Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Physiology - Paris 97 (2003) 517–528
Journal of Physiology Paris
www.elsevier.com/locate/jphysparis

Local neurons play key roles in the mammalian olfactory bulb

Armen Saghatelyan, Alan Carleton, Samuel Lagier, Antoine de Chevigny, Pierre-Marie Lledo *

Laboratory of Perception and Memory, Centre National de la Recherche Scientifique, Unité de Recherche Associée 2182, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris, France

Abstract

Over the past few decades, research exploring how the brain perceives, discriminates, and recognizes odorant molecules has received a growing interest. Today, olfaction is no longer considered a matter of poetry. Chemical senses entered the biological era when an increasing number of scientists started to elucidate the early stages of the olfactory pathway. A combination of genetic, biochemical, cellular, electrophysiological and behavioral methods has provided a picture of how odor information is processed in the olfactory system as it moves from the periphery to higher areas of the brain.

Our group is exploring the physiology of the main olfactory bulb, the first processing relay in the mammalian brain. From different electrophysiological approaches, we are attempting to understand the cellular rules that contribute to the synaptic transmission and plasticity at this central relay. How olfactory sensory inputs, originating from the olfactory epithelium located in the nasal cavity, are encoded in the main olfactory bulb remains a crucial question for understanding odor processing. More importantly, the persistence of a high level of neurogenesis continuously supplying the adult olfactory bulb with newborn local neurons provides an attractive model to investigate how basic olfactory functions are maintained when a large proportion of local neurons are continuously renewed. For this purpose, we summarize the current ideas concerning the molecular mechanisms and organizational strategies used by the olfactory system to encode and process information in the main olfactory bulb. We discuss the degree of sensitivity of the bulbar neuronal network activity to the persistence of this high level of neurogenesis that is modulated by sensory experience. Finally, it is worth mentioning that analyzing the molecular mechanisms and organizational strategies used by the olfactory system to transduce, encode, and process odorant information in the olfactory bulb should aid in understanding the general neural mechanisms involved in both sensory perception and memory. Due to space constraints, this review focuses exclusively on the olfactory systems of vertebrates and primarily those of mammals.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: GABA; Glutamate; Granule cells; Mitral cells; Odors; Reciprocal synapses; Sensory deprivation; Synchronization

1. Introduction

Animals can recognize and discriminate chemical signals from the environment that not only provides essential information for survival, but also profoundly influence their behavior. While chemosensation involves both taste and smell in higher organisms, most animals rely on olfaction as the principal chemosensory modality. The mammalian olfactory system regulates multiple complex functions such as food localization, reproductive and/or maternal functions, neuroendocrine regulation, recognition of conspecifics, emotional states, and social information concerning the family, the clan or outsiders. To accomplish this, odor molecules are recognized by two anatomically and functionally distinct sensory organs in mammals, the olfactory epithelium and the vomeronasal organ. The former is thought to recognize odor molecules that provide information about the world at large and can induce measured behavioral responses. It is connected with the main olfactory bulb referred to in this review as the olfactory bulb. In contrast, the latter has traditionally been implicated in the recognition of pheromones, odorants that provide information about the social and sexual status of other conspecifics. This sensory organ projects to the accessory olfactory bulb. In the present review, we only examine recent findings concerning the mammalian

* Corresponding author. Tel.: +33-1-45-68-88-03; fax: +33-1-45-68-83-69.
E-mail address: pmlledo@pasteur.fr (P.-M. Lledo).

0928-4257/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jphysparis.2004.01.009

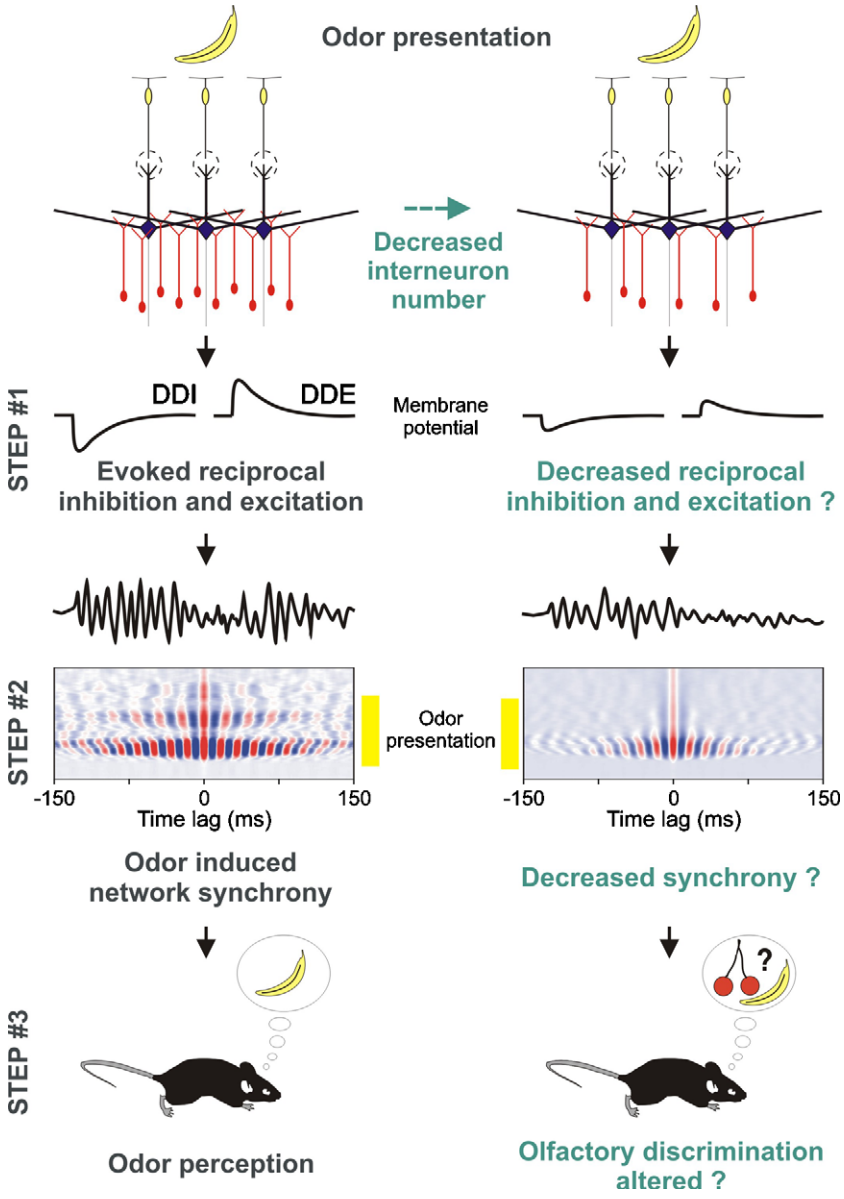

Fig. 1. Model of olfactory information processing in the olfactory bulb. In normal conditions (left column), odor presentation leads to the activation of olfactory receptors and the increase of sensory neuron firing (yellow). At the glomerular level, mitral cells (blue) are depolarized by the glutamate released from olfactory nerve terminals. (Step 1) The activation of mitral cells increases the feedback release of both GABA (dendro-dendritic inhibition, DDI) and glutamate (dendro-dendritic excitation, DDE) from granule cells (red). The result of such interactions is a synchronization of mitral cell firing activity (Step 2) as seen by oscillations of the local field potential (black trace) and the colored stripes in the sliding auto-correlogram. This temporal network activity may represent the basis of olfactory perception coding. (Step # 3) Note that for clarity, the information processing within the olfactory cortex was omitted. If this model is true, one may predict that the function of interneurons is crucial for sensory information processing. Thus, a reduction in the number of interneurons (right column) when adult neurogenesis is challenged, should reduce both excitatory and inhibitory dendro-dendritic responses that would be lead to less synchrony in the network. Although under these conditions, a mouse could detect odor, its discrimination abilities should be impaired.

continuous renewal during an animal's lifetime, will affect network synchrony and olfactory perception.

4. Bulbar neurogenesis

Although the majority of the cells in the nervous system are born during the embryonic and early postnatal period, newborn neurons continue to be generated within at least two areas of the adult mammalian brain for a recent review, see [32,33] (Fig. 2). The first region concerns the subgranular zone of the dentate gyrus that gives rise to granule cells [34,35] (Fig. 2B). The second is the subventricular zone (SVZ) located in the wall of the lateral ventricles [36–38], and to a less extent the rostral extension of the SVZ [39] (Fig. 2C), where steadily

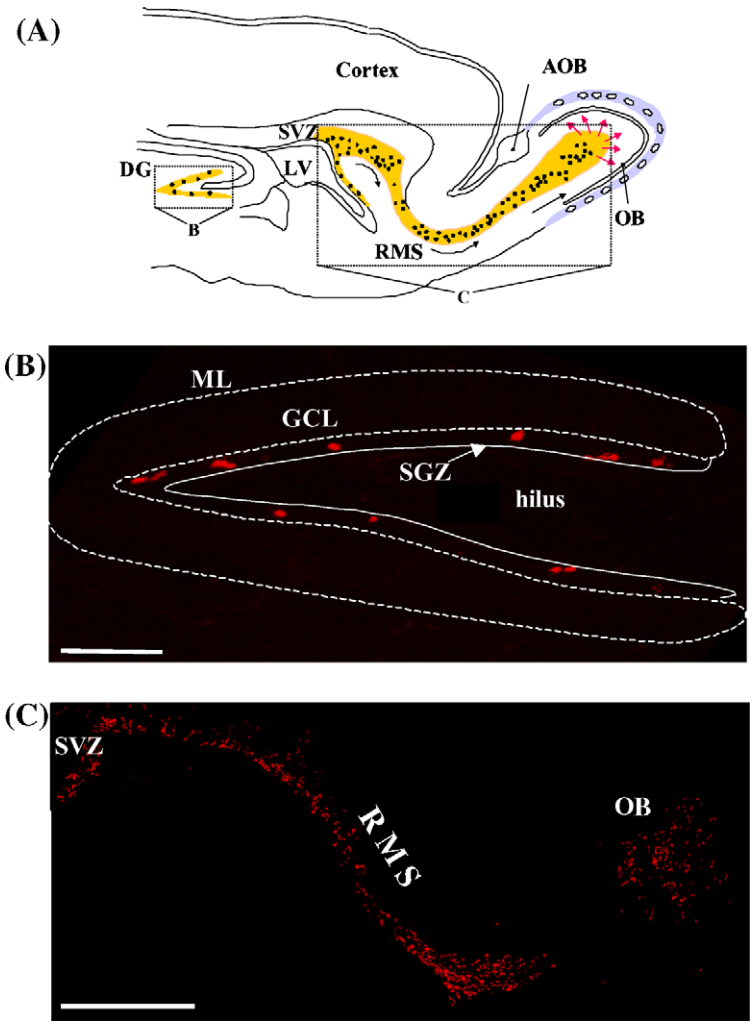

Fig. 2. Neurogenic areas in the adult brain. (A) Schematic drawing of a sagittal section of the mouse brain. Two neurogenic areas are marked in yellow. Black dots correspond to dividing cells. In the olfactory system, black arrows reflect tangential migration and pink arrows reflect radial migration. (B) Immunofluorescence anti-BrdU on a sagittal section of the brain at the level of the hippocampus. (C) Immunofluorescence anti-bromodeoxyuridine (BrdU) on a sagittal section of the forebrain. The mouse was perfused 4 h after BrdU injection. Each red dot is a BrdU-positive cell.

dividing stem cells and progenitors that produce new neurons are harbored. These neuronal progenitors then migrate through the so-called rostral migratory stream to populate the olfactory bulb, where they differentiate into local interneurons and establish connections with their neuronal targets [40,41]. Although this ongoing neurogenesis as well as the migration process has been extensively characterized, its function remains unknown. Moreover, despite the knowledge we had regarding the source and migration path of neurons generated in the adult SVZ, the cellular physiology of these newborn cells has not yet been described. It remains unclear whether all migrating cells give rise to newly formed neurons capable of surviving and being integrated into already functional neuronal circuits. Filling this gap has been impeded largely by the inability to distinguish between previously generated and newly generated neurons in living brain tissue.

In order to answer to this question, we developed an in vivo method based on retrovirus-mediated gene transfer for labeling large numbers of SVZ progenitor cells and combined this approach with simultaneous morphological analyses and patch-clamp recordings [42]. We showed that newborn interneurons displayed the typical morphology of a mature neuron: complex arborization and presence of dendritic spines. Moreover, they expressed neuronal markers such as NeuN and the specific enzyme for GABA production, GAD67. Finally, we determined that the cells became electrically active and were synaptically connected to the bulbar network. In agreement with other reports in adult birds and the adult mammalian hippocampus, these observations demonstrate that the newborn cells in adult MOB differentiate into mature neurons [43–46].

Thus, if granule cells produced in adulthood are necessary for olfactory bulb function, then a defect in

the rostral migration leading to a reduction of the new bulbar interneuron population should alter olfactory bulb functions. The tangential migration of precursors from the SVZ cells into the olfactory bulb is associated with the expression of the neural cell adhesion molecule (NCAM) in its highly polysialylated form (PSA-NCAM) [47]. Hence, NCAM-knockout mice, which have been shown to be also almost totally devoid of PSA, show a defect in the rostral migration of SVZ precursors, resulting in an accumulation of precursors along the pathway and finally in a size-reduction of the olfactory bulb [48–50]. We are currently using this animal model to evaluate the impact of altering interneuron number.

According to our model (see Fig. 1), the efficiency of synaptic transmission received by mitral cells may show a dramatic decrease in both evoked excitatory and inhibitory synaptic responses in NCAM-mutant mice. This would lead to less synchronization of mitral cell output patterns. Finally, we propose that the mouse will have difficulties characterizing odorants presented as shown by our behavior analysis [51a]. This model gives a hint to a specific role for adult-generated neurons in bulbar coding of odorant information. It proposes a specific role for this interneuron population in downstream coding of olfactory information. Since the olfactory bulb is also involved in consolidation processes associated with long-term odor memory [51b], a modulation in the number of interneurons by continual neurogenesis may also participate in certain forms of olfactory learning.

5. Implication of newly generated interneurons in the olfactory bulb circuitry

The persistence of a high level of neuron production in adulthood and its conservation throughout evolution suggest that this process is of fundamental biological significance. The relevance of adult neurogenesis has been suggested by our observation that survival of newly generated neurons in the olfactory bulb can be prolonged by sensory experience and this has important implications in the olfactory memory processes [52]. How might an increase in the population of interneurons turn on the neuronal remodeling underlying the improvement of olfactory performance? First, inhibition may spatially and/or temporally filter sensory responses to create appropriate signals for recruiting plasticity mechanisms. A second possibility, in line with our results, is that experience-dependent induced changes involve recruitment of simultaneous excitatory and inhibitory circuits provided by newborn neurons. Different spatial arrangement of these inputs to the principal cells could lead to the compartmentalization of mitral cell activity that may be instrumental for the refinement of odor molecule tuning. These possibilities suggest that high levels of interneuron production may not be responsible for keeping neuronal plasticity closed but instead may promote neuronal remodeling onset. Thus, the large-scale production of immature neurons into the olfactory bulb [53] provides a substrate by which neural regulatory factors and olfactory bulb activity could, in the adult animal, direct the organization of developing neural connections.

The olfactory bulb is an important brain structure because it plays a central role for odor encoding and discrimination [54]. In addition to acquisition, some studies have provided evidence that the olfactory bulb has a transient role in memory and shows a high degree of structural plasticity [55]. Our recent findings demonstrated that modulation of the neurogenesis level in adult animals could have profound and robust effects on the short-term odor memory [52]. However, it is still not clear whether increased level of newly generated interneurons affects processing of olfactory cues or their transient storage within the olfactory bulb. Whatever the exact mechanism is going to be, the existence of continuous renewal of local bulbar interneurons, their capability to excite mitral cells by the release of glutamate and their participation in the synchronization of principal neuron activities points to an unexpected degree of complexity in the neuronal network of the olfactory bulb and forces us to revise current concepts about the way the granule cells regulate olfactory bulb outputs.

6. Activity-dependent modulation of neurogenesis

It is well documented that during development of sensory systems environmental-induced activity shapes the establishment of appropriate patterns of neuronal circuitry [56,57]. The experience-induced activity is particularly influential during the sensitive period of development, called the ''critical period'', and maturation of GABAergic synaptic transmission during that period is believed to be one of the essential factors contributing to functional remodeling [58,59]. In contrast to other sensory systems, the constant neurogenesis of the interneuronal network of the olfactory bulb raises the possibility for a ''never-ending critical period'' in the olfactory system. This unique feature thus provides an ideal opportunity to investigate the role of sensory activity in refining adult neuronal circuitry.

It has been previously shown that proliferation and migration of neuronal precursors were not affected by removal of olfactory bulb [60], transection of olfactory peduncle [61] or unilateral nostril occlusion but see also [62,63]. In support of this, we also have demonstrated that bi-directional modification of sensory activity either by odor-enriched environment or after unilateral nostril

occlusion leave proliferation of neuroblasts unaffected [52,64]. In contrast, elevated neuronal death in the olfactory bulb was documented in odor-deprived animals [62,65,66] and odor enrichment increased the number of newly generated granule cells in pups [67] and adults [52]. This indicates that sensory driven activity may play an important role in the survival of progenitors and/or in their guidance from the rostral migratory stream to the olfactory bulb. As a result, this leads to net changes in the number of newly generated interneurons that may modify odor processing by affecting the synchronization of mitral cells [68]. However, the exact contribution of newly generated interneurons in the olfactory bulb circuitry, their role in synchronizing activity of output neurons and their functional significance in term of olfactory behaviors still remain elusive. To shed some light on this issue, we were able to demonstrate that an increase in number of newly generated interneurons in the olfactory bulb improved olfactory memory [52]. More particularly, animals raised in odor-enriched environment were able to recognize familiar odor longer and stronger (e.g., resistance to retroactive interference) as compared to animals raised in standard conditions [52]. This effect was specific to the olfactory bulb as the second neurogenic zone of the adult CNS, the dentate gyrus of the hippocampus, that is also implicated in several forms of olfactory memory remained unaltered after the enrichment. Although in these experiments, the potential consequences of odor enrichment on the synaptic efficacy and/or synchronization of mitral/tufted cells were not explored, overall these results support a link between the population of newborn interneurons and short-term olfactory memory.

In line with this, our electrophysiological investigations showed that a reduction in the number of newly generated granule cells following olfactory deprivation correlated with the decrease in the frequency of miniature inhibitory postsynaptic currents (IPSCs). To our surprise, action-potential dependent spontaneous and evoked IPSCs, as well as synchronization of mitral cells remained largely unaffected by the sensory deprivation [64]. This result led us to explore two forms of GABAergic inhibition exerted on mitral/tufted cells: the tonic form revealed by measuring the input membrane resistance and the evoked form induced by mitral cell firing. As a first approach to assess the degree of plasticity of these two forms, intracellular recordings of mitral/tufted cell firing activity were performed in control and occluded bulbs. In response to current injection, mitral/tufted cells generated trains of action potentials, the frequency of which increased with amplitude of the injected current (Fig. 3A and B). Changes in the input membrane resistance induced by the $GABA_A$ receptor antagonist, BMI, revealed the degree of the tonic form of GABAergic inhibition. Furthermore, since GABA release from granule cells could also be triggered by mitral cell firing via dendro-dendritic reciprocal synapses, the slope of the relationship between the injected current intensity and the firing frequency of mitral/tufted cells (i.e., input–output function) was used to assess the degree of evoked inhibition. Interestingly, if BMI-induced changes of input membrane resistance were more important in control than in occluded bulbs (Fig. 3D), BMI application altered similarly the slope of the input–output functions obtained from control and odor-deprived bulbs (Fig. 3C). These results indicated that sensory deprivation might decrease the tonic, but not the evoked, form of GABAergic inhibition exerted on mitral/tufted cells. In agreement with this, we have also found unaltered reciprocal dendro-dendritic inhibition and excitation following sensory deprivation leading to a normal level of mitral/tufted cell synchronization [64]. Thus, if it is clear that odor-deprivation reduced the number of newly generated neurons in the olfactory bulb, some compensatory processes maintain the constant level of evoked inhibition required for mitral/tufted cell synchronization. In agreement with this hypothesis, olfactory responses assessed at the behavioral level were unaltered after odor-deprivation [69]. These results are very intriguing since activity-dependent change in the number of newly generated interneurons seems to have different functional implication in olfactory performances. An increase in the number of newborn neurons induced by elevation of olfactory activity ameliorated olfactory memory [52], whereas odor-deprivation triggered decrease in the number of newly generated interneurons leaving behavioral responses unaltered [69]. Such apparent contradiction could result from the different ages of animals used during the course of these experiments. Results in odor enrichment were obtained from adult mice while neonatal animals were used for odor deprivation paradigms. It is conceivable that compensatory mechanisms revealed by occlusion experiments may operate during development stages but not in adulthood. Additionally, the olfactory system is the only system that normally experiences a reduction of sensory activity, for instance during nasal infections, and it has even been proposed that the olfactory system may have evolved compensatory mechanisms to develop relatively normally even when sensory inputs are dramatically reduced [70].

7. Functional consequence of challenging the adult neurogenesis

As with development of the immature brain, neurogenesis that takes place in the adult brain includes cell division, differentiation, migration, synapse formation and programmed cell death. In central sensory pathways, all these processes depend on external stimuli that