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Is Adult Neurogenesis Essential for Olfaction?

Françoise Lazarini^{1,2} and Pierre-Marie Lledo^{1,2}

¹ Institut Pasteur, Laboratory for Perception and Memory, F-75015 Paris, France.

² CNRS, URA2182, F-75015 Paris, France.

Corresponding author: Lledo, P.-M. (pmlledo@pasteur.fr)

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Abstract

In mammals, new neurons are recruited into restricted brain areas throughout life. Adult-born neurons produced in the subventricular zone migrate towards the olfactory bulb. Although thousands neurons reach this central structure every day, the functional impact of their integration into mature circuits remains debated. Recent investigations have revealed no striking sensory deficit *per se* when adult bulbar neurogenesis is challenged. **However**, cognitive functions, such as perceptual learning or olfactory memory, are clearly impaired. In this review, we highlight the role of network activity in shaping ongoing neurogenesis and in turn, how the integration of adult-born neurons refines preexisting network function, and consequently olfactory behavior.

Introduction

New neurons are continuously generated in two discrete areas of the adult brain, the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone of the hippocampus [1]. The latter give rise to new granule neurons, which mature locally in the dentate gyrus, while the former produces neuroblasts undergoing a long migration along the rostral migratory stream (RMS) *en route* to the olfactory bulb (OB), the first olfactory relay in the CNS. Neural progenitors finish rostral migration in the core of the OB where they begin radial migration and mature into interneurons [2]. Despite extensive cellular characterization of individual adult-born neurons, the impact of adult neurogenesis on OB circuit function and olfactory behavior is still unclear. It has been argued that ongoing adult neurogenesis is essential for structural maintenance of the OB circuit since blocking adult neurogenesis depletes the bulb of interneurons [3]. This review will focus on new insights that indicate the function of

adult bulbar neurogenesis goes beyond the mere maintenance of neuronal circuits. In particular, we detail the functional characteristics of new neurons and how they shape mitral cell function and olfactory behavior.

Functions for old and new neurons

In rodents, olfaction is a key chemosensory modality that enables diverse essential functions such as food selection, danger detection and conspecific interactions. To fulfill this repertoire of functions, the olfactory system has to detect and discriminate odorants from a rich and varied olfactory environment, and then preserve this information in the form of memories. Remarkably, these tasks are highly flexible and adjustable by behavioral states (*i.e.*, the context of sensory exposure), particularly by attention and motivation. **Separate** microcircuits in the OB achieve these functions (**Figures 1 and 2**). For the vast majority of odorants, sensory detection triggers a behavioral response that is dictated by experiential associations. However, for a subset of odorants, stereotyped behaviors are induced both between individuals and upon repeated exposure for a given subject [4].

Sensory information processing

In mammals, chemical stimuli are transduced into spike trains by olfactory receptor neurons. These sensory neurons release glutamate onto the distal dendrites of mitral and tufted cells, the two relay neurons of the OB circuit. Because the flow of information goes from the external world to the brain, this sensory input represents the **so-called bottom-up pathway (Figure 3)**. **However, the OB circuit is more than a mere relay station acting as a feedforward filter of sensory inputs. This** circuit actively processes and refines sensory information using two classes of local inhibitory

interneurons: Periglomerular cells and granule cells [5] (**Figure 2**). Periglomerular cells (GABAergic and dopaminergic interneurons), project to one or a few glomeruli and interact with olfactory nerve terminals and/or the primary dendrites of relay neurons [6]. Granule cells contribute to the network activity of the OB by inhibiting mitral cell dendrites via reciprocal dendrodendritic synapses, and by facilitating both mitral cell synchronization and network oscillations [7-9].

Odor discrimination, learning and memory are thought to depend on the synaptic interplay between interneurons and mitral cells [10-12]. Local synaptic inhibition is essential for odor-discrimination performance [13] **and for olfactory perceptual learning [14] (see glossary)**. Granule cells might achieve this function by enabling the discharge patterns of mitral cells to diverge from similar and confusing sensory input patterns imposed by sensory receptor neurons (a task called pattern segregation).

Bulbar output patterns

After being processed and refined, odor information in the OB is transmitted to higher-order brain structures in the primary and accessory olfactory cortex. These centers receive excitatory input from mitral cells and presumably from associations between OB output patterns and stimuli in the environment. The behavioral state modulates the OB activity *via* the centrifugal fibers entering the bulbar circuit (**Figures 1 and 2**). These centrifugal inputs **are comprised of cortical, subcortical and modulatory projections [15]:** 1) glutamatergic fibers from frontal cortex and olfactory cortex [16,17], 2) modulatory projections from the locus coeruleus (norepinephrin), the horizontal limb of the diagonal band of Broca (acetylcholine), and the dorsal raphe nucleus (serotonin) [18,19]. **Centrifugal inputs shape the function of the bulbar**

network on various timescales (from milliseconds to days) and various contexts (see below). Interestingly, most of the centrifugal connections target granule cells [20], and tune the dendrodendritic inhibition of mitral cells [21,22]. On the other hand, centrifugal serotonergic fibers preferentially target periglomerular cells and modulate odor input at the beginning of odor information processing, acting indirectly on olfactory receptor neuron terminals through a modulation of periglomerular inhibitory cells [23].

By modulating the activity of local interneurons, centrifugal inputs shape the spatial and temporal firing patterns of mitral cell activity, and thus are important for producing dynamic odor representation in the context of different behavioral states [23-27]. Much of the sensory input that enters the brain does so because we actively seek for sensory information through sniffing and others means, and because sensory investigation can often be triggered by environmental cues. In this case, centrifugal inputs to the OB provide the contextual information required for sensory formatting of odor representations, learning and memory. In short, this *top-down* pathway represents a mechanism by which the brain makes sense of the olfactory content (**Figure 3**). Olfactory bulb activity, and thus odor perception, is shaped by the interaction between the *bottom-up* and the *top-down* pathways. Experiments that manipulate the *top-down* pathway have been shown to influence olfaction. For instance, changing the impact of centrifugal cholinergic inputs to the OB circuit affects olfactory perception by changing rodents' ability to differentiate between perceptually similar odorants [28,29]. Similarly, experimental manipulation of noradrenergic activity in the OB has resulted in changes in olfactory perception and memory formation [30] and olfactory habituation memory [31]. Decreasing glutamatergic feedback from the olfactory peduncle to the OB has revealed a key role for

centrifugal inputs in changing the formation of odor–reward associations, but not primary bulbar odor representations [32]. In this context, it is noteworthy that the first connections of adult-born neurons are established with centrifugal fibers, which can provide excitation to these young neurons soon after they arrive and integrate within the OB circuit [2,33,34]. Centrifugal afferents offer a pathway by which new neuron activity, and thus olfactory function, can be shaped by the behavioral state and olfactory experience, including learning. Further studies will aim at characterizing the detailed synaptic mechanisms that links adult-generated neurons to centrifugal projections.

As we shall discuss below, both *bottom-up* and *top-down* pathways may regulate adult neurogenesis in a tightly orchestrated manner following temporal constraints defined by the progression of neuroblast maturation. According to this hypothesis, new neurons act as coincident detectors by appearing at the nexus between two key events: the spatial dimension and developmental stages (Figure 4).

Wiring adult-generated neurons into preexisting circuits

Thousands adult-born neurons arrive to the bulb per day and integrate into the existing neural circuit within just a few days [35]. About 95% of the new cells differentiate into granule cells, less than 3% become periglomerular cells [36], and a very small proportion give rise to glutamatergic juxtglomerular neurons [37]. Each subtype of new bulbar interneuron originates from specific neural stem cells located in distinct regions of the SVZ-RMS system [38-40]. Adult-born neurons express GABA and glutamate receptors as soon as they start migrating [41] and when entering the deeper layers of the OB [34,42,43] before gradually increasing their responsiveness to both GABA and glutamate. A recent study on the dynamic pattern

of synaptogenesis onto newborn neurons entering the OB has revealed the unexpected contribution of centrifugal afferents to new neuron maturation [34]. By observing that proximal synaptic structures on granule cell soma and dendrites form much earlier than the dendrodendritic contacts with resident mitral cells, this work shed light on the early function of centrifugal fibers on adult neurogenesis, consistent with a previous report [44]. The source of the centrifugal inputs that form early connections remains to be determined. Revealing their identity will be critical toward further understanding on the contribution of the top-down process in controlling OB adult neurogenesis.

The development of input synapses in adult-formed OB neurons is mostly complete by four weeks after the cell birth, and is sensitive to neuronal activity during a restricted period [33,45]. One-half of new neurons die during their process of dendritic branching and synaptogenesis between 15 and 40 days after cell birth, [45-51]. Most adult-generated neurons that survive this period of cell death will survive for several months and become indistinguishable from pre-existing neurons [46,48,49,52-54], but see [47].

Having established that **not only thousands** adult-born neurons integrate into the existing OB circuit **per day**, but also **about half of them** survive, it was important to determine if the integration of new neurons influences OB-related behaviors. To address this issue, a popular strategy consists in ablating adult neurogenesis using systemic or genetic techniques. Unfortunately, no available technique can specifically reduce the population of adult-generated OB neurons. All reported ablation techniques present both advantages and undesired off-target effects, which may explain why discrepancies have emerged between these efforts (see **Box 1**). Further

studies using selective and reversible ablation of adult neurogenesis should help elucidate the respective roles of neurogenic systems to animal behavior.

Interactions between sensory experience and adult neurogenesis

To elucidate the role of sensory inputs on adult neurogenesis many studies have used passive stimulation paradigms [55-57]. Passive olfactory experiences include either transient odorant exposure or olfactory enrichment lasting several days. As described below, recent findings demonstrate that some passive olfactory experiences, but not all, impact bulbar neurogenesis (see **Box 2**).

Effects of passive odorant exposure

Activity-dependent factors regulate many aspects of bulbar neurogenesis including synapse formation and the survival of new neurons [45,47,50,55,56,58,59]. Whereas passive/short-term exposure to odorants does not change the survival of new neurons [50,58,60,61], passive/long-term exposure to odorant-enriched environment enhances both SVZ proliferation [40] and the survival of new granule cells [55,59]. Conversely, long-term sensory deprivation reduces the survival of adult-born granule cells [45,56,59,62]. Interestingly, new cells that survive after sensory deprivation display an increased density of proximal input synapses in the unbranched apical dendrite [33]. It remains to be established whether adult-born granule cells survive in the absence of sensory input because they are able to compensate for their excitatory drive by enhancing their synapse density. Similarly, adult-born periglomerular neurons display activity-dependent survival. Sensory deprivation decreases [56], whereas olfactory enrichment [55] increases the survival of distinct populations of adult-born periglomerular neurons, supporting the hypothesis that their

integration rate is dependent on the magnitude of sensory activity [59]. Also, sensory enrichment accelerates the development of their glutamatergic input synapses as visualized by genetic synaptic markers [63].

Impact of olfactory learning

Odor sampling behavior reflects not only current perception but also expectation following learning rules [24,64,65], highlighting once again the importance of the *top-down* pathway in processing the sensory information in the OB circuit. Olfactory perception is highly dependent on experience, as repeated exposures to similar odorants can improve olfactory discrimination (a task called olfactory perceptual learning) in rodents and humans [66,67]. Not surprisingly, adult neurogenesis also constitutes a substrate to the context-dependent modulation of odor responses (see **Box 2**). For instance, bulbar neurogenesis increases following olfactory perceptual learning [68]. Moreover, adult-generated neurons are preferentially involved in processing of experienced odorants rather than naïve odorants [68]. Similarly, bulbar neurogenesis increases following an active sensory experience such as associative olfactory learning (conditioned mice), but not by the simple passive exposure to odorants [50,58,60,61]. Moreover, olfactory learning in mice induces clustering of newborn cells in specific regions of the granule cell layer, 5 days post-conditioning, in an odorant-specific way [61]. In the time window when learning increases survival (*i.e.*, 18-30 days after neuron birth), new granule cells begin receiving glutamatergic synaptic contacts. This suggests that the survival of adult-generated granule cells might be sensitive to experience during the period of early synaptogenesis. Interestingly, olfactory learning induces a spatial distribution of newborn neurons, which is conserved after 30 days but not after 90 days post-conditioning, suggesting

that cell death might occur in 30 to 90 day-old newborn cells [61]. Since learning to discriminate between two odorants reduces the survival of 38/45-day-old granule cells [50,69], but not 65-days-old neurons [50], it is possible that olfactory learning promotes the survival of specific 18-30 day-old neurons and simultaneously induces cell death of other 38-45-day-old neurons. This would explain why the previous olfactory learning tasks observe no net increase in granule cell survival.

Recent experiments demonstrate that adult-born granule cell synaptogenesis is sensitive to changes in synaptic input and have suggested that both synaptogenesis and neuronal survival occur after the neuron receives a minimum threshold of excitation from local glutamatergic inputs or centrifugal fibers [2,33,70]. Further studies will have to clarify the respective functions of sensory-driven inputs and centrifugal fibers on the regulation of newborn neuron survival and synaptogenesis. Particularly, it will be important to decipher the mechanism by which some adult-generated neurons are "primed" to survive following a learning task **that involves the *top-down* pathway.**

Functions of adult-born neurons in the olfactory bulb circuit

The potential functions of adult-born neurons in the OB can be summarized into four main tasks: (1) maintenance of the OB circuit; (2) shaping sensory information processing; (3) supporting learning processes, and/or (4) mediating odor memory. Below we discuss these non-exclusive options.

The contribution of adult neurogenesis to circuit maintenance

In the developing OB, most newborn granule cells reside in the superficial granule cell layer and can survive for the lifetime of the animal [48]. This phenomenon is

reversed during adulthood although estimates for the precise rate of neuronal turnover remain debated. Some studies estimate that only a small proportion (~ 15%) of the total population of interneurons turnover in the granule cell layer [71,72]. Others suggest that most granule cells located deeper in the granule cell layer and half of the superficial ones are subjected to continuous turnover [3,73]. These inconsistencies may result from different methodologies and should be solved in the future using different models. Interestingly, cell survival seems to depend on the quantity of newly produced neurons that reaches the OB: When bulbar neurogenesis is reduced after SVZ irradiation, the survival rate of the newborn neurons reaching the bulb is significantly prolonged [51], but see [73]. This observation could reflect a competition of adult-generated neurons for survival factors released by the neural targets, just as they compete with existing neurons for their innervations with synaptic projections.

Impact of adult neurogenesis on shaping sensory information processing

Adult-born neurons target mitral and tufted cells in the OB providing an additional inhibitory function to the bulbar circuit. However, adult-born neurons also can target local inhibitory interneurons yielding disinhibitory function in the OB circuit (Bardy et al., the Society For Neuroscience, 2009). Thus, the contribution of adult-generated neurons to bulbar physiology remains unclear and conflicting results have been reported regarding the functional outcomes of adult neurogenesis for olfaction [73-75]. One study found no impact of neurogenesis ablation on bulbar activity following efficient suppression of neurogenesis by irradiation [73]. In contrast, another study based on intraventricular infusion of the antimetabolic agent Arac-C reported strong changes in mitral and tufted cell activity induced by depleting adult-born neurons,

leading to impairment of short-term olfactory memory [75]. The contrasting results obtained in the two aforementioned studies highlight the need for further investigations to decipher the role of adult-generated neurons in modulating mitral cell activity. Finally, it is worth noting that a correlation between the turnover rate of adult bulbar neurogenesis and olfactory discrimination time was recently established [74]. This study used a pan-caspase inhibitor to impair the natural elimination of adult-born granule cells, and found that the time required to discriminate between two similar odorants was subsequently increased. Therefore, the continuous recruitment of adult-born neurons may lead to changes in the strength of the temporal binding of signals originating from different odorant receptors. This might be critical not only for adjusting odor discrimination, but also for perceptual learning **as described above** [76].

Impact of adult neurogenesis on learning processes

Previous studies have demonstrated that active sensory processing results in perceptual learning, associative learning, and memory formation, through changes in OB network activity [60]. Adult-generated neurons dedicated to the OB circuit seem not to be involved in perception *per se* (**see Box 3**), but rather in cognitive function (**see Box 2**). For instance, manipulation of bulbar neurogenesis revealed that the supply of new neurons is not necessary for odorant detection, discrimination and acquisition of an associative olfactory learning [3,51,61]. On the other hand, adult OB neurogenesis is required for olfactory fear conditioning [73], olfactory perceptual learning [68] and long-term olfactory memory of an associative olfactory learning [51,61].

Developing adult-generated interneurons are unique in that their connectivity adapts to the degree of neuronal activity in the host circuit [2,33,52,77,78]. They also trigger unique responses during odorant familiarization [79], suggesting a key role in olfactory processing and plasticity. This idea has received recent support from an electrophysiological study [80]. It was found that soon after synaptic integration, adult-born neurons displayed robust long-term potentiation not found in preexisting neurons. This ability is progressively lost as the cells mature over time. These findings are similar to those reported for adult-generated dentate gyrus neurons, which exhibit synaptic plasticity fading with time [81]. Interestingly, the synaptic plasticity of young adult-born OB interneurons occurs during a time window when new neurons are initially added to the circuit indicating that they might play a role in circuit plasticity required for learning. Although synaptic development is mostly completed by four weeks after cell birth [43,44,47,49,52], spines may still undergo rearrangement after this period indicating that mature OB interneurons still maintain some capacity for synaptic modification [52]. Thus, the precise stages at which new OB neurons play important role for olfaction remains to be characterized.

Role of adult neurogenesis in olfactory memory

The recruitment of new neurons in the OB circuitry might allow the association of otherwise **temporally** distinct input signals originating from bottom-up and/or top-down connections (**Figure 4**). Interestingly, newly-formed neurons receive first synapses from centrifugal fibers before establishing contact with afferent inputs from mitral cells [34,44]. Through these early contacts with the *top-down* pathway, newborn neurons are positioned to provide a *top-down* context to olfactory information. In addition, these early contacts may support memory based on

integrating recursive signaling from the olfactory cortex and the OB. In line with this **hypothesis**, a correlation has been found between the degree of bulbar neurogenesis and short-term olfactory memory [55,82,83]. **Moreover, blocking bulbar neurogenesis before and during odor enrichment prevents olfactory perceptual learning, and thus memory formation of experienced odorants in mice [68]. Early contacts with top-down fibers offer a mechanism by which odor experience increases newborn granule cell responsiveness and improves olfactory discrimination learning [14,58,68]. Altogether, mounting evidence supports the hypothesis that adult-generated neurons are required in the formation and/or shaping of olfactory memory circuits.**

Halting adult neurogenesis by radiation, or Ara-C treatment, also modifies long-term odor associative memory tasks [51,61]. In contrast, long-term memory has been shown to be unaffected in mice producing a neuron-specific enolase-diphtheria toxin [3], and in mice treated with Ara-C [75] despite the significant neurogenic reduction in the two latter studies. Details of the experimental design might have contributed to these discrepancies, including the strains of animal models used, the off-target effects of ablation methods and the behavioral paradigm used. Considering the latter option, it is worth noting that **some authors** [3, 75] employed a non-operant task whereas **we** [51] and **others** [61] used operant tasks. Operant conditioning differs from non-operant conditioning in that the animal is motivated to acquire a particular behavior to complete its task. Non-operant and operant conditioning paradigms for investigating olfactory abilities often lead to different conclusions, even from the same animal model, indicating they might support different olfactory functions [84-86]. The discrepancy between the two learning paradigms might originate from the participation of top-down centrifugal fibers brought into play during operant conditioning **only**.

Together, these data indicate that adult-born neurons are required for long-term memory of associative olfactory tasks involving active learning [51,61], but not for non-operand associative training [3,75], but see [73]. These findings also indicate that the need for adult-born neurons might depend on the nature of the task. A recent study shows that the procerebrum of invertebrates, which is analogous to the mammalian OB, represents the storage site for associative olfactory memories and is directly involved in the memory retrieval process [87]. In the OB, adult-generated neurons may be the main targets of learning-induced changes originating from sensory inputs (the sensory space) and centrifugal fibers (the internal state), acting as key elements in the recall of memory traces (**Figure 4**). Similarly, a role for adult hippocampal neurogenesis in memory storage, rather than in learning set, was proposed in rodents [88]. **Interestingly, as in the OB, developing immature hippocampal neurons might serve as pattern separators [89]. This function is particularly important for the accuracy of memory encoding. Pattern separation is also essential for the formation and use of memories derived from stimuli that are close in space or time.**

Concluding remarks

Olfactory sensory processing begins in sensory neurons that transform chemical signaling into electrical patterns. This stage is unlikely to change over time during animal's experience. Then, mitral cells distribute neural activity widely to downstream structures **and are influenced by a combination of sensory input** and the behavioral state. This second transformation step constitutes a target not only for adult neurogenesis but also for *top-down* connections. Because the ability to process sensory information depends on the functional architecture and synaptic connectivity

of the OB, adult neurogenesis has a particular impact on information processing. Understanding the precise functions of adult bulbar neurogenesis will require elucidating the mechanisms that control the development of **both** the synaptic inputs and outputs of the adult-born OB interneurons, and how these precise sequence impact olfactory behavior.

Activity-dependent regulation of neurogenesis and experience-dependent participation of new neurons in olfactory information processing both highlight the relationship between adult neurogenesis and olfactory abilities. In this review, we have highlighted the potential functions of new neurons as the following: 1) Maintaining the OB circuit. Newborn neurons might replace dying interneurons in the OB, thus providing a structural role for the maintenance of the network. 2) Tuning information processing. Adult-born neurons target mitral cells in the OB thus providing sufficient inhibitory activity necessary for sharp pattern segregation. In some circumstances, it might be vital to decorrelate and modify the odor representation that is going to be ultimately sent to downstream brain structures. 3) Role in learning. Because adult-born neurons express unique synaptic plasticity, these neurons are more suited than mature neurons to encoding new information. 4) Role in memory. **As we propose**, newborn neurons might be able to provide significant context to the information content **due to their role as coincidence detectors**. Even in the absence of sensory inputs, newborn neurons could be driven by centrifugal fibers, a feature that could represent part of a memory process.

Accumulating evidence suggests that modifications to adult neurogenesis alter olfactory processing in diverse ways. It will be of great interest to explore precisely at what stage of development the adult-generated OB neurons contribute to olfactory perception, odor memory, or fear responses. Are new neurons functionally important

when they are young and more excitable? Or are they most important later when they are mature and fully integrated into the existing network? Also, future studies will have to be designed to selectively target distinct populations of adult-born interneurons (periglomerular cells *versus* granule cells). So far, the role for new periglomerular cells in olfactory information processing remains unknown. Finally, further studies on the relationship/cross-talk between adult-generated neurons and centrifugal projections will be certainly helpful to unravel the cellular and molecular mechanisms of odor discrimination and those involved in forming and restituting olfactory traces after learning. Clarification of these issues will highlight not only how OB neurogenesis impacts olfaction, but will inform the future development of cellular therapies for neurological disorders **(Box 4)**.

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Box 1. Strategies employed for challenging adult neurogenesis.

Most of the approaches today aim at impairing neurogenesis using radiation, generic anti-mitotic [91] or anti-proliferative [92] drug treatment, genetic manipulation or aging mice models. Studies using traditional knockout of genes essential for neurogenesis are interesting, but often result in conflicting data on olfactory behavior [84,93-95]. Their interpretations are often limited by abnormalities of the whole brain structure or compensatory effects elicited during development.

Another alternative model of decreased neurogenesis is aging [93]. However, it is difficult to interpret unambiguously whether the sensory deficit found in this model results from the specific decrease of bulbar neurogenesis or to other impairment in the aging brain.

Treatments with antimetabolic drugs, such as SVZ infusion of the antimetabolic drug cytosine arabinosine (Ara-C), are also useful to halt adult SVZ neurogenesis [61,68,75]. However, these treatments are unspecific since they target all dividing cells, including neurovascular cell types and oligodendrocyte precursors cells [96]. In addition, Ara-C is also toxic for non-dividing cells, such as oligodendrocytes [96] and post-mitotic neurons [97]. Although no side effects were observed at low doses [68,75], slightly higher doses cause weight loss, bone marrow suppression, cerebellar neurotoxicity and reduction in motor coordination. In addition, antimetabolic treatment probably reduces cell division throughout a large portion of the brain, even when the drug is focally infused into the parenchyma of the SVZ or into the cerebrospinal fluid of lateral ventricle. For instance, the effects of Ara-C after intraventricular infusion are therefore not limited to the SVZ, as recently shown by the subsequent 30-50 % reduction in hippocampal neurogenesis [61,75]. Remarkably, strong cognitive deficits **have been reported** to result from a similar reduction in the

hippocampal neurogenesis [98]. Finally, treatment with antimitotic agents might disrupt the continuous production of olfactory sensory neurons necessary for olfactory function perception [99].

An alternative way to disrupt adult neurogenesis uses focal irradiation. Region-restricted irradiation is more specific than focal infusion of antimitotic drugs, as only the irradiated area, but not the neighboring ones, exhibits a decrease in neurogenesis [51,73,89,100]. However, irradiation affects locally all dividing cells, including oligodendrocyte cell precursors [101], and some subtypes of neurovascular cells [102]. It also induces strong microglial activation, a hallmark of neuroinflammation, lasting for two months [103]. Thus, it is crucial to perform behavioral tests several months after irradiation.

Finally, adult neurogenesis can be suppressed by inducible recombination [98] or conditional transgenic ablation of neural stem cells [3,104,105] or neuronal precursor cells [106]. These genetic approaches are less specific than focal irradiation or antimitotic drug treatment, since bulbar and hippocampal neurogenesis are both ablated, limiting thus the interpretation of behavioral analyzes.

Box 2. Operant associative olfactory learning versus non-operant olfactory learning.

Learning can be divided into distinct categories including **non-associative** forms such as habituation and sensitization, in which an animal learns about the properties of a single stimulus, and **associative** forms, in which an animal learns about the relationship between two stimuli (classical conditioning) or between a stimulus and the animal's own behavior (operant conditioning).

If when an animal first responds to a stimulus, it is neither rewarding nor harmful, the animal reduces subsequent responses. For example, a short-term exposure of a adult rat to an odorant, in the absence of any paired reinforcement, leads to a reduction in the proportion of mitral cells responding to that odorant with increased firing rates. This process called **habituation** reflects the progressive diminution of behavioral response probability with repetition stimulus.

Sensitization is another example of non-associative learning in which the progressive amplification of a response follows repeated administrations of a stimulus. Sensitization is part of adaptive as well as maladaptive learning processes in the organism.

Operant conditioning is a situation that leads to modify the occurrence and expression of a given behavior. Operant conditioning is distinguished from classical conditioning in that operant conditioning concerns changes of voluntary behavior.

Classical conditioning was first demonstrated by Ivan Pavlov (1927) [107]. It involves repeatedly pairing an unconditioned stimulus (which unfailingly evokes a reflexive response) with another previously neutral stimulus (which does not normally evoke the response). Following conditioning, the response occurs both to the unconditioned stimulus and to the other, unrelated stimulus.

Today, a central question in experimental Psychology is whether these different forms of learning are related to adult neurogenesis. When applied to olfaction, do the associative and non-associative forms of learning require the continuous arrival of newborn olfactory neurons? It seems to be the case (**Figure I**). For instance, increasing newborn neuron survival in the OB by non-associative olfactory experience, leads to stronger short-term olfactory memory [55]. Similarly, enrichment to odors improves olfactory discrimination in adult rats [108] through a non-associative process called perceptual learning that requires intact adult neurogenesis [68]. Also, an olfactory memory deficit was reported in a spontaneous short-term memory paradigm after suppression of bulbar neurogenesis [75].

Adult-born neurons are also at play in associative olfactory learning. When adult neurogenesis is suppressed in the adult cricket, both olfactory learning and memory tested in an operant olfactory conditioning are impaired [82]. Similarly, correlative studies performed in rodents indicate a function of adult neurogenesis in associative learning [50,58,69]. Also, blocking of neurogenesis by irradiation or anti-mitotic treatment in mice impairs long-term retention of an associative olfactory task but not its acquisition [51,61]. Once selected to survive by learning, newborn neurons might change the neural representation of the learned odorants through shaping the physiology of the OB network. Following this view, it can be postulated that newborn

neurons might be able to retain a memory trace of previous olfactory experience during an associative learning task. Finally, no deficit in olfactory memory was reported when adult neurogenesis was reduced by genetic ablation [3]. This discrepancy might result from technical issues (specific context) and/or from the degree of difficulty of the discrimination tasks that has been used. Further experiments are required to clarify the relationship between the degree of adult neurogenesis and the complexity of discrimination learning task.

Box 3. Role of adult neurogenesis in olfactory discrimination

Olfactory discrimination is a primary function of OB granule cells [13]. As adult-generated granule cells are a significant component of bulbar interneuron population, it was not surprising to find a link between the turnover rate of adult bulbar neurogenesis and the olfactory reaction time [74]. But surprisingly, most experiments aimed at manipulating adult bulbar neurogenesis failed to demonstrate a direct role of adult neurogenesis in odorant discrimination [3,51,73,75]. Olfactory discrimination is clearly impaired in animals in which bulbar neurogenesis has been altered during embryogenesis [84,93,95] but not during adulthood [3,51,73,75], suggesting that this task is not mediated by adult-generated neurons. The function of newborn neurons in odorant discrimination might be difficult to unveil because of their relatively small proportion compared to the population size of mature interneurons (but see [3]). It might also be due to the eventual development of compensatory effects such as synaptic changes in mature neurons, or to the current methods used to challenge adult neurogenesis (see **Box 1**). Designing experiments that selectively activate or inhibit new neurons within the adult OB during odorant discrimination tasks of diverse difficulty are required to solve this still unsolved question.

Box 4: Outstanding questions

- At what stage of development do adult-generated OB neurons contribute to olfactory perceptual learning, odor memory, and fear responses?
- How does the orchestrated development of the synaptic inputs onto adult-born bulbar neurons impact olfactory abilities?
- Are new neurons functionally important when they are young and more excitable? Or are they most important later when they are mature and fully integrated into the existing network?
- Is there any role for new periglomerular cells in forming odor memories?
- What is the relationship/cross-talk between adult-generated neurons and centrifugal projections?
- What are the cellular and molecular mechanisms underlying olfactory perceptual learning and odor discrimination?
- What are the mechanisms involved in forming and restituting olfactory traces after learning?
- What is the contribution of adult neurogenesis to pheromone-related behaviors such as mating, social recognition and maternal behavior?

- What is the contribution of the constitutive adult bulbar neurogenesis to brain repair occurring during brain trauma, ischemia, neurodegenerative or inflammatory diseases?

Glossary

Bottom-up pathway: The classical view of cortical processing proposes that sensory information evolves through a feed-forward hierarchy. In the first step of the olfactory system, the bottom-up pathway includes sensory signals from the olfactory epithelium to the olfactory bulb.

Coincidence detection: A process by which a neuronal element can encode information by detecting the incidence of simultaneous yet spatially segregated events. Coincidence detection enables a combinatorial code to achieve outcomes that cannot be generated by isolated stimuli and to increase the number of results attainable with a limited stimulus set. Coincidence detection, long appreciated to play a role in strengthening synaptic connections in mature nervous systems, can be important for establishing synaptic connections during neural maturation.

Long-term memory: Memory lasting several days or months.

Long-term potentiation: An increase in size of a synaptic response that indicates plasticity in synaptic connections, lasting one hour or more.

Non-operant associative olfactory task: Passive pairing of a context with an odorant stimulation that does not necessitate the learning of a specific behavior to get a reward.

Olfactory associative memory: Memory of an association between a given odorant stimulus and a reward.

Olfactory enrichment: Repeated exposure to novel odorants for several days in unique housing.

Olfactory fear conditioning: Learning of an association between a neutral stimulus (odorant) and an aversive stimulus. The animal cannot choose to escape the conditioned stimulus upon testing.

Olfactory habituation/spontaneous discrimination task: Assessment of non-associative short-term memory, of spontaneous discrimination among dissimilar or similar odorants, unaltered by previous reinforcement.

Olfactory habituation: Progressive decrease in investigating repeated similar odorant stimuli.

Operant associative olfactory learning: A form of learning consisting of the acquisition of a certain behavior (for instance go and lick in a go-no go procedure) by pairing the neutral odorant stimulus with a reward.

Pattern segregation: The separation of neuronal cell discharge patterns allowing divergence from similar and confusing input patterns.

Perceptual learning: A form of implicit memory that can be defined as an increased sensitivity to stimulus parameters that improves perceptual acuity due to experience.

Reinforced olfactory discrimination learning: Acquisition of a specific behavior in response to an odorant stimulus, paired with a positive reinforcer (reward) in a specific context (called conditioning: water or food deprivation for water or food reward, respectively).

Short-term memory: Memory lasting several minutes or hours.

Top-down pathway: Propagates information from higher (olfactory cortex, modulatory centers) to lower (olfactory bulb) levels.

Figure Legends

Figure 1. Schematic illustration of the OB structure and its connections. The OB is divided into six main layers: (1) the glomerular layer, (2) the external plexiform layer, (3) the mitral cell layer, (4) the internal plexiform cell layer, (5) the granule cell layer and (6) the rostral migratory stream at the core (RMS). The OB receives inputs from olfactory sensory neurons (OSN) and from centrifugal fibers. The OB output is provided by mitral/tufted cells, which project to several parts of the olfactory cortex. OSN project axons to glomeruli (white disks), in which they activate mitral/tufted cells and periglomerular cells. Centrifugal inputs modulate periglomerular and granule cell activities, which in turn modulate mitral cell activity.

Figure 2. A canonical olfactory bulb circuit. The olfactory system includes the olfactory sensory neurons (OSN) located in the sensory epithelium, the relay neurons (mitral and tufted cells) of the OB, and cortical neurons in downstream structures, comprising the primary and accessory olfactory cortex. These centers include the anterior olfactory nucleus, which connects the OB through a portion of the anterior commissure, the olfactory tubercle, the piriform cortex (considered to be the primary olfactory cortex), the cortical nucleus of the amygdala and the entorhinal area [16,21,77]. The OB is the first relay of olfactory processing and encoding by three main types of neurons: (1) OSN detects odorants and transmits action potentials to the OB where it forms glutamatergic synapses with the dendrites of (2) relay neurons, called mitral cells, and (3) local interneurons in target area called glomeruli.

Mitral/tufted cells are glutamatergic neurons which project to the olfactory cortex. The activity of mitral/tufted cells is modulated by glutamatergic input from OSNs, GABAergic input from interneurons called granule cells (GC), GABAergic and dopaminergic input from interneurons called periglomerular cells (PG), as well as centrifugal innervations (centrifugal fibers: CF). Unlike most central nervous system regions, inhibitory interneurons in the OB far outnumber principal cells by at least 50:1 [90]. The granule cells account for about 90% of all bulbar interneurons [90]. Periglomerular and granule cells are the primary targets of the centrifugal inputs to the OB. Sensory experience modulates the activity of glomeruli, of mitral/tufted cells and of granule cells. Centrifugal inputs play a key role in odor detection, discrimination and learning/memory by regulating sensory information. Newly generated interneurons continuously integrate into the existing neuronal circuit of the adult OB where they provide a source of plasticity that may allow behavioral adaptation to an ever-changing environment. Adult-born neurons target mitral cells in the OB thus providing an inhibitory function. Learning modulates the survival of newborn granule cells probably via centrifugal inputs. In this case, newborn granule cells might support long-term memory after learning.

Figure 3. Regulation of the olfactory bulb activity. The neuronal activity of the OB circuit depends on three intermingled processes (open arrows): Sensory inputs from the olfactory epithelium (*the bottom-up pathway*), centrifugal fibers (*the top-down pathway*) and adult neurogenesis. Centrifugal fibers contribute to the context-dependent modulation of the circuit activity (*i.e.*, attention, reward, learning, and memory). The olfactory epithelium provides sensory information to the OB circuit

from two different behavioral contexts: Passive odorant exposure or active sensing. Challenging OB network activity influences the recruitment of new neurons (black arrow), which in return will affect the ongoing activity of the host circuit. Note that adult neurogenesis is also sensitive to olfactory experience (*i.e.*, sensory deprivation or odor-enriched environment) and to the behavioral state (*i.e.*, learning to discriminate between two odorants) (black arrows).

Figure 4. Coincidence detection in the olfactory bulb. Within the OB network, the relay neurons (mitral and tufted cells) are spatially activated by both sensory inputs from olfactory sensory neurons (OSN) and by the centrifugal fibers (CF). Soon after their arrival to the OB circuit, adult-born neurons receive first inputs from centrifugal fibers before establishing synapses with afferent inputs (mediated by the mitral cells). Thus, newborn neurons are able to associate a context to the information content at a particular stage of their maturation (temporal dimension). **Information** processing and refining within the OB circuitry might depend on both **spatial and temporal dimensions**, owing to the detection of coincidence by adult-generated neurons. This process might participate to the encoding of the odor representation, which is then sent to the olfactory cortex.