *Brain Res Bull.* 49:221–243.
- Peteanu L, Alvarez-Buylla A. 2002. Maturation and death of adult-born OB granule neurons: role of olfaction. *J Neurosci.* 22:6106–6113.
- Rakic P. 2002. Adult neurogenesis in mammals, an identity crisis. *J Neurosci.* 22:614–618.
- Rochefort C, Gheusi G, Vincent JD, Lledo P-M. 2002. Enriched odor exposure increases the number of newborn neurons in the adult OB and improves odor memory. *J Neurosci.* 22:2679–2689.
- Rogelien N, Ericson C, Lundberg C. 2005. In vivo labeling of neuroblasts in the subventricular zone of rats. *J Neurosci Methods.* 142:285–293.

- Rubinson DA, Dillon CP, Kwiatkowski AV, Sievers C, Yang L, Kopinja J, Rooney DL, Ihrig MM, McManus MT, Gertler FB, et al. 2003. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. *Nat Genet.* 33:401–406.
- Saghatelian A, Roux P, Migliore M, Rochefort C, Charneau P, Shepherd G, Lledo P-M. 2005. Activity-dependent adjustments of the inhibitory network in the adult OB following early postnatal deprivation. *Neuron.* 46:103–116.
- Schmidt M. Forthcoming 2007. The olfactory pathway of decapod crustaceans—an invertebrate model for life-long neurogenesis. *Chem Senses.* 10.1093/chemse/bjm008.
- Schmidt-Hieber C, Jonas P, Bischofberger J. 2004. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature.* 429:184–187.
- Scotto-Lomassese S, Strambi C, Strambi A, Aouane A, Augier R, Rougon G, Cayre M. 2003. Suppression of adult neurogenesis impairs olfactory learning and memory in an adult insect. *J Neurosci.* 23:9289–9296.
- Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A. 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J Neurosci.* 21:7153–7160.
- Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, Cross JC, Weiss S. 2003. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science.* 299:117–120.
- Spergel DJ, Kruth U, Shimshek DR, Sprengel R, Seeburg PH. 2001. Using reporter genes to label selected neuronal populations in transgenic mice for gene promoter, anatomical, and physiological studies. *Prog Neurobiol.* 63:673–686.
- Theer P, Hasan MT, Denk W. 2003. Two-photon imaging to a depth of 1000 microns in living brains by use of a Ti:Al₂O₃ regenerative amplifier. *Opt Lett.* 28:1022–1024.
- Tiscornia G, Singer O, Ikawa M, Verma IM. 2003. A general method for gene knockdown in mice by using lentiviral vectors expressing small interfering RNA. *Proc Natl Acad Sci USA.* 100:1844–1848.
- Tramontin AD, Garcia-Verdugo JM, Lim DA, Alvarez-Buylla A. 2003. Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. *Cereb Cortex.* 13:580–587.
- Van der Borght K, Wallinga AE, Luiten PG, Eggen BJ, Van der Zee EA. 2005. Morris water maze learning in two rat strains increases the expression of the polysialylated form of the neural cell adhesion molecule in the dentate gyrus but has no effect on hippocampal neurogenesis. *Behav Neurosci.* 119:926–932.
- Van Praag H, Christie BR, Sejnowski TJ, Gage FH. 1999. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA.* 96:13427–13431.
- Wang S, Scott BW, Wojtowicz JM. 2000. Heterogenous properties of dentate granule neurons in the adult rat. *J Neurobiol.* 42:248–257.
- Washbourne P, McAllister AK. 2002. Techniques for gene transfer into neurons. *Curr Opin Neurobiol.* 12:566–573.
- Weiss S, Reynolds BA, Vescovi AL, Morshead C, Craig CG, van der Kooy D. 1996. Is there a neural stem cell in the mammalian forebrain? *Trends Neurosci.* 19:387–393.
- Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG. 2002. Long-term survival and cell death of newly generated neurons in the adult rat OB. *Eur J Neurosci.* 16:1681–1689.
- Yuste R, Lanni F, Konnerth A. 2000. Imaging neurons: a laboratory manual. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
- Zhang J, Campbell RE, Ting AY, Tsien RY. 2002. Creating new fluorescent probes for cell biology. *Nat Rev Mol Cell Biol.* 3:906–918.

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