

The role of neurogenesis in olfaction-dependent behaviors

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Abstract

Newly born neurons continuously migrate into the main and accessory olfactory bulbs and modulate the output of projection neurons. Despite some contradictory results, it is becoming clear that these newly born neurons play an important role in the response to some odorant cues. In this minireview, we discuss the recent findings surrounding the functional significance of adult neurogenesis in olfaction-dependent behaviors.

Introduction

There are many neural stem cells in two brain regions of adult mammals, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal formation [23,36,38]. These neural stem cells are mostly quiescent under the control of Notch, Shh and BMP signaling [1,3,6,8,16,24,28] but occasionally divide to give rise to transit-amplifying cells, which proliferate and generate many neurons. Neurons born in the SVZ migrate via the rostral migratory stream into the olfactory bulb (Fig. 1), while neurons born in the SGZ migrate into the hippocampal dentate gyrus [23,38]. The functional significance of adult neurogenesis in hippocampal-dependent learning and memory has been well documented [9,38], but the role of such neurogenesis in olfactory activity is rather obscure.

The olfactory bulb consists of two structures, the main olfactory bulb and the accessory olfactory bulb (Fig. 1). The main olfactory bulb is involved in the cognitive response to chemical cues detected by the main olfactory epithelium, while the accessory olfactory bulb is involved in the instinctive response to pheromonal cues detected by the vomeronasal organ, although this functional difference between the main and accessory olfactory bulbs is not absolute [4,10]. The vast majority of neurons born in the SVZ differentiate into granule cells while others become periglomerular cells in the main olfactory bulb [23,36,38]. Some neurons born in the SVZ migrate into the accessory olfactory bulb (Fig. 1) [29]. All these neurons are local interneurons that modulate the output of projection neurons (mitral and tufted cells) [36]. Interestingly, newly born neurons exhibit different synaptic plasticity from that of mature neurons: theta-burst stimulation induces long-term potentiation in newly born neurons but not in mature neurons [27]. Furthermore, aged mice, which have more mature neurons and fewer newly born neurons than young adult mice, are impaired at fine olfactory discrimination [11,25]. These data suggest that newly born neurons might be required

for optimal olfactory activity. However, the precise roles of adult neurogenesis in odor processing remain to be determined.

To understand the functional significance of adult neurogenesis in olfactory activity, researchers have used many methods to ablate newly born neurons in animals, and these animals have been used for behavioral analyses [9]. However, there are some discrepancies among the published reports of neurogenesis-dependent odor-associated behaviors, complicating the interpretation of the significance of neurogenesis. These discrepancies may be due to different ablation methods and different behavioral tests. In this minireview, we discuss the recent data about the functional significance of adult neurogenesis in olfactory activity.

Approaches for ablating newly born neurons

Ablation of newly born neurons has been attempted to understand their functions in the adult brain [9]. The most common ablation methods are treatments with γ -ray irradiation or anti-mitotic drugs, such as methylazoxymethanol (MAM), to kill dividing neural stem/progenitor cells. For γ -ray treatment, a brain area encompassing the SVZ or SGZ is exposed to irradiation while the other brain region is protected with lead shields (Fig. 2a). This method allows region-specific inhibition of neurogenesis, although the long-term effect on the SGZ neurogenesis by the SVZ irradiation was not analyzed. For anti-mitotic drug treatment, an appropriate dose is injected subcutaneously or intraperitoneally. This method inhibits neurogenesis in both the SVZ and SGZ. Drug can be also specifically applied to the SVZ by implanting an osmotic minipump into the lateral ventricle (Fig. 2b), although neurogenesis in the SGZ is also affected to a lesser extent [5,34]. It was reported that about 60-90% of neurogenesis in the SVZ is reduced by γ -ray treatment or anti-mitotic drugs [5,12,20,34,35]. Although these methods are effective, the efficiency for inhibition of neurogenesis could be variable depending on treatment protocols, and neurogenesis could recover later because quiescent neural stem cells are rather resistant to γ -ray irradiation and anti-mitotic drugs. In addition, these treatments also cause severe side effects, such as inflammation, on mature neurons, which might affect the mood and behavior of the animals. These disadvantages may lead to inconsistency in behavior defects observed after such treatments.

To overcome such disadvantages, genetic methods for ablating newly born neurons have been developed. One such method uses Nestin-CreER^{T2} mice, which

express tamoxifen-inducible Cre in neural stem/progenitor cells under the control of the Nestin promoter/enhancer (Fig. 2c). These mice were crossed with NSE-loxP-Stop-loxP-DTA (NSE-DTA) mice, in which Cre induces loxP-mediated deletion of a stop cassette and allows the neuron-specific enolase (NSE) promoter to drive expression of diphtheria toxin fragment A (DTA, Fig. 2b) [14,15]. In Nestin-CreER^{T2};NSE-DTA mice, Cre becomes active in neural stem/progenitor cells after tamoxifen administration, but DTA is not expressed in these cells because the NSE promoter is inactive (Fig. 2c). However, when the cells begin neuronal differentiation, the NSE promoter becomes active and induces expression of DTA, which kills the cells (Fig. 2c). Thus, in these mice, neural stem cells in the SVZ and SGZ do not die, but only newly born neurons are efficiently ablated after tamoxifen treatment without any noticeable side effects on mature neurons: this method achieved about 96% reduction of neurogenesis in the main olfactory bulb compared to controls, forming spaces void of neurons [15]. While neurogenesis in the SVZ and SGZ is simultaneously blocked in these mice, more restricted ablation is also attempted by using region- or neuronal subtype-specific promoters instead of the NSE promoter [our unpublished data]. These transgenic mice can be used for behavior tests, and it will be important to compare the results obtained from such genetic methods and those obtained from γ -ray irradiation or anti-mitotic drug treatments to reconcile inconsistent results.

Behavioral tests for the response to chemical cues

Odor experiences affect the survival of newly born neurons, suggesting that neurogenesis is involved in odor-associated activities [2,36]. To understand the significance of neurogenesis in olfaction, various olfactory behavioral tests have been conducted in mice following manipulation of neurogenesis. One such test is to determine odor detection threshold by measuring the sniffing time of various odors. Untreated mice spent more time investigating some odors at 10^{-4} dilution than they did mineral oil, while mice whose neurogenesis was blocked by continuous infusion of the antimitotic drug cytosine arabinoside (AraC) did not [5]. Thus, these mice could not differentiate between odors at 10^{-4} dilution and mineral oil, suggesting that odor detection was less sensitive in the absence of neurogenesis. At higher concentrations such as 10^{-3} dilution, there was no difference in investigation time for detection of odors between treated and untreated mice. However, when the same odors at this

concentration were exposed twice for 5 min each, with a 60-min interval (habituation-dishabituation tests), untreated mice spent significantly less time investigating during the second exposure, whereas treated mice spent similar lengths of time during the two exposures [5]. Because it is well known that mice spend less time investigating familiar odors, these results suggest that odor memory is lost during a 60-min interval without neurogenesis. Another study reported similar results [26]. Thus, continuous neurogenesis is required for optimal odor detection threshold and for short-term odor memory. In agreement with this idea, it was reported that newly born neurons preferentially expressed the immediate early gene *Zif268*, an indicator of activation, in response to odor stimulation, and that blocking neurogenesis reduced the number of newly born *Zif268*-expressing neurons [26]. Together, these findings suggest that newly born neurons are preferentially involved in processing odor memory.

However, not all findings have supported this. In odor reward threshold test, tamoxifen-treated Nestin-CreER^{T2};NSE-DTA mice showed apparently normal sensitivity [30]. Furthermore, in habituation-dishabituation tests, Mice treated with γ -ray irradiation to block neurogenesis showed no significant differences from untreated mice [20], suggesting that spontaneous odor discrimination is not affected in these mice. Similar results were observed in Bax-null mice with reduced programmed cell death and interrupted migration of newly born neurons into the olfactory bulb [18]. Although these mice had very few newly born olfactory bulb neurons at 12 months, they displayed normal behaviors in habituation-dishabituation tests, suggesting that neurogenesis is not required for odor discrimination and short-term odor memory [18]. The discrepancies between these two findings may be due to different ablation methods or efficiencies, but further analysis is required to clarify this issue.

An odor-reward association memory test in the previously described, tamoxifen-treated Nestin-CreER^{T2};NSE-DTA mice suggested that continuous neurogenesis is not required for odor memory. Two related odors (enantiomers), one mixed with sugar and the other without sugar, were given to food-restricted mice four times a day; after four days of training, each mouse was placed in a cage, in which both odors, without sugar, were placed under the bedding at separate sites (odor-reward association memory test). Both wild-type and mutant mice spent significantly more time digging at the site of the sugar-associated odors than at the site of the non-sugar-associated odors. The same behavior was observed one day, one week, and even two

months after training [15], strongly indicating that continuous neurogenesis is not required to discriminate similar odors or for either short- or long-term retention of the odor-associated memory (Table 1). The apparently conflicting results between habituation-dishabituation and odor-reward association memory tests may be due to differences in motivation. The former test is based on spontaneous behaviors, while the latter test provides mice with strong motivation to get a reward, and mutant mice with a strong motivation may overcome any disadvantage resulting from a lack of neurogenesis.

However, conflicting results were also reported following similar motivation-dependent tests. Although the mice treated with γ -ray irradiation learned sugar-associated versus non-sugar-associated odors as well as the untreated mice, 30 days after training they made more errors (they chose non-sugar-associated odors more frequently) than untreated mice, suggesting that long-term olfactory memory is impaired in these mice [20]. In another reward-associated odor memory test, mice were trained on a 2-hole board apparatus; one hole had both odor and reward while the other had neither odor nor reward. Mice treated with continuous infusion of AraC to block neurogenesis were able to learn sugar-associated odors as well as untreated mice; however, while untreated mice retained the odor-associated memory for at least five days, the treated mice did not [34], suggesting that neurogenesis is required for retention of the odor-associated memory. Fear conditioning tests were also used to examine odor memories. Mice that received odor stimulation and footshock (odor-cued fear conditioning) exhibited freezing behavior in response to the odor. Although γ -ray irradiated mice showed normal acquisition of odor-cued fear conditioning, they froze less in response to the odor one day later [35], suggesting that olfactory memories are not properly retained without continuous neurogenesis, even when the mice are strongly conditioned. The precise reason for these discrepancies is currently unknown. One possibility is that irradiation and antimitotic drug treatment, which not only kill dividing progenitors but also damage mature neurons, result in severer defects in olfactory memory than genetic ablation of newly born neurons, because mice with genetically inhibited neurogenesis (Bax-null mice and tamoxifen-treated Nestin-CreERT2;NSE-loxP-Stop-loxP-DTA mice) displayed no apparent defects in either habituation-dishabituation or odor-reward association memory tests (Table 1) [15,18].

Olfactory behavior was also examined in mice lacking the neural cell

adhesion molecule NCAM. The migration of neurons to the olfactory bulb is impaired in these mice, leading to about 40% reduction in the granule cell layer of the olfactory bulb [13]. In habituation-dishabituation tests, wild-type mice spent more time investigating a novel odor than a familiar odor, whereas mutant mice did not investigate novel odors longer than familiar ones, suggesting that odor discrimination is affected in NCAM-null mice [13]. However, NCAM-null mice were able to discriminate different odors and learn the odor reward-associated task. Both wild-type and NCAM-null mice spent significantly more time digging at the site of sugar-associated odors than at the site of non-sugar-associated odors [31], indicating that NCAM-null mice can learn odor-associated tasks and retain the odor-associated memory at least for one day. It is likely that in this test, the mice were well motivated to learn the odor discrimination task. These results strongly suggest that reduced numbers of newly born neurons in the olfactory bulb does not affect odor discrimination or short-term retention of strongly conditioned olfactory memory.

Behavioral tests for the response to pheromonal cues

In addition to the cognitive recognition of smell, the instinctive response to pheromonal cues is also processed by the main and accessory olfactory systems. Recent studies indicate that neurogenesis plays an essential role in pheromone-associated activities. Olfactory activities are very important for the maintenance of pregnancy [7,17,32], and pregnancy induces biphasic stimulation of neurogenesis in the SVZ, leading to a biphasic increase in the production of both granule cells and periglomerular cells in the olfactory bulb [33]. The first peak of neurogenesis occurs around gestation day 7, neurogenesis returns to baseline at gestation day 14, and the second peak occurs around postpartum day 7. This pregnancy-induced stimulation of neurogenesis is mediated by prolactin, which directly activates proliferation and neuronal differentiation of the SVZ neural stem cells [19,33]. Reduced prolactin levels following the administration of the dopamine D2-receptor agonist bromocriptine led to decreased neurogenesis and impaired maternal behaviors [19]. In support of this observation, pregnant mice treated with MAM showed defects in maternal behaviors [19]. Neurogenesis in females is also induced by dominant male pheromones and seems to be important for sexual behaviors [21,29]. In agreement with this idea, we have recently found that tamoxifen-treated Nestin-CreER^{T2};NSE-DTA mice, in which neurogenesis is genetically blocked, exhibit

severe deficits in pheromone-associated behaviors [our unpublished data], suggesting that continuous neurogenesis is essential for such gender-specific activities.

However, conflicting results were also reported (Table 1). Mice treated with γ -ray irradiation in the SVZ, which showed 63% reduction of neurogenesis in the main olfactory bulb, seemed to display normal sexual and maternal behaviors [12]. The discrepancy between this study and others remains to be clarified, but one possibility is that in this γ -ray irradiation experiment the remaining ability for neurogenesis could be sufficient for prolactin-dependent functions. Another possibility is that neurogenesis in the dentate gyrus is also involved in sexual and maternal behaviors, because in the γ -ray irradiation study, neurogenesis was inhibited only in the SVZ while in other studies neurogenesis was blocked in the SVZ and SGZ. It would be interesting to compare sexual and maternal behaviors when neurogenesis is inhibited only in the SGZ.

Induced neurogenesis is also observed in male mice. When they remain with their female partners during pregnancy and post-partum, they exhibit retrieval behavior toward their pups. Furthermore, these males can discriminate between their offspring and those of others, even after they are separated from their offspring for three weeks [22]. In these males, neurogenesis is significantly activated, and without such increased neurogenesis, they fail to discriminate between their offspring and non-offspring [22]. This increased neurogenesis appears to depend on the odor of their offspring and is mediated by prolactin [22]. These results suggest that neurogenesis is important for males to perform offspring recognition.

The above results indicate that olfactory neurogenesis is significantly enhanced when sexual and maternal behaviors are required, and suggest that neurogenesis plays a role in such pheromone-associated behaviors, although there are some conflicting results. To clarify the inconsistencies, the functional significance of adult neurogenesis in pheromone-associated behaviors should be further examined by using various ablation methods.

Concluding remarks

While treatments with γ -ray irradiation or anti-mitotic drugs are very effective, these treatments cause severe side effects, resulting in some inconsistent results. Recently developed genetic methods are very efficient, and it will be important to compare the results obtained from the conventional methods and those obtained from the genetic

methods to reconcile inconsistent results. It will be also important to standardize behavior test protocols to avoid laboratory-specific variations.

In spite of some discrepancies, genetic ablation experiments clearly showed that continuous neurogenesis is mostly dispensable for discrimination of similar odors and short- and long-term odor memory, at least when strongly motivated, suggesting that olfactory functions are well maintained in the absence of continuous neurogenesis. Neurogenesis is significantly induced when sexual and maternal behaviors are required, and at least some studies reported that such behaviors depend on continuous neurogenesis. Odor memory of new partners and pups and proper behaviors to such odors are essential for species preservation, and it is reasonable that newly born neurons in the adult brain play more important roles in sexual and maternal behaviors to new olfactory memories than in discrimination and memory of chemical odors. However, the mechanisms by which newly born neurons are integrated into the preexisting neural circuit and of how they regulate odor-associated behaviors, particularly pheromone-dependent instinctive behaviors, are largely unknown. Further analysis, such as region- and neuronal subtype-specific ablation, will be required to address these issues.

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Figure legends

Figure 1. Adult neurogenesis and the olfactory system. Neurons born in the subventricular zone (SVZ) of the lateral ventricles (LV) migrate via the rostral migratory stream (RMS) into the olfactory bulb. The olfactory bulb consists of two structures, the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB). The main and accessory olfactory bulbs receive signals from the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), respectively.

Figure 2. Methods for inhibition of adult neurogenesis. (a) Inhibition of neurogenesis by γ -ray irradiation. A brain area encompassing the SVZ or SGZ is exposed to irradiation while the other brain region is protected with lead shields. (b) Inhibition of neurogenesis by administration of anti-mitotic drugs with an osmotic minipump. (c) Genetic ablation of newly born neurons. In Nestin-CreERT2;NSE-loxP-Stop-loxP-DTA mice, Cre is activated in neural stem/progenitor cells after tamoxifen administration, but DTA is not expressed in neural stem/progenitor cells because the NSE promoter is inactive in these cells. However, when these cells start neuronal differentiation, the NSE promoter becomes active and induces expression of DTA, which kills cells. Thus, in these mice, only newborn neurons are efficiently ablated after tamoxifen treatment. DTA, diphtheria toxin fragment A; NSE, neuron-specific enolase.

Table 1. Olfaction-dependent behavior phenotypes of treated or mutant mice.

Behavior tests	Treatments/ Genotypes	Affected regions	Phenotypes	References
Habituation-dishabituation	γ -ray irradiation	SVZ	Normal short-term memory	20
	AraC	SVZ	Defective short-term memory	5,26
	Bax KO	SVZ	Normal short-term memory	18
	NCAM KO	SVZ	Defective odor discrimination	13
Odor-reward association	γ -ray irradiation	SVZ	Defective long-term memory	20
	AraC	SVZ	Defective long-term memory	34
	Nestin-CreER ^{T2} ; NSE-DTA	SVZ SGZ	Normal short- and long-term memory	15
	NCAM KO	SVZ	Normal short- and long-term memory	31
Maternal behaviors	γ -ray irradiation	SVZ	Normal	12
	MAM	SVZ SGZ	Defective	19
	Nestin-CreER ^{T2} ; NSE-DTA	SVZ SGZ	Defective	30

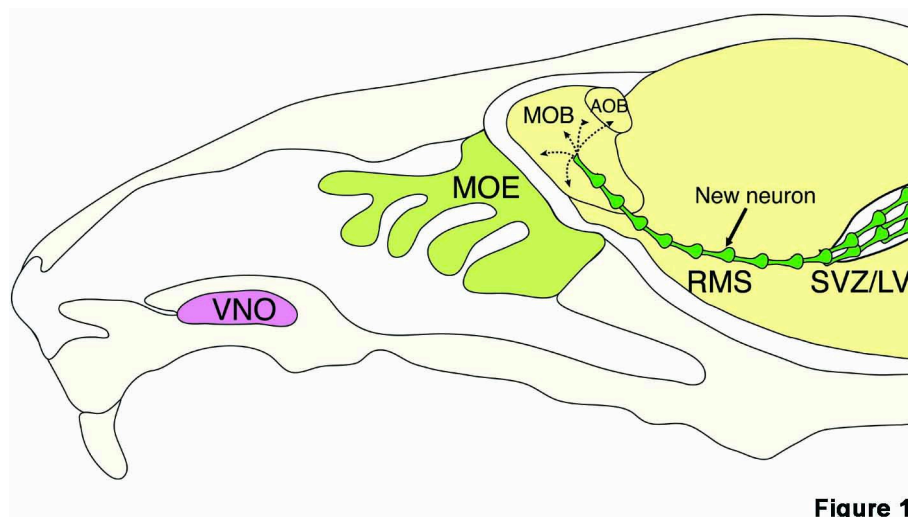


Figure 1

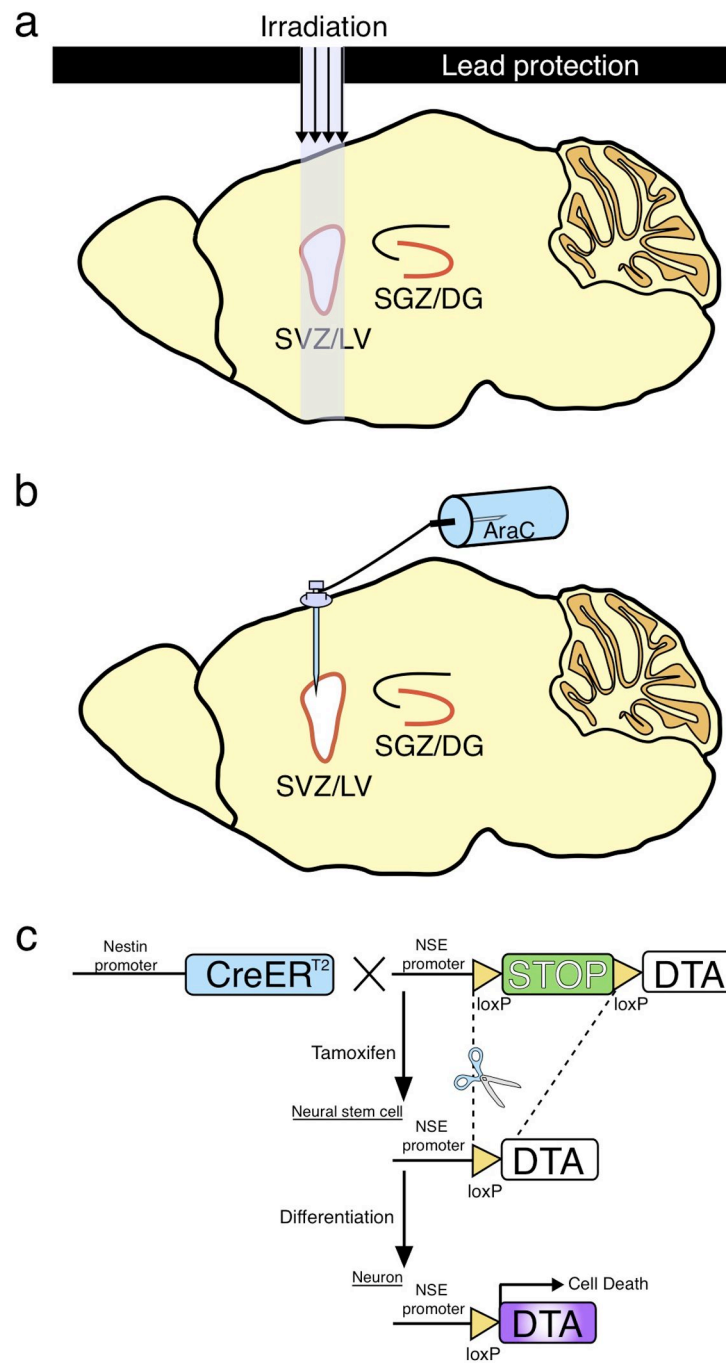


Figure 2