CMLS, Cell. Mol. Life Sci. 58 (2001) 531–537
1420-682X/01/040531-7 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Targeting of olfactory neurons

J. Strotmann

Institute of Physiology, University Stuttgart-Hohenheim, Garbenstrasse 30, 70593 Stuttgart (Germany),
Fax: +49 711 459 3726; e-mail: strotman@uni-hohenheim.de

Abstract. Olfactory sensory neurons detect an enormous variety of small volatile molecules with extremely high sensitivity and specificity. The actual recognition and discrimination of odorous compounds is accomplished by specific receptor proteins located in the ciliary membrane of the sensory neurons. Axonal connections into the olfactory bulb, the first relay station for odor processing in the brain, are organized such that all neurons expressing the same odorant receptor converge their axons onto common glomeruli which are located at similar positions in all individuals from one species. For the establishment of this precise targeting of olfactory axons to their appropriate glomeruli, combinatorial functions of axon-associated cell adhesion molecules and odorant receptor proteins appear to be required. Odorants that stimulate distinct receptor cell populations will thereby activate a specific combination of glomeruli in the bulb; this characteristic activity pattern may be used by the system to encode the quality of a particular odorant.

Key words. Axon guidance; coding; gene targeting; glomerulus; odorant receptor; olfaction; olfactory bulb; projection.

Introduction

Odorants for terrestrial animals are typically hydrophobic, volatile organic compounds. Subtle alterations in their molecular structure can lead to profound changes in the perceived odor quality. An immense variety of odorous molecules are recognized and discriminated by the sense of smell. The discriminatory capacity is supposed to derive from precise information processing at different levels of the olfactory system, which begins in the chemosensory epithelium of the nose, where odorous molecules stimulate distinct sets of olfactory neurons, followed by the glomeruli of the main olfactory bulb (MOB), which receives the sensory information and relays it to higher brain centers like e. g. the piriform cortex and the entorhinal cortex.

The initial steps in odor reception occur in olfactory neurons. These bipolar nerve cells reside in the olfactory epithelium that lines the walls of the posterior nasal cavity, including the nasal septum [1, 2]. Millions of neurons are distributed over the wide and thin sheet of nasal epithelium which covers the surface of an intricate array of complex convolute structures of the nasal cavity, called turbinates. Each neuron extends a single dendrite to the surface of the epithelium; the apical dendritic knob extends into several ciliary processes bathing in the mucus layer that covers the olfactory epithelium. These cilia are the specialized sensory compartments of the olfactory neurons comprising odorant receptors as well as the molecular machinery for signal transduction [3]. Odor molecules entering the nasal cavity by the stream of inhaled air dissolve in the mucus and are supposed to bind to specific odorant receptors in the ciliary membrane. In mammals, the large repertoire of odorant receptor types is encoded by about a thousand different genes, each gene encoding a distinct seven-transmembrane domain receptor protein [4]. Individual olfactory neurons are believed to express only one of the thousand receptor types [5]. Upon binding of an appropriate ligand, the receptor triggers an intracellular reaction cascade mediating the chemoelectrical signal transduction process that generates the receptor potential which elicits action potentials conveyed to the MOB. Distinct odorants are supposed to activate various subpopulations of olfactory neurons to a different extent, thus eliciting a characteristic pattern of neural activity.

Thus, by the chemoelectrical transduction process, the chemical structures of odor molecules are mapped in neural space within the large population of olfactory neurons. Whereas in the visual and somatosensory system, sensory cells encode spatial attributes of the stimulus by virtue of their exact position in the sensory epithelium,

the olfactory system does not encode external spatial cues. It has therefore been proposed that neural space may map molecular properties, based upon binding of odorous ligands to odorant receptors of distinct sensory cell populations [6]. Approaching the question whether neurons activated by a given odor and expressing a given receptor type are distributed randomly in the olfactory epithelium, or whether they are grouped in spatial patterns led to conflicting findings. Whereas physiological investigations have provided evidence that particular odors elicit localized responses ('activity hot spots') in the nasal epithelial sheet [7–9], in situ hybridization studies have indicated that neurons expressing a specific receptor type are segregated within one of several broad but circumscribed anterior-posterior zones within the epithelium [10–12]. The current data indicate that within one of the zones most olfactory neuron subpopulations are randomly distributed. It is presently unclear how the rather broad topographical distribution of chemospecific neurons and the localized physiological activity patterns can be reconciled. However, for a small receptor subfamily it was found that the expressing neurons are organized in clusters [13–15].

Zone-to-zone topography

Each olfactory neuron projects an unmyelinated axon to the brain; the axon penetrates the cribriform plate and terminates in the MOB within discrete anatomical structures called glomeruli. Glomeruli are spherical conglomerates of complex neuropil, formed by the axonal processes of olfactory neurons and the dendritic trees of MOB neurons (mitral/tufted cells). Since the axons of olfactory neurons neither have collaterals within the epithelium nor terminal branches projecting to multiple glomeruli [16, 17], it is conceivable that an olfactory neuron conveys its information to a single glomerulus. Whether the olfactory neurons project their axons from the epithelium to the bulb in a diffuse or a topographically organized manner, has been a long-standing issue in the field of olfaction [18]. Although the apparently random arrange-

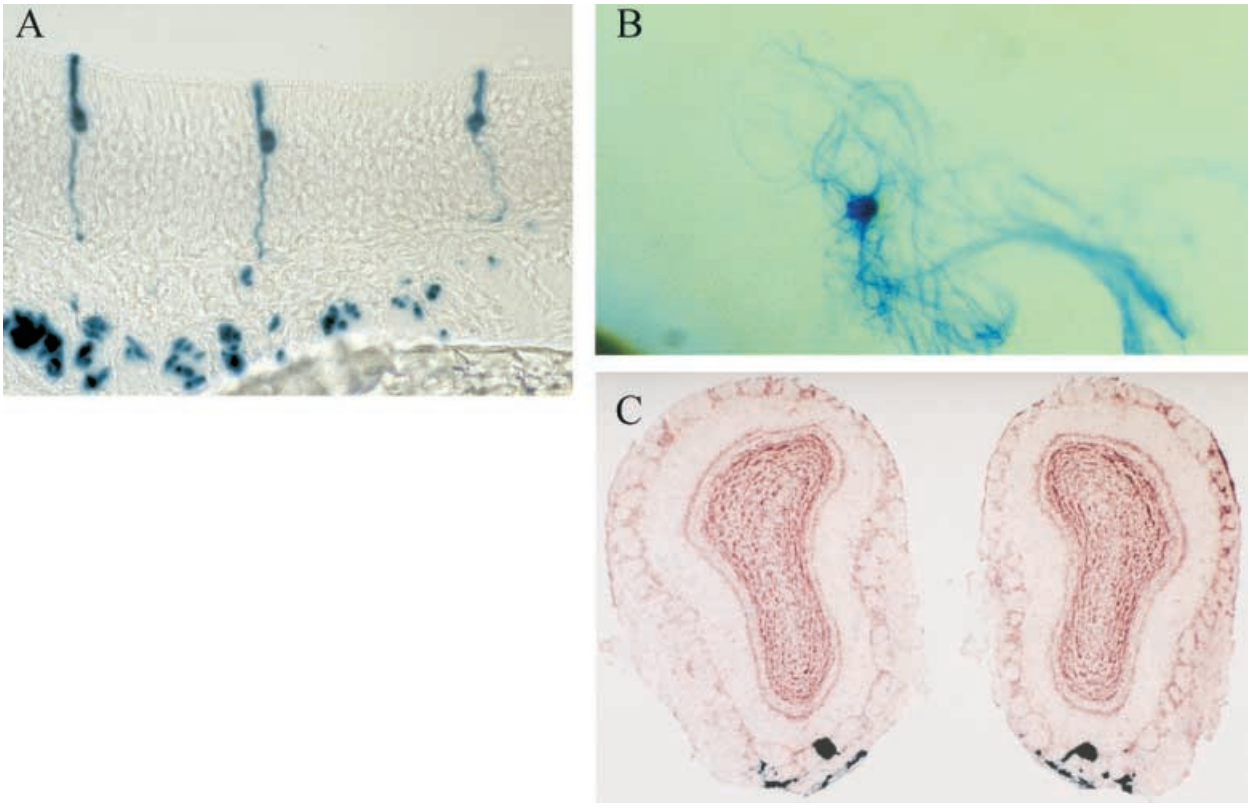

Figure 1. (*A*) Cross-section through the olfactory epithelium of an *OR-IRES-tau-lacZ* mouse after X-gal staining. Cell body, dendrite and axon of neurons expressing the mutated receptor gene are stained blue. (*B*) Whole-mount view of an olfactory bulb after X-gal staining. Axons from neurons expressing *tau-lacZ* under control of a distinct receptor type converge onto the same glomerulus. The axons approach this point of convergence from different directions. (*C*) Cross-section through the olfactory bulbs after X-gal staining. Counterstaining with neutral red shows the outlines of all glomeruli present on this particular section. Only a single glomerulus in each bulb is intensely stained blue due to the incoming axons from *tau-lacZ* expressing neurons. The location of the glomeruli is bilaterally symmetric in the two bulbs from one individual.

ments of nerve bundles in the olfactory nerve layer of the bulb suggested little topographical organization, recent anatomical, functional and molecular studies have provided experimental evidence indicating topographical specificity.

Various tract-tracing approaches, including the Nauta degeneration technique and horseradish peroxidase retrograde transport, revealed that distinct areas of the olfactory epithelium are topographically related to defined regions of the MOB [19–23]. Comparing the locations of neurons that were found to project to different regions of the bulb in those studies with the spatial zones based on expression of receptor types suggested that neurons in a specific expression zone project their axons selectively to a characteristic region in the MOB.

Recent studies try to identify molecules that might be involved in this regional patterning. So far, the best-characterized molecule is the olfactory-specific cell adhesion molecule (OCAM), which is an integral protein of the axonal membrane [24]. It appears to be selectively expressed in olfactory neurons located in the three ventro-lateral zones of the sensory epithelium, but not in neurons residing in the dorsomedial zone. The two neuron populations show a clearly segregated projection of their axons: OCAM-positive cells project onto glomeruli in the ventro-lateral regions, and OCAM-negative cells project onto glomeruli in the dorso-medial regions of the bulb. It has been proposed that OCAM may contribute in guiding olfactory axons to their appropriate target region in the bulb by selective fasciculation of zonal subsets of olfactory axons [24]. It is assumed that probably OCAM-related molecules are present in the OCAM-negative dorsal zone. A subset of olfactory neurons in the dorso-medial zone can be visualized by a monoclonal antibody CC2; these cells project to a corresponding dorso-medial region of the bulb [25].

Subpopulations of olfactory neurons which are intermingled in the nasal epithelium not only have to find their target region in the bulb but also their specific target glomerulus. This raises the question how axons of a neuron population which are widely scattered throughout an epithelial zone specifically terminate in exactly the same glomerulus. Based on the discovery that subpopulations of olfactory neurons carry distinct cell surface carbohydrates, it has been proposed that these molecules may play a role in targeting axons to their specific glomeruli [26]. Recently, the first experimental evidence has been provided supporting this notion; the lectin *Dolichus biflorus agglutinin* (DBA) specifically labels a subset of neurons which are dispersed throughout the epithelium, but their axons coalesce into discrete fascicles in the nerve fiber layer of the bulb before terminating in select glomeruli [26]. In mutant mice that lack the endogenously expressed lectin galectin-1 [27], a subpopulation of the DBA-positive neurons that normally project to the dorso-caudal region of the bulb, now fails to do so. The lectin-carbohydrate interaction may mediate selective fasciculation of axon subpopulations, e.g. by cross-linking carbohydrate moieties on their axonal surface.

Axonal convergence of neurons expressing the same odorant receptor

Based on histochemical observations and physiological recordings from the MOB, it was postulated that olfactory neurons expressing the same odorant receptor may converge their axons onto a few common target glomeruli [28–30]. Experimental proof for this model has recently been provided by means of molecular approaches. Using an ultrasensitive in situ hybridization method, messenger RNAs (mRNAs) for distinct odorant receptors were detected at a few sites of the bulb [31, 32] which appear to be glomeruli. These observations suggest that receptor mRNAs transcribed in the somata of olfactory neurons in the nasal epithelium may reach the axon terminals. It is currently unclear whether this occurs by anterograde transport or passive diffusion; also, the functional relevance of mRNAs in the axon terminals is unknown. The massive convergence of axons onto a distinct spot provides levels of mRNA sufficient for visualization. Using this approach, the targets for distinct sensory cell populations have been identified: two loci in each bulb, one located on the medial and one on the lateral side of each bulb. These positions were found to be bilaterally symmetrical between the left and the right bulb and roughly invariant among individuals from one species. These experimental data support the model of convergence for axons from olfactory neurons expressing the same odo-

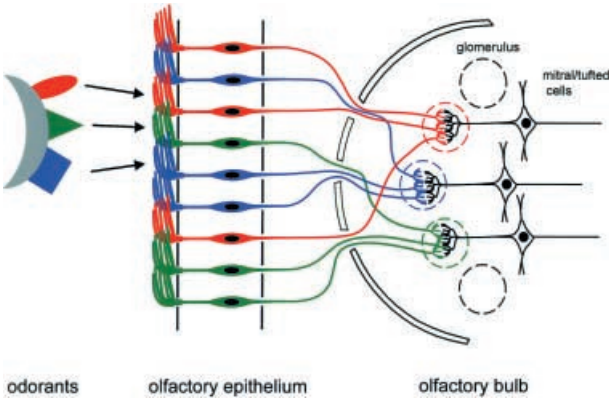

Figure 2. Axonal convergence. The seemingly random distribution of neurons within the epithelium expressing a given receptor is turned into an ordered projection towards the bulb. Axons of neurons expressing the same receptor type converge onto the same glomerulus. Odorous molecules which stimulate different receptor subtypes will thereby activate a characteristic pattern of glomeruli, which may be used by the system to encode the quality of a particular odor.

rant receptor. However, the demonstration that the axons from a large number of olfactory neurons expressing a given receptor converge onto common glomeruli did not exclude the possibility that axons from certain neurons of this population diverge to other targets and may thus be below the detection level.

Analysis of projection by axonal markers

Mombaerts et al. [33] have developed a much more precise labeling technique allowing single-axon resolution. They designed a genetic strategy in mice to follow the projection of neurons expressing the same receptor. The tau-lacZ gene, producing a fusion protein of the axon-targeting signal of the microtubule-associated protein tau and the bacterial β-galactosidase, was inserted immediately 3′ the stop codon of the odorant receptor gene *P2*. Inclusion of an internal ribosome entry site (IRES) ensured that the tau-lacZ reporter was expressed in the exact subset of neurons that express this particular receptor gene, without disrupting the coding region (*P2-IRES-taulacZ*). By a simple enzymatic reaction employing the β-galactosidase substrate X-gal, *P2*-expressing neurons including their axonal processes were stained blue (fig. 1 a). This elegant approach provided direct demonstration that all neurons expressing *P2-IRES-taulacZ*, which are broadly distributed throughout zone III of the epithelium, converge their axons onto two glomeruli in each bulb, one on the medial side and one on the lateral side (fig. 1b). The glomeruli were found in fixed positions in the bulb in all individuals (fig. 1 c), confirming the results obtained by in situ hybridization. The convergence of a large number of functionally equivalent sensory neurons is supposed to greatly enhance the sensitivity of the olfactory system.

Role of the odorant receptor in axon guidance

The tight linkage between the receptor choice of an olfactory neuron and the site of its projection within the MOB led to the concept that the receptor itself may be involved in the targeting process. To test this hypothesis, mice were generated in which the receptor gene *P2* was modified in a way that neurons which activate this gene locus express only tau-lacZ but not the receptor [34]. It was found that the population of neurons which lacked the P2 receptor still extended their axons from the epithelium toward the bulb; however, the axons did not enter into the glomerular layer, but remained broadly dispersed in the outer nerve layer. This observation strongly suggested that the presence of a functional receptor protein is required for axonal convergence and raised the question, what role does the receptor play in this process? It seemed conceivable that a functional receptor protein, independent of its exact primary structure, may be necessary to correctly target the axon. An alternative model suggested that the receptor protein may be present in the axon membrane, directly interacting with guidance cues and thereby helping to navigate the growth cone to its appropriate target. To scrutinize these models, Mombaerts et al. [33] have genetically substituted the gene encoding the odorant receptor *P2* by the coding region of odorant receptor gene *M12*. Neurons expressing the *M12* receptor normally project to glomeruli far distant from those expressing *P2*. If the receptor proteins guide the axons to their destination in the bulb, *M12*-expressing *P2* neurons should now project their axons onto the wild-type *M12* glomeruli. It was found that *P2* neurons expressing M12 receptors converged onto two glomeruli, but surprisingly these glomeruli were normally not innervated by either of the two receptors [33]. This finding indicated that the receptor protein indeed seems to contribute to the targeting of the axons, but the precise destination requires additional guidance cues, e. g. zone-specific surface molecules, like OCAM. This idea was favored by the observation that replacement of the *P2* sequence by a receptor expressed in the same zone caused a projection to target sites next to the wild-type glomeruli [34]; the slight incorrectness in targeting was explained by subtle sequence changes in the ‘swapped’ receptor which were necessary to generate the new allele. A coordinated interplay of zone-specific guidance molecules and odorant receptors thus may determine the precise destination of projection. In fact, based on the location of the novel convergence points, it has been hypothesized that zone-specific guidance cues may determine the positioning of projection within the dorsal-ventral axis, whereas the receptor proteins dictate the positioning of the glomeruli along the anterior-posterior axis of the bulb [34]. Odorant receptor proteins seem to play a dual functional role: detecting volatile ligands at the level of the cilia and recognizing guidance cues at the level of the axon.

Guidance labels in the bulb

Whereas some potential guidance molecules have been identified for the olfactory neurons, possible interaction partners in the bulb are still elusive. Such molecules are supposed to be located on the dendritic membrane of the mitral/tufted cells, the synaptic partners of the incoming axons. However, recent studies have provided evidence which seems to argue against such a model; when *P2-IRES-taulacZ* mice were crossed with mutant mice that lack mitral and tufted cells [35], it was observed that convergence of olfactory axons still occurred; furthermore, even the topographic location of the targeted glomeruli was indistinguishable from the wild type. Similar results

were obtained with mice that lack the major GABA-ergic interneurons (periglomerular and granule cells) of the bulb; convergence of axons onto glomerular targets seemed not to be impaired [35]. These results leave the question open, which cell type of the olfactory bulb provides the guidance cues for the incoming axons of olfactory neurons. Also, the molecular nature of the guidance cues is elusive. It has recently been proposed that target cells in the MOB may also express olfactory receptors [36]; in this scenario, a homophilic interaction of identical or similar receptor proteins would provide the required specificity for targeting.

Projection pattern in the VNO

Pheromones are thought to be received by chemosensory neurons of the vomeronasal organ (VNO); these neurons transmit their information to the accessory olfactory bulb (AOB). The chemosensory epithelium of the VNO, unlike that of the main olfactory epithelium, is not divided into anterior-posterior zones, but rather into apical and basal layers of neurons. They are defined by the complementary expression of two distinct putative pheromone receptor families [37–40], as well as two distinct G-protein α subunits, $G_{i2\alpha}$ and $G_{o\alpha}$, respectively [41, 42]. Previous immunohistochemical studies have revealed that the layered organization of the epithelium is preserved at the level of the AOB: neurons from the apical layer specifically project to the rostral part of the AOB, whereas those from the basal layer target glomeruli in the caudal part [43].

To get further insight into the organizational principles underlying the sensory map of the VNO, receptor genes expressed in distinct subsets of VNO neurons were analyzed using the transgenic technology of expressing axonal markers in these cells. In two different studies [44, 45], VNO receptor genes were tagged with tau-lacZ; the knock-in constructs were specifically expressed in small numbers of neurons in the VNO epithelium. Interestingly, the population of VNO neurons expressing a particular receptor type projected to many, between 20–30 glomeruli, in the AOB. This is in sharp contrast to the main olfactory system. Clusters of glomeruli were found in several regions, suggesting that the AOB is divided into several distinct subdomains for axon targeting. Furthermore, the projection patterns were surprisingly variable among different individuals and even between the two bulbs from one individual. Nevertheless, comparing the projection patterns of two VNO neuron populations revealed characteristic patterns for each population [45]. The fact that neurons expressing a particular receptor type targeted about 20–30 different glomeruli implies that there is far less convergence in the vomeronasal map than in the olfactory map; it was calculated that only 10–20 VNO neurons converge their axons onto individual glomeruli [44], in contrast to the several thousand in the MOB. Although glomeruli in the AOB are small and difficult to define morphologically, glomeruli were detected that receive input from more than one VNO neuron population [45], indicating that individual mitral cells may receive input from more than one type of receptor neuron, which is another striking difference to the MOB.

The question whether VNO receptors are involved in guiding axons to their glomerular targets was addressed by generating mice in which particular VNO receptor genes were deleted. VNO neurons lacking a functional receptor projected into the AOB, but dispersed randomly, indicating that a VNO receptor seems to be required for normal targeting. Rodriguez et al. [44] performed an intriguing 'swap experiment': they replaced the coding region of a VNO receptor gene with that of a receptor gene normally expressed in the main olfactory epithelium. When this odorant receptor was expressed in VNO neurons, their axons still projected into the AOB; interestingly, they targeted many glomeruli, which seemed comparable to the wild-type situation. However, the projection pattern was distinct from neurons expressing the original VNO receptor.

The VNO map thus apparently is more complex than the simple spatial map characteristic of the main olfactory system. It was proposed that this may be due to the nature of pheromonal stimuli, which are usually blends of compounds whose individual components are present in several species. To recognize the correct pheromone cocktail, the VNO may focus on the exact composition of the mixture rather than individual components, losing the enormous discriminatory power generated in the MOB.

Conclusion

The emerging principle of axonal convergence has defined the glomerulus as an important site for integrating information from olfactory neurons expressing the same odorant receptor type (fig. 2). The application of novel approaches, like gene targeting, has provided fundamentally new insights into the molecular mechanisms underlying the precise wiring of olfactory sensory neurons. It is anticipated that detailed knowledge about the functional organization of the olfactory system will ultimately lead to a better understanding of the principles underlying olfactory coding and information processing.

1 Allison A. C. (1953) The morphology of the olfactory system in the vertebrates. Biol. Rev. **28:** 195–244
2 Graziadei P. P. C. (1977) Functional anatomy of the mammalian chemoreceptor system. In: Chemical Signals in Vertebrates, pp. 435–454, Müller-Schwarze D. and Mozell M. M. (eds), Plenum Press, New York
3 Menco B. M., Bruch R. C., Dau B., and Danho W. (1992) Ultractructural localization of olfactory transduction components: the G protein subunit Golf alpha and type III adenylyl cyclase. Neuron **8:** 441–453
4 Buck L. and Axel R. (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell **65:** 175–187
5 Malnic B., Hirono J., Sato T. and Buck L. B. (1999) Combinatorial receptor codes for odors. Cell **96:** 713–723
6 Shepherd G. M. (1985) The olfactory system: the uses of neural space for a non-spatial modality. Prog. Clin. Biol. Res. **176:** 99–114
7 Kauer J. S. and Moulton D. G. (1974) Responses of olfactory bulb neurones to odour stimulation of small nasal areas in the salamander. J. Physiol. **243:** 717–737
8 Mackay-Sim A. and Kesteven S. (1994) Topographic patterns of responsiveness to odorants in the rat olfactory epithelium. J. Neurophysiol. **71:** 150–160
9 Youngentob S. L., Kent P. F., Sheehe P. R., Schwob J. E. and Tzoumaka E. (1995) Mucosal inherent activity patterns in the rat: evidence from voltage-sensitive dyes. J. Neurophysiol. **73:** 387–398
10 Ressler K. J., Sullivan S. L. and Buck L. B. (1993) A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell **73:** 597–609
11 Strotmann J., Wanner I., Helfrich T., Beck A., Meinken C., Kubick S. et al. (1994) Olfactory neurones expressing distinct odorant receptor subtypes are spatially segregated in the nasal neuroepithelium. Cell Tissue Res. **276:** 429-438
12 Vassar R., Ngai J. and Axel R. (1993) Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. Cell **74:** 309–318
13 Strotmann J., Wanner I., Krieger J., Raming K. and Breer H. (1992) Expression of odorant receptors in spatially restricted subsets of chemosensory neurones. Neuro Report **3:** 1053–1056
14 Strotmann J., Wanner I., Helfrich T., Beck A. and Breer H. (1994) Rostro-caudal patterning of receptor-expressing olfactory neurones in the rat nasal cavity. Cell Tissue Res. **278:** 11–20
15 Strotmann J., Hoppe R., Conzelmann S., Feinstein P., Mombaerts P. and Breer H. (1999) Small subfamily of olfactory receptor genes: structural features, expression pattern and genomic organization. Gene **236:** 281–291
16 Shepherd G. M. and Greer C. A.(1990) Olfactory bulb. In: The Synaptic Organization of the Brain, pp. 133–169, Shepherd G. M. (ed.), Oxford University Press, New York
17 Scott J. W., Wellis D. P., Riggott M. J. and Buonviso N. (1993) Functional organization of the main olfactory bulb. Microscopy Research and Technique **24:** 142–156
18 Shepherd G. M. (1994) Discrimination of molecular signals by the olfactory receptor neuron. Neuron **13:** 771–790
19 Pedersen P. E., Jastreboff P. J., Stewart W. B. and Shepherd G. M. (1986) Mapping of an olfactory receptor population that projects to a specific region in the rat olfactory bulb. J. Comp. Neurol. **250:** 93–108
20 Stewart W. B. and Pedersen P. E. (1987) The spatial organization of olfactory nerve projections. Brain Res. **41:** 248–258
21 Saucier D. and Astic L. (1986) Analysis of the topographical organization of olfactory epithelium projections in the rat. Brain Res. Bull. **16:** 455–462
22 Astic L., Saucier D. and Holley A. (1987) Topographical relationships between olfactory receptor cells and glomerular foci in the rat olfactory bulb. Brain Res. **424:** 144–152
23 Schoenfeld T. A., Clancy A. N., Forbes W. B. and Macrides F. (1994) The spatial organization of the peripheral olfactory system of the hamster. Part I: Receptor neuron projections to the main olfactory bulb. Brain Res. Bull. **34:** 183–210
24 Yoshihara Y., Kawasaki M., Tamada A., Fujita H, Hayashi H., Kagamiyama H. et al. (1997) OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons. J. Neurosci. **17:** 5830–5842
25 Schwarting G. A. and Crandall J. E. (1991) Subsets of olfactory and vomeronasal sensory epithelial cells and axons revealed by monoclonal antibodies to carbohydrate antigenes. Brain Res. **547:** 239–248
26 Key B. and Akeson R. A. (1993) Distinct subsets of sensory olfactory neurons in mouse: possible role in the formation of the mosaic olfactory projection. J. Comp. Neurol. **335:** 335–368
27 Puche A. C., Poirier F., Hair M., Bartlett P. F. and Key B. (1996) Role of galectin-1 in the developing mouse olfactory system. Dev. Biol. **179:** 274–287
28 Stewart W. B., Kauer J. S. and Shepherd G. M. (1979) Functional organization of rat olfactory bulb analyzed by 2-deoxyglucose method. J. Comp. Neurol. **185:** 715–734
29 Lancet D., Greer C. A., Kauer J. S. and Shepherd G. M. (1982) Mapping of odor-related neuronal activity in the olfactory bulb by high-resolution 2-deoxyglucose autoradiography. Proc. Natl. Acad. Sci. USA **79:** 670–674
30 Imamura K., Mataga N. and Mori K. (1992) Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. J. Neurophysiol. **68:** 1986–2002
31 Ressler K. J., Sullivan S. L. and Buck L. B. (1994) Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell **79:** 1245–1255
32 Vassar R., Chao S. K., Sitcheran R., Nunez J. M., Vosshall L. B. and Axel R. (1994) Topographic organization of sensory projections to the olfactory bulb. Cell **79:** 981–991
33 Mombaerts P., Wang F., Dulac C., Chao S. K., Nemes A., Mendelsohn M. et al. (1996) Visualizing an olfactory sensory map. Cell **15:** 675–686
34 Wang F., Nemes A., Mendelsohn M. and Axel R. (1998) Odorant receptors govern the formation of a precise topographic map. Cell **93:** 47–60
35 Bulfone A., Wang F., Hevner R, Anderson S., Cutforth T., Chen, S. et al. (1998) An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons. Neuron **21:** 1273–1282
36 Dreyer W. J. (1998) The area code hypothesis revisited: olfactory receptors and other related transmembrane receptors may function as the last digits in a cell surface code for assembling embryos. Proc. Natl. Acad. Sci. USA **95:** 9072–9077
37 Dulac C. and Axel R. (1995) A novel family of genes encoding putative pheromone receptors in mammals. Cell **83:** 195–206
38 Matsunami H. and Buck L. B. (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell **90:** 775–784
39 Herrada G. and Dulac C. (1997) A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell **90:** 763–773
40 Ryba N. J. P. and Tirindelli R. (1997) A new multigene family of putative pheromone receptors. Neuron **19:** 371–379
41 Halpern M., Shnayder Shapiro L. and Jia Ch. (1995) Differential localization of G proteins in the opossum vomeronasal system. Brain Res. **677:** 157–161
42 Berghard A. and Buck L. B. (1996) Sensory transduction in vomeronasal neurons: evidence for $G_{\alpha o}$, $G_{\alpha i2}$, and adenylyl cyclase II as major components of a pheromone signaling cascade. J. Neurosci. **16(3):** 909–918

43 Jia C. and Halpern M. (1996) Subclasses of vomeronasal receptor neurons: differential expression of G proteins ($G_{i\alpha 2}$ and $G_{o\alpha}$) and segregated projections to the accessory olfactory bulb. Brain Res. **719:** 117–128
44 Rodriguez I., Feinstein P. and Mombaerts P. (1999) Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. Cell **97:** 199–208
45 Belluscio L., Koentges G., Axel, R. and Dulac C. (1999) A map of pheromone receptor activation in the mammalian brain. Cell **97:** 209–220