

Making scents of olfactory neurogenesis

Alan Carleton, Christelle Rochefort, Javier Morante-Oria, David Desmaisons,
Jean-Didier Vincent, Gilles Gheusi, Pierre-Marie Lledo*

*C.N.R.S., UPR 2197, Unité “Développement, Evolution, Plasticité du Système Nerveux”, Avenue de la Terrasse,
91198 Gif-sur-Yvette Cedex, France*

Abstract

Olfaction was long considered to belong more to the realm of art than to that of science. As a result, how the brain perceives, discriminates, and recognizes odorant molecules is still a mystery. Recent progress has nonetheless been made at early stages of the olfactory pathway when olfactory studies entered into the molecular era to elucidate the first contact of an odor molecule with a receptor. Our group focuses on the analysis of odor information in the olfactory bulb, the first processing relay in the mammalian brain. Using this model, we are attempting to decipher the code for odorant information. Furthermore, the olfactory bulb also provides an attractive model to investigate neuronal proliferation, differentiation, migration, and neuronal death, processes involving an interplay between genetic and epigenetic influences. Finally, our goal is to explore the possible consequences of the olfactory bulb plasticity, in olfactory performance. For these purposes, we aim to combine morphological, electrophysiological and behavioral approaches to investigate: (1) how the olfactory bulb processes odor molecule information, (2) how neural precursors differentiate into olfactory bulb interneurons, (3) how these newly-generated neurons integrate into an operational neural network, (4) what role they play in the adult olfactory bulb, and (5) how are basic olfactory functions maintained in such a sensory system subjected to continuous renewal of a large percentage of its neuronal population. These questions should provide new fuel for the molecular and cellular bases of sensory perception and shed light onto cellular bases of learning and memory. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Olfactory bulb; Neurogenesis; Glutamate; GABA; Granule cells; Reciprocal synapses

1. Introduction

Animals can recognize and discriminate chemical signals in the environment, which provide essential information for survival and profoundly influence their behavior. Chemical cues are not only necessary to detect and assess food, prey, predators and mating partners but also for intraspecific communication. In higher organisms, while chemosensation involves both taste and smell, most animals rely on olfaction as the principal chemosensory modality. Olfaction plays a critical role in food selection, as the perception of flavors results from the integration of olfactory and gustatory signals. This can have important consequences for the survival

of the organism, in as much as rotten food is easily perceived by the olfactory system and unpleasant sensations are memorized and associated with bad food-related experiences. Moreover, the mammalian olfactory system has been shown to regulate multiple complex functions such as sexual behavior (i.e. reproductive and/or maternal functions), neuroendocrine regulation, recognition of conspecifics, emotional states (i.e. attention, enthusiasm, aggression), and social information concerning the family, the clan or outsiders. Furthermore, while the power of olfactory stimuli in memory and the control of animal behavior have long been recognized, the neural mechanisms underlying this phenomenon are poorly understood. Basic to the investigation of these and other olfactory functions is an understanding of the anatomical and neurochemical organization of the olfactory system and its links to other parts of the brain. Before embarking on a systematic account of olfactory neuroanatomy and physiology, an overview of the principal neural circuitry is presented below.

* Corresponding author. Tel.: +33-1-69-82-4309; fax: +33-1-6907-0538.

E-mail address: lledo@iaf.cnrs-gif.fr (P.-M. Lledo).

2. Organization of the vertebrate olfactory system

The basic anatomy of the olfactory system is well known [1–3]. In mammals, initial odor detection takes place in the olfactory epithelium of the nasal cavity at the posterior end of the nose (Fig. 1). This area is exquisitely tuned to discriminate an immense variety of odor molecules of differing shapes and sizes which are often present in minuscule quantities in the environment. The discriminatory capacity of this system arises from a series of information-processing steps that occur in anatomically distinct structures. The first step takes place at the olfactory epithelium of the nose. This tissue is comprised of olfactory sensory neurons which detect odor molecules (odorants). Odor molecules activate receptors and a well-characterized signal transduction cascade in the olfactory neurons located in the epithelial surface of the nasal cavity. Odorant-evoked action potentials propagate along the axons of these sensory neurons to the central nervous system where the signals are relayed by projecting neurons (Fig. 1). Hence, the second step for olfactory processing occurs in the main olfactory bulb which receives sensory neuron projections to encode these sensory informations. The final processing occurs in higher-order brain structures com-

prising the primary and accessory olfactory cortex that correspond to the piriform and the entorhinal cortex, respectively. It is noteworthy that the preponderance of evidence indicates that the outputs from the olfactory bulb do not have the kind of point-to-point topographical projections to their target structures characteristic of all other sensory systems. As a result, odor information seems to be encoded by activity across the entire neuronal network.

3. Sensory transduction in the olfactory epithelium

Mature sensory neurons of the mammalian olfactory epithelium are the primary transducers of odorant signals from the external world to the central nervous system. Hence, the initial events in olfactory perception take place in these neurons that are embedded in a pseudo-stratified columnar epithelium that, in mammals, lines the posterior nasal cavity. This epithelium contains three major cell types: sensory neurons, support cells and olfactory stem cells. Sensory neurons are unusual in that they are short-lived cells that exist for only 30–60 days and are continually replaced by the stem cells. These small bipolar cells extend a single

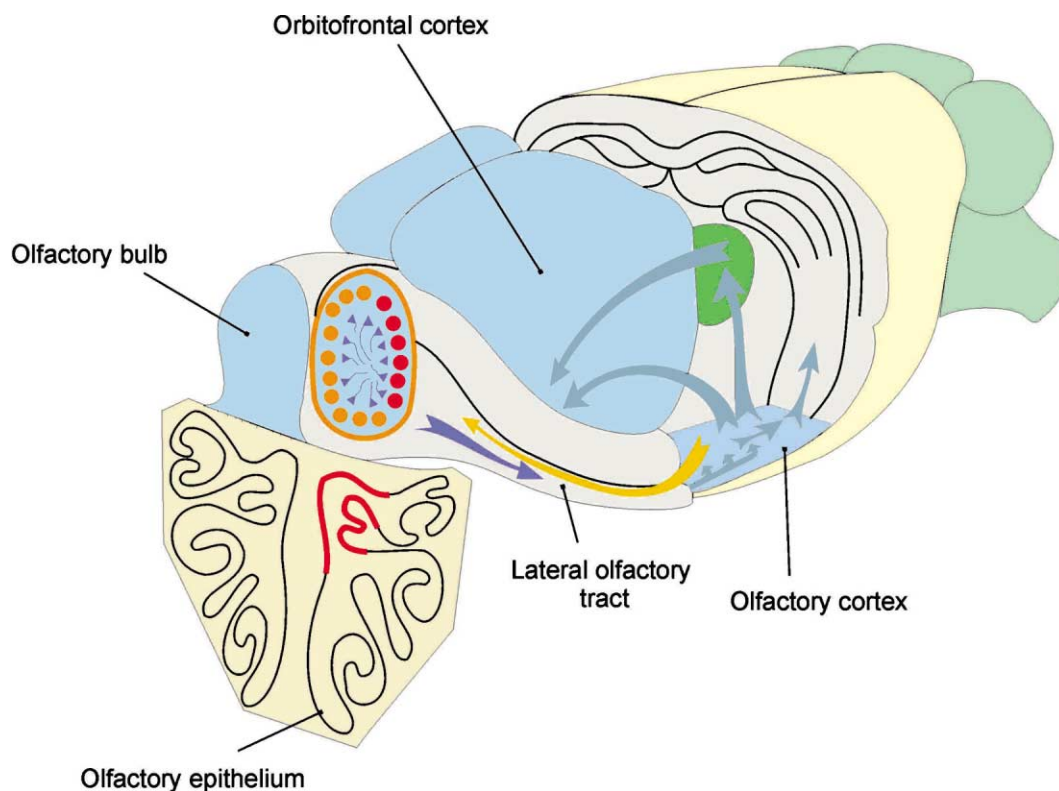


Fig. 1. The olfactory neural pathway. Coronal view of the nasal fossae shows about 2 cm² olfactory sensory epithelium projecting directly to the olfactory bulb of the central nervous system. Odor molecules and environmental substances enter through the nostrils and interact with sensory neurons located in olfactory epithelium. The axons of mitral and tufted cells form the lateral olfactory tract and synapse on neurons in the olfactory cortex. This cortex then project to other olfactory cortical regions and to the medial dorsal nucleus of the thalamus (green). Together, these cortical and thalamic regions are thought to be involved in conscious perception of odors. (adapted with permission from [33]).

dendrite to the epithelial surface from the apical pole. Numerous cilia protrude from this dendrite and extensively invade the mucus lining of the nasal cavity. Odor molecules that dissolve in the nasal mucus bind to specific receptors on the cilia of olfactory sensory neurons. These receptors, which have been cloned recently, are members of the G protein-coupled seven-transmembrane proteins [4]. Their activation induces a cascade of intracellular events culminating in the generation of a receptor potential in the soma of the sensory neuron. From its basal pole, the neuron sends a single axon to the olfactory bulb. The unmyelinated sensory neuron axons merge to form the olfactory nerve, which transmits the electrical signals to the main olfactory bulb. Each olfactory neuron achieves receptive specificity by expressing only one of about 1000 odorant receptor genes. Additionally, olfactory neurons expressing the same odorant receptor, which are randomly dispersed in one of the four zones of the olfactory epithelium, converge axons into a small number of topographically fixed glomeruli within the bulb (Fig. 2). Olfactory neurons are replaced continuously throughout life, but the neuronal population maintains a conserved connection pattern to the olfactory bulb.

4. Sensory information inputs to the bulb

The olfactory nerve projects to the primary output neurons of the olfactory bulb, the mitral/tufted (M/T) cells. Axonal termini of olfactory sensory neurons, unlike those of other sensory neurons, synapse directly

onto second-order neurons within the forebrain [5–7]. Here, the olfactory nerve forms excitatory synapses in regions of the main olfactory bulb known as glomeruli [8] and recent evidence shows that these synapses are glutamatergic [9]. The topography of these connections has been the subject of extensive studies (for review see [10]), which reveal that sensory neurons expressing a given receptor project to a given subset of glomeruli (Fig. 3). These data suggest that the processing of olfactory information adheres to a certain spatial distribution, at least between sensory neurons and the olfactory bulb itself.

Since the olfactory system can detect extremely faint signals [11], it is reasonable to suppose that synaptic transmission between the olfactory nerve and mitral cells should be extremely reliable. Indeed, we have found that at the first level of sensory processing in the olfactory system, the distal dendrites of M/T cells receive highly reliable excitatory synaptic inputs from the olfactory nerve. We and others have shown that these synaptic events are comprised of both AMPA/kainate and NMDA receptor-mediated components [9,12] (Fig. 4A). The NMDA component is particularly prolonged and may play an important role in the output from the olfactory bulb by maintaining a pattern of sustained discharge in mitral cells. We have also found that glutamate release from olfactory sensory axons can be down- but not up-regulated, suggesting a high probability of glutamate release from sensory neurons (Fig. 4B). Taken together these data suggest that the olfactory bulb amplifies sensory signals coming from olfactory sensory cells.

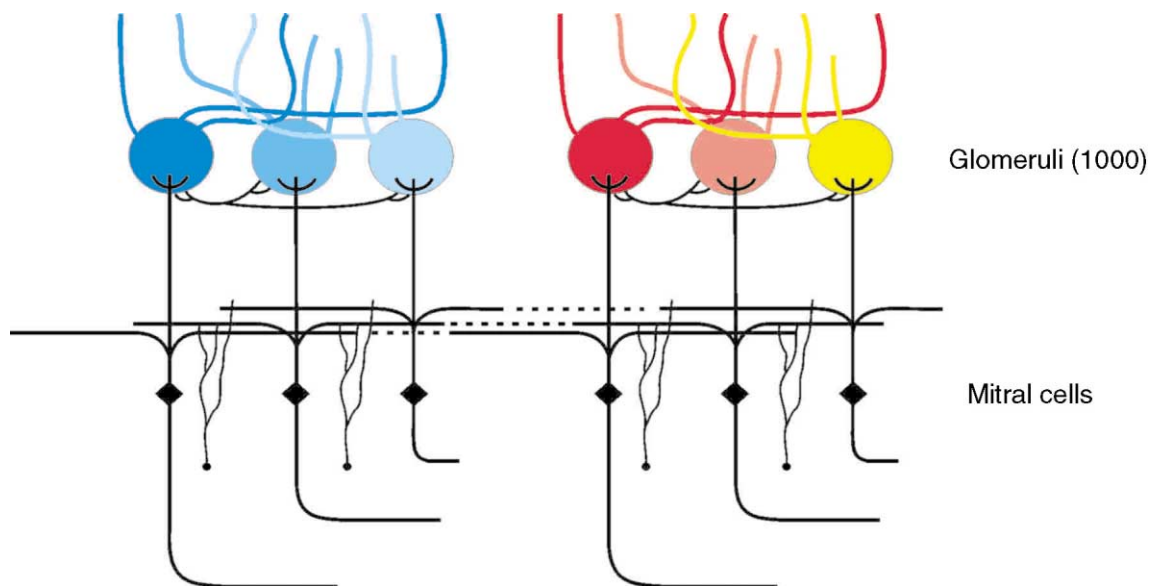


Fig. 2. Patterns of neurons can help the brain interpret a smell. Neurons that contain a particular type of receptor (indicated here by color) are arranged randomly throughout the epithelium, but their axons converge on localized regions of the olfactory bulb known as glomeruli. An odor can therefore be identified by a characteristic pattern of activity in the glomeruli.

5. Sensory information processing in the main olfactory bulb

Sensory information received by the olfactory bulb is likely to be processed and refined prior to its transmission to the olfactory cortex. This transmission is accomplished by intrabulbar circuits involving two classes of interneurons; periglomerular cells and granule cells. In addition, centrifugal inputs to the olfactory bulb from several other brain regions may modulate intrabulbar circuits and thereby influence signal transmission from the olfactory bulb [13,14].

The first potential site of signal processing in the olfactory bulb is the glomerulus. Here, periglomerular cells, which receive excitatory input from sensory axons

and form dendro-dendritic synapses with M/T neurons, may exert two types of inhibition. First, GABAergic periglomerular cells inhibit M/T cells via dendro-dendritic synapses. This type of inhibition is mediated by postsynaptic GABA_A receptors. Second, the presence of dopamine in other periglomerular cells and the expression of D₂ receptors on olfactory sensory axons lead us to propose a modulatory role for dopamine at this synapse.

We indeed found that dopamine receptor activation in the olfactory bulb significantly depresses synaptic transmission at the first relay between olfactory receptor neurons and mitral cells [15]. This depression results from the activation of the D₂ subtype of the dopamine receptor. A change in paired-pulse modulation during

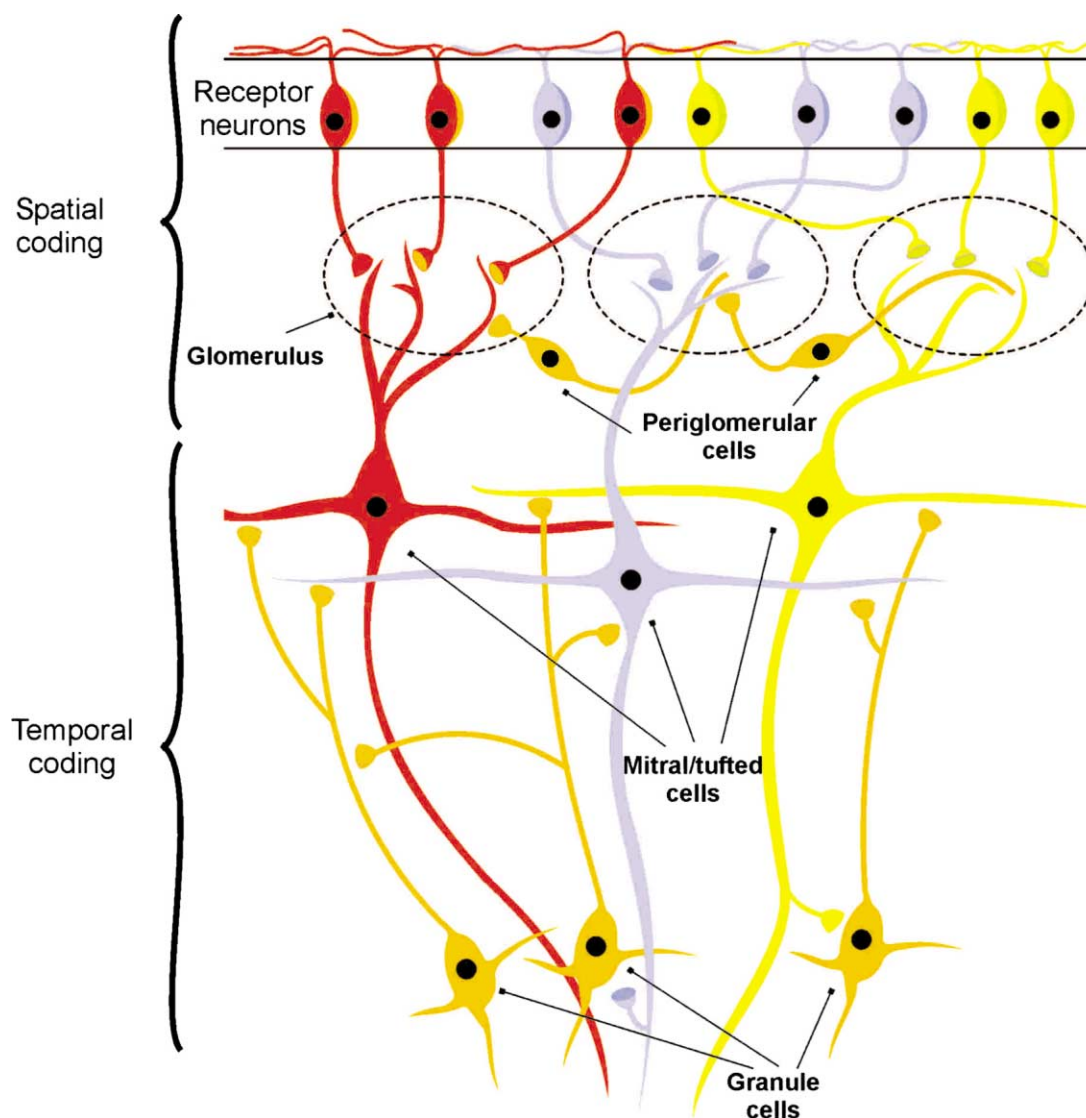


Fig. 3. Synaptic organization of the main olfactory bulb. Diagram of a possible arrangement of neurons in the olfactory pathway for representing odor molecules in neural space. Top, different types of receptor neurons each express a different member of the olfactory receptor family. Middle, olfactory glomeruli primarily receive converging input from neurons expressing one receptor molecule type (indicated by a color code). Bottom, mitral and tufted cells (deutoneurons) send the output of the olfactory bulb to the olfactory cortex after having received inhibitory inputs from granule cells. (adapted with permission from [35]).

the depression suggests a presynaptic locus for this dopamine action. This synaptic depression, which is independent of synaptic activity, provides the first evidence for dopaminergic control of olfactory inputs to the main olfactory bulb.

A second potential site for signal modulation lies deeper within the bulb. In addition to their primary dendrites which are confined to a single glomerulus (at least in mammals), individual M/T cells extend long basal dendrites laterally within the external plexiform layer underlying the glomeruli. These basal dendrites form reciprocal dendro-dendritic synapses with the dendrites of granule cells, the main group of bulbar interneurons. Granule cell dendrites are depolarized by glutamate released from mitral cells, and, in turn, inhibit M/T cells mediated by the release of GABA. Thus the interaction between M/T cells and granule cells at the reciprocal synapse regulates mitral cell firing activity by negative feedback. Because each granule cell contacts the dendrites of numerous M/T cells, it has been proposed that these connections might mediate lateral inhibition between M/T cells that innervate different glomeruli [16,17].

Both anatomical and functional analyses support the existence of lateral inhibitory mechanisms by which activity in M/T cells innervating one glomerulus may lead to suppression of activity in M/T cells innervating neighboring glomeruli [1,3,16]. Interestingly, these studies also suggest a role for these interactions in the refinement of sensory information. Examination of responses of individual M/T cells to inhalation of aliphatic aldehydes reveals that many individual cells are excited by one subset of these odorants, inhibited by another subset, and unaffected by yet a third subset [18]. The inhibitory responses were shown to be suppressed by agents that block reciprocal synapses between M/T cells and granule cells. Remarkably, the odorants that excited any given M/T cell were found to have numerically consecutive carbon chain lengths whereas those that inhibited the cell differed by one to several carbons. On the basis of these observations, it was postulated that each glomerular unit has the potential to respond to a wide range of related odorants but also receives inhibitory inputs from neighboring glomerular units through lateral inhibition. Weak responses to a given odorant in one glomerular unit may be thereby suppressed by strong responses in another unit. The predicted outcome is that although the glomerular unit receives signals pertaining to many related odorants, it relays information to the cortex concerning only a subset of these odorants. Thus, at the level of the main olfactory bulb, the quality of the odor stimulus is encoded by a specific combination of activated glomerular modules.

Another important feature which seems to play a key role in olfaction is the generation of synchronized net-

work oscillations which emerge in the olfactory bulb [19,20]. We have recently shown that this coordinated activity may result from interactions between the intrinsic properties of mitral cells and feedback inhibition exerted by granule cells at dendro-dendritic synapses [12]. These data provide a new role for inhibition as an efficient propagator of synchronous activity within the olfactory bulb (Fig. 5).

6. Granule cells neurogenesis

The olfactory bulb is one of the few structures in the mammalian central nervous system in which there is a continuous supply of newly generated neurons [21,22]. These progenitor cells originate from the subventricular zone (SVZ) of the lateral ventricle which produces a proliferative population of stem cells throughout the life of the organism (for a recent review [23]). Their progeny can either die or give rise to neuronal progenitors that migrate along a tangential pathway, called the rostral migratory stream, into the core of the main olfactory bulb [24,25] (Fig. 6). Once in the bulb, the precursor cells turn radially to invade the granule and periglomerular layers, where they differentiate primarily into local interneurons [21,26]. Although this ongoing neurogenesis and

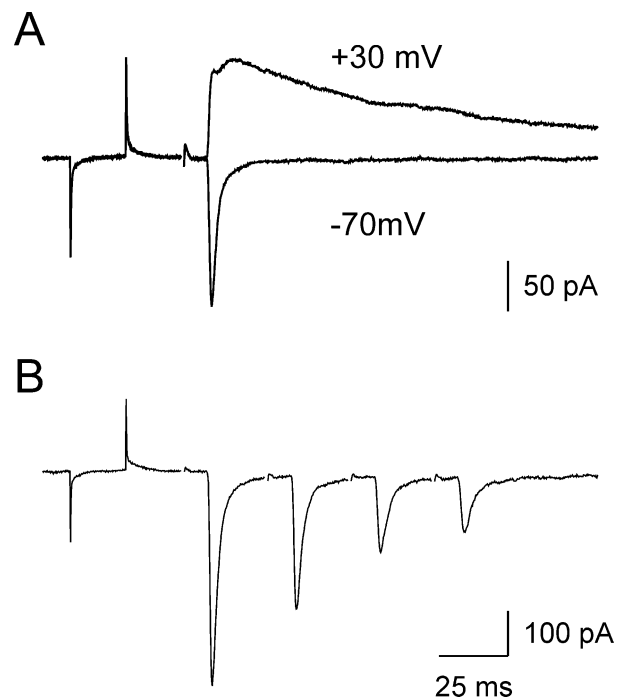


Fig. 4. Olfactory nerve stimulation activates mitral cells via NMDA and non-NMDA receptors. A, Excitatory synaptic currents recorded at two membrane potentials from the same mitral cell. Trace obtained at -70 mV revealed the non-NMDA component while at $+30$ mV, two components were clearly observed (e.g. NMDA and non-NMDA currents). B, A train of four stimulations indicated a strong depression indicating a high glutamate release from olfactory nerve terminals.

migration has been extensively documented, its function remains unknown.

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). 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. This defect is manifest by both an accumulation of precursors along the pathway and a reduction in the size of the olfactory bulb [27,28]. A similar defect is observed in neonatal animals treated with an enzyme that specifically cleaves the polysialic acid moiety from NCAM [29]. Based on these findings we suggest that interneuron populations are modulated at different stages of cell development.

As described earlier, inhibitory synapses from granule cells shape the temporal discharge patterns of olfactory bulb output neurons [12]. This temporal coding is

thought to be responsible for synchronization of these principal neurons, which, at least in insects is crucial for odor processing [30,31; for review see 32]. Thus, if granule cells generated in adulthood are necessary for olfactory bulb function, then a defect in the rostral migration leading to a reduction of the recruitment of new bulbar interneurons should alter olfactory behavioral responses. We tested this hypothesis by quantifying the level of migration and the size of interneuron populations in NCAM-mutant mice. We found that the dramatic reduction in granule cells in knockout mice was accompanied by impaired odor discrimination, with no change in odor detection or olfactory memory [34]. This suggests a specific role for this interneuron population in downstream coding of olfactory information. We propose that a critical level of inhibition mediated by the activation of GABA_A receptors localized on the secondary dendrites of M/T cells, is crucial to olfactory processes, at least at the first relay of the system. Since the olfactory bulb is also involved in consolidation

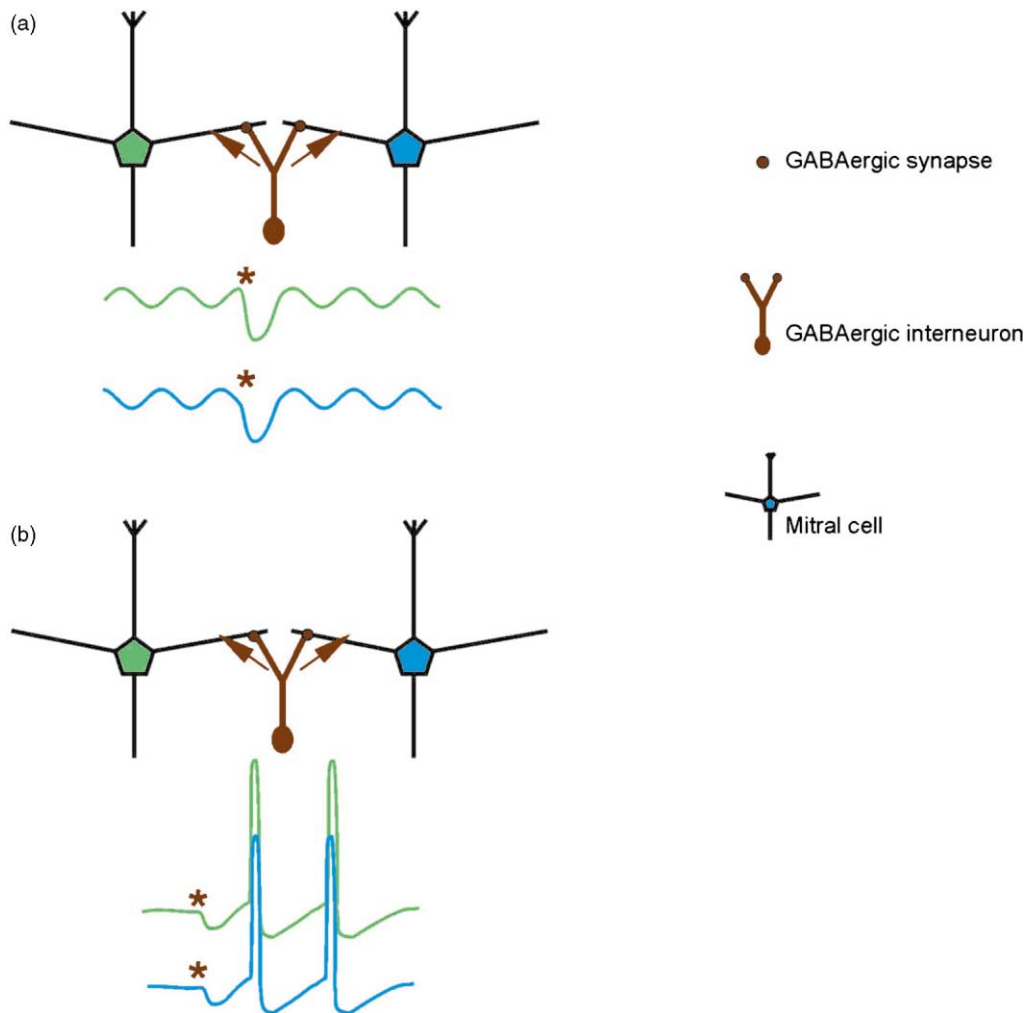


Fig. 5. Models for synchronization of mitral cells involving intrinsic subthreshold oscillations of the membrane potential with inhibitory synaptic inputs. A, Synchronization of subthreshold oscillations resulting from two IPSPs in distinct mitral cells coming from the same granule cell. B, For lower membrane potential values, IPSPs can trigger 'rebound' spikes with similar timing in the two distinct mitral cells.

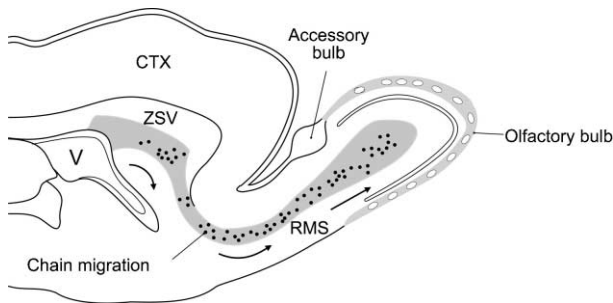


Fig. 6. Parasagittal section of the rodent forebrain. Newly-generated neurons (dots) originate from the subventricular zone (SVZ) located in the wall of the lateral ventricle. Here, steadily dividing stem cells and progenitors produce neuroblasts. These neuronal progenitors then migrate through the so-called rostral migratory stream (RMS) to populate the main olfactory bulb, where they differentiate into local inhibitory interneurons and establish connections with their neuronal targets.

processes associated with long-term odor memory, we propose that a modulation in the number of GABAergic interneurons by continual neurogenesis may participate in certain forms of olfactory learning, for example, the memorization of the odor of a lamb by its mother.

7. Concluding remarks

The basic circuit underlying excitatory and inhibitory interactions in the olfactory bulb reveals a wide range of synaptic connections. This diversity amplifies the possibilities for differential pathways through the relay neurons and for the number and variety of microcircuit interactions with periglomerular and granule cells. In the olfactory bulb, information is thought to be encoded across neuron assemblies that cannot be extracted by averaging the firing frequency. GABAergic inhibition is therefore important for olfactory coding, but within a framework that differs from conventional inhibition rules described for other sensory systems. The persistence of a high level of inhibitory interneuron production within the olfactory bulb, throughout adulthood and its conservation throughout evolution suggest that this process is of fundamental biological significance. We propose that a combination of both cell addition and cell elimination events may create conditions under which the odor discrimination level may be regulated.

Acknowledgements

This work was supported in part by Centre National de Recherche Scientifique, a grant from the French Ministère de la Recherche et de l'Enseignement (A.C.) and from Ministère de l'Éducation Nationale, de la Recherche et de la Technologie (ACI Biologie du Développement et Physiologie Intégrative 2000) to P.-M.L.

References

- [1] M.T. Shipley, M. Ennis, Functional organization of olfactory system, *J. Neurobiol.* 30 (1996) 123–176.
- [2] S.L. Sullivan, L. Dryer, Information processing in mammalian olfactory system, *J. Neurobiol.* 30 (1996) 20–36.
- [3] G. Shepherd, *The Synaptic Organization of the Brain*, Fourth Edition, Oxford University Press, Oxford, 1998.
- [4] L. Buck, R. Axel, A novel multigene family may encode odorant receptors: a molecular basis for odor recognition, *Cell* 65 (1991) 175–187.
- [5] P.P. Graziadei, J.F. Metcalf, Autoradiographic and ultrastructural observations on the frog's olfactory mucosa, *Z. Zellforsch. Mikrosk. Anat.* 116 (1971) 305–318.
- [6] D.G. Moulton, Dynamics of cell populations in the olfactory epithelium, *Ann. N. Y. Acad. Sci.* 237 (1974) 52–61.
- [7] R.M. Costanzo, E.E. Morrison, Three-dimensional scanning electron microscopic study of the normal hamster olfactory epithelium, *J. Neurocytol.* 18 (1989) 381–391.
- [8] K. Mori, M.C. Nowicky, G.M. Shepherd, Analysis of synaptic potentials in mitral cells in the isolated turtle olfactory bulb, *J. Physiol. Lond.* 314 (1981) 295–309.
- [9] M. Ennis, L.A. Zimmer, M.T. Shipley, Olfactory nerve stimulation activates rat mitral cells via NMDA and non-NMDA receptors in vitro, *Neuroreport* 7 (1996) 989–992.
- [10] L.B. Buck, Information coding in the vertebrate olfactory system, *Annu. Rev. Neurosci.* 19 (1996) 517–544.
- [11] A. Menini, C. Picco, S. Firestein, Quantal-like current fluctuations induced by odorants in olfactory receptor cells, *Nature* 373 (1995) 435–437.
- [12] D. Desmaisons, J.D. Vincent, P.M. Lledo, Control of action potential timing by intrinsic subthreshold oscillations in olfactory bulb output neurons, *J. Neurosci.* 19 (1999) 10727–10737.
- [13] N. Halasz, G.M. Shepherd, Neurochemistry of the vertebrate olfactory bulb, *Neuroscience* 10 (1983) 579–619.
- [14] F. Macrides, B. Davis, *Olfactory bulb*. In: P.C. Emson (Ed.), *Chemical Neuroanatomy*, Raven Press, New York, 1983, pp. 391–426.
- [15] A.Y. Hsia, J.D. Vincent, P.M. Lledo, Dopamine depresses synaptic inputs into the olfactory bulb, *J. Neurophysiol.* 82 (1999) 1082–1085.
- [16] M. Yokoi, K. Mori, S. Nakanishi, Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb, *Proc. Natl. Acad. Sci. USA* 92 (1995) 3371–3375.
- [17] J.S. Isaacson, B.W. Strowbridge, Olfactory reciprocal synapses: dendritic signaling in the CNS, *Neuron* 20 (1998) 749–761.
- [18] K. Mori, H. Nagao, Y. Yoshihara, The olfactory bulb: coding and processing of odor molecule information, *Science* 286 (1999) 711–715.
- [19] E.D. Adrian, The electrical activity of mammalian olfactory bulb, *Electro. Clin. Neurophysiol.* 2 (1950) 377–388.
- [20] W.J. Freeman, Spatial properties of an EEG event in the olfactory bulb and cortex, *Electroencephalogr. Clin. Neurophysiol.* 44 (1978) 586–605.
- [21] A. Alvarez-Buylla, Mechanism of migration of olfactory bulb interneurons, *Semin. Cell Dev. Biol.* 8 (1997) 207–213.
- [22] M.B. Luskin, Neuroblasts of the postnatal mammalian forebrain: their phenotype and fate, *J. Neurobiol.* 36 (1998) 221–233.
- [23] S. Temple, A. Alvarez-Buylla, Stem cells in the adult mammalian central nervous system, *Curr. Opin. Neurobiol.* 9 (1999) 135–141.
- [24] M.B. Luskin, Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone, *Neuron* 11 (1993) 173–189.
- [25] C. Lois, A. Alvarez-Buylla, Long-distance neuronal migration in the adult mammalian brain, *Science* 264 (1994) 1145–1148.
- [26] N.A. O'Rourke, Neuronal chain gangs: homotypic contacts support migration into the olfactory bulb, *Neuron* 16 (1996) 1061–1064.

- [27] H. Tomasiewicz, K. Ono, D. Yee, C. Thompson, C. Goridis, U. Rutishauser, T. Magnuson, Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system, *Neuron* 11 (1993) 1163–1174.
- [28] H. Cremer, R. Lange, C. Cristoph, M. Plomann, G. Vopper, J. Roes, R. Brown, S. Baldwin, P. Kraemer, S. Scheff, D. Barthels, K. Rajewsky, W. Wille, Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning, *Nature* 367 (1994) 455–459.
- [29] K. Ono, H. Tomasiewicz, T. Magnuson, U. Rutishauser, N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid, *Neuron* 13 (1994) 595–609.
- [30] G. Laurent, M. Wehr, H. Davidowitz, Temporal representations of odors in an olfactory network, *J. Neurosci.* 16 (1996) 3837–3847.
- [31] M. Stopfer, S. Bhagavan, B. Smith, G. Laurent, Impaired odour discrimination on desynchronization of odour-encoding neural assemblies, *Nature* 390 (1997) 70–74.
- [32] G. Laurent, Dynamical representation of odors by oscillating and evolving neural assemblies, *Trends Neurosci.* 19 (1996) 489–496.
- [33] A. Holley, G. Sicard, Les récepteurs olfactifs et le codage neuronal du l'odeur, *Médecine/Sciences* 10 (1994) 1091–1098.
- [34] G. Gheusi, H. Cremer, H. McLean, G. Chazal, J.D. Vincent, Pierre-Marie Lledo, Importance of newly generated neurons in the adult olfactory bulb for odor discrimination, *PNAS* 97 (2000) 1823–1828.
- [35] E.B. Keverne, Olfactory learning, *Current Opinion in Neurobiology* 5 (1997) 482–488.