

Role of Axonal Odorant Receptors in Olfactory Topography

Claudia Lodovichi^{1,2,3} 

¹Neuroscience Institute CNR, Department of Biomedical Science, Padova, Italy. ²PNC, Padova Neuroscience Center, Padova, Italy. ³VIMM, Veneto Institute of Molecular Medicine, Padova, Italy.

Neuroscience Insights
Volume 15: 1–4
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/2633105520923411



ABSTRACT: A unique feature in the organization of the olfactory system is the dual role of the odorant receptors: they detect odors in the nasal epithelium and they play an instructive role in the convergence of olfactory sensory neuron axons in specific loci, ie, glomeruli, in the olfactory bulb. The dual role is corroborated by the expression of the odorant receptors in 2 specific locations of the olfactory sensory neurons: the cilia that protrude in the nostril, where the odorant receptors interact with odors, and the axon terminal, a suitable location for a potential axon guidance molecule. The mechanism of activation and function of the odorant receptors expressed at the axon terminal remained unknown for almost 20 years. A recent study identified the first putative ligand of the axonal odorant receptors, phosphatidylethanolamine-binding protein 1, a molecule expressed in the olfactory bulb. The distinctive mechanisms of activation of the odorant receptors expressed at the opposite locations in sensory neurons, by odors, at the cilia, and by molecules expressed in the olfactory bulb, at the axon terminal, explain the dual role of the odorant receptors and link the specificity of odor perception with its internal representation, in the topographic map.

KEYWORDS: Axonal odorant receptor, topographic map, olfactory system, real-time imaging

RECEIVED: April 5, 2020. **ACCEPTED:** April 10, 2020.

TYPE: Commentary

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Claudia Lodovichi, Neuroscience Institute CNR and VIMM, Via Orus 2, Padova 35129, Italy. Emails: claudia.lodovichi@unipd.it; lodovichi.c@gmail.com

COMMENT ON: Zamparo I, Francia S, Franchi SA, et al. Axonal odorant receptors mediate axon targeting. *Cell Rep.* 2019;29(13):4334-4348.e7. doi:10.1016/j.celrep.2019.11.099. PubMed PMID: 31875544; PubMed Central PMCID: PMC6941231. <https://www.ncbi.nlm.nih.gov/pubmed/31875544>

The odorant receptors (ORs) play a key part in the organization of the olfactory system. They detect odors in the olfactory epithelium and also define the targets of olfactory sensory neuron (OSN) axons in the olfactory bulb, the first relay station of the olfactory system. The dual role of the OR began to emerge more than 20 years ago, during the first investigations on the topographic organization of the olfactory system. Each OSN expresses only one in a repertoire of more than 1000 OR genes. The OR is a G protein-coupled receptor, which, upon binding odors, leads to an increase in cAMP (cyclic adenosine monophosphate) and Ca²⁺ (Figure 1). In the olfactory epithelium, neurons expressing a given OR are confined within overlapping zones along the dorsoventral axis, randomly intermingled to neurons expressing different receptors.¹ Spatial order, however, is achieved in the olfactory bulb, where neurons expressing the same OR converge to make synapses with the postsynaptic cells in invariant positions, i.e., glomeruli, on the medial and on the lateral side of each olfactory bulb.² This spatial segregation of sensory afferents provides the topographic map that defines the quality and the intensity of sensory stimuli (Figure 2). From the character of this organization emerges that, unlike other sensory modalities, the spatial distribution of sensory afferents does not reflect the spatial relation among neighbor OSNs in the periphery, whereas it is based on the identity of the OR. This concept was corroborated by elegant genetic experiments, the results of which clearly indicated that alterations of the OR sequence result in altered convergence of OSNs.³ These data provided compelling evidence that the ORs play an instructive role in the formation of the sensory map and posed the question

on the mechanism by which the ORs could regulate sensory axon segregation. To direct OSN axons to their target, ORs should be expressed at the axon terminal, as any axon guidance molecules, to detect cues in the target area. A few years later, this hypothesis was confirmed by the evidence that the ORs are locally expressed and translated at the axon terminal.^{4,5} It is worth noticing that the ORs were found to be expressed specifically and exclusively in 2 locations, the cilia and the axon terminal-growth cone, suggesting distinct functions for the ORs in the respective locations.

The expression and the local translation of the OR at the axon terminal is in line with a number of evidence⁶ that indicates the axon terminus as an autonomous compartment where molecules involved in axon guidance are locally translated and expressed. The major advantage of the axon as an autonomous compartment that modulates locally protein synthesis and expression is to endow the axon with the ability to respond promptly to cues encountered in the environment along the path to the proper target.

However, whether the OR expressed at the axon terminal was involved in guiding sensory neurons to their specific locations in the olfactory bulb remained unknown. The first evidence in support of such a possible role came from the work of Maritan and collaborators⁷ that investigating, with real-time imaging, the spatiotemporal dynamics of cAMP and Ca²⁺ upon the axonal OR activation, demonstrated that the ORs expressed at the axon terminal are functional and coupled to localized increases of cAMP and Ca²⁺ (Figure 1). These results were of relevance for the potential role of the axonal ORs as



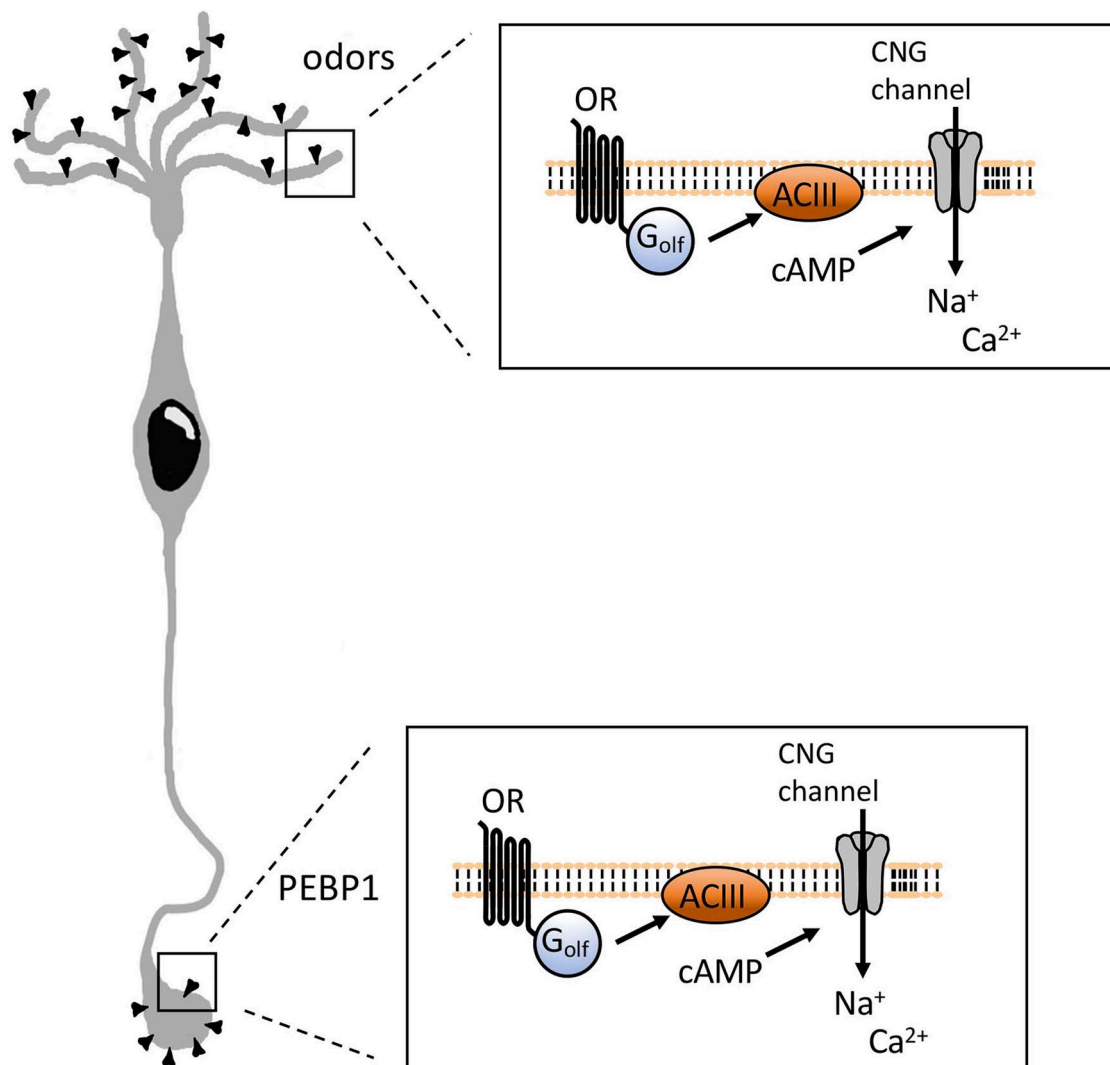


Figure 1. Schematic of an olfactory sensory neuron. The odorant receptors (ORs) are expressed at the cilia, where they bind odors, and at the axon terminus, where the ORs interact with cues elaborated in the olfactory bulb, such as PEBP1, the first putative ligand. OR activation is coupled to local increases of cAMP and Ca²⁺.

ACIII indicates adenylyl cyclase III; cAMP, cyclic adenosine monophosphate; CNG, cyclic nucleotide gated channel; Golf, G protein; PEBP1, phosphatidylethanolamine-binding protein 1.

axon guidance molecules, as these second messengers are known to play an important role in regulating axonal motility in many systems, including the olfactory system. The importance of the OR-signaling in the formation of the sensory map was unraveled by Imai et al⁸ that generated a defective OR, unable to couple G protein and therefore to synthesize cAMP. This manipulation did not allow to identify whether the OR expressed at the cilia or the OR expressed at the axon terminal was involved in the axonal convergence, although it made possible to understand the importance of the OR signaling in the sensory map formation. OSNs expressing the mutant OR, failed to converge to form glomeruli in the olfactory bulb. The disrupted convergence could be ascribed to altered axon terminal-growth cone dynamics and/or to aberrant gene expression, associated with the lack of cAMP synthesis. Imai et al⁸ investigated the latter and reported that cAMP modulated the expression of molecules involved in axon guidance, such as

Neuropilin-1 that, in turn, dictates the position of glomeruli along the anteroposterior axis of the olfactory bulb. The results obtained by Maritan and colleagues⁷ provided evidence of the dual action of the OR-derived cAMP, identifying the synthesis of the cAMP locally at the axon terminal and visualizing the nuclear translocation of the cAMP-activated protein kinase A (PKA), upon activation of the axonal OR. At the nuclear level, PKA could then trigger the expression of guidance molecules, such as Neuropilin-1, that direct axon targeting.

The critical question that remained open was the mechanism of activation of the OR expressed at the axon terminal, its possible ligands. The classical paradigm of axon guidance implies that cues elaborated in the target area activate molecules expressed on the growth-cone to instruct axon targeting. In this scenario, the ORs expressed at the axon terminal are supposed to recognize molecular cues elaborated in the olfactory bulb. This hypothesis, formulated more than 20 years ago,³

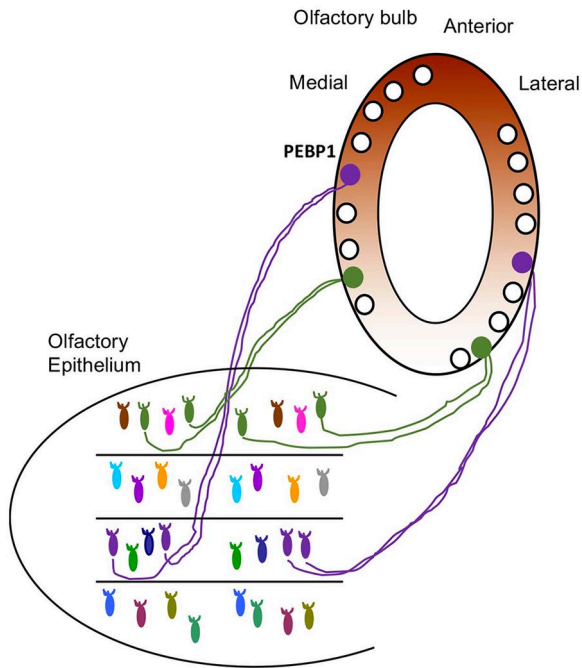


Figure 2. Schematic of the topographic organization of the olfactory system. In the olfactory epithelium, olfactory sensory neurons (OSNs) expressing the same odorant receptor (same color) are confined within broad areas, intermingled to OSNs expressing different types of receptors. Spatial order is achieved in the olfactory bulb, where like axons converge to form glomeruli in specific locations. Molecules elaborated in the olfactory bulb, such as PEBP1, the first putative ligand of the axonal OR, play a key role in directing sensory axons to their final destination. PEBP1 indicates phosphatidylethanolamine-binding protein 1.

remained open until recently, when Zamparo et al,⁹ through an unbiased screen, characterized protein extract from the olfactory bulb able to elicit Ca^{2+} responses when locally applied to OSN axons. Further support that Ca^{2+} rise was due to the OR activation was obtained by transfecting human embryonic kidney (HEK) cells expressing specific ORs. Only HEK cells expressing distinct ORs exhibited Ca^{2+} rise in response to olfactory bulb proteins and to the corresponding odor ligand. Ca^{2+} responses were not observed in HEK cells not expressing OR. The physiological relevance of these findings was deduced by time-lapse imaging, in which it was shown that the active pool of molecules from the olfactory bulb can modulate OSN axon turning, in a classic turning assay.⁹

Finally, by mass spectrometry of the active pool of molecules, Zamparo and collaborators identified the first putative ligand of the OR expressed at the axon terminal: phosphatidylethanolamine-binding protein 1 (PEBP1). PEBP1 is a small molecule, ~21 kDa, widely expressed in the brain and in nonbrain tissue, the function of which remains elusive. The small molecular weight, the ability to be secreted, although in a nonclassical way, and to modulate G protein-coupled receptor made PEBP1 a suitable candidate. Furthermore, it was reported that mice carrying a null mutation in PEBP1 exhibited olfactory deficits.¹⁰ Its role as a putative ligand of the

axonal receptor was strengthened by its expression around the glomeruli, in periglomerular cells, an ideal location to interact with the incoming sensory neuron axons.⁹ As for the pattern of expression in the bulb, PEBP1 is expressed in a global gradient along the anteroposterior axis (Figure 2), although at the local level, glomeruli with high expression of PEBP1 are intermingled with glomeruli in which PEBP1 can be hardly detected.⁹ This pattern resembles the expression of Neuropilin-1, another molecule involved in directing the position of glomeruli along the anteroposterior axis.¹¹

PEBP1 was reported to activate a subset of axonal OR and regulate their axon turning behavior, in a classic turning assay, *in vitro*. The physiological relevance of PEBP1 was obtained by *in vivo* experiments. Mice carrying a null mutation for PEBP1 exhibited a deeply perturbed sensory map, characterized by the displacement of the main glomerulus along the anteroposterior axis and by the presence of additional heterogeneous glomeruli. The fundamental principles that define the olfactory map, namely the formation of homogeneous glomeruli (i.e., formed by axons expressing the same OR) in a specific location of the olfactory bulb, resulted to be subverted in the absence of PEBP1. Noteworthy, the alterations in the convergence of sensory axons were observed only for OSNs expressing OR responsive to PEBP1 in *in vitro* experiments and not for OSNs expressing OR not responsive to PEBP1, *in vitro*, corroborating the role of PEBP1 in directing a distinct subset of OSNs to their targets.⁹

The fact that PEBP1 activates only a subset of ORs suggested that other candidates remain to be unraveled. Two scenarios can be envisioned. First, the number of ligands in the olfactory bulb is the same than the number of ORs. Second, a few cues elaborated in the olfactory bulb can bind, with different affinity, a subset of ORs. The rather broad expression of the first putative ligand and its interaction with several ORs strengthen the second hypothesis. Based on this evidence, we favor a model in which a given cue elaborated in the OB guides a subset of the sensory axons in a specific area of the olfactory bulb. Within this district, the different affinity of distinct ORs for the ligand directs the formation of specific glomeruli.

It is known since the early studies³ that although the OR plays a key role in the formation of the sensory map, it is not the only determinant. Other molecules, the expression of which is highly correlated to and/or regulated by the OR, such as the ephrin-A¹² and Neuropilin-1,⁸ contribute to the formation of the sensory map. How can this ensemble of cues orchestrate axon targeting? In agreement with a previous hypothesis³ and according to the recent evidence,⁹ we favor a model in which each OSN expresses at the axon terminal-growth cone the OR along with a unique combination of cues, which, interacting with specific molecules in the olfactory bulb, provide sensory axons with information to reach the proper targets. This model is in line with the classic

paradigm of axon targeting, in which an array of molecules expressed on the axon terminal interacts with positional cues in the target areas to reach the proper location.

Despite the fact that the topography of the olfactory bulb hinges on OR identity, odor-evoked activity does not have a significant impact on the formation of the sensory map. Spontaneous afferent activity, on the contrary, plays an important role in the refinement and in the maintenance of the topographic organization of the olfactory bulb.¹³ Interestingly, the identity of the OR modulates not only the evoked but also the spontaneous firing of OSNs.¹⁴ It was, therefore, suggested that ligand-independent activation of the OR could trigger the increase of OR-derived cAMP required for axon targeting.¹⁵ Due to the high variability of spontaneous firing even among neurons expressing the same receptor, it is difficult to conceive how specificity could be achieved in these conditions.

The role of the OR expressed at the axon terminal unravels a very elegant mechanism by which Nature solved the challenging problem to create spatial order in a population of more than 1000 different types of neurons, by assuming the same OR identity as the discriminating factor that defines the range of odors a given neuron is responsive to and the target the same neuron projects to. After more than 20 years from the characterization of the role of the OR in the topographic organization of the olfactory bulb, the identification of the first putative ligand of the axonal OR allowed to begin to understand the mechanism that links the OR identity to its internal representation. This finding opens a new field of investigation to unravel the complete set of axonal OR ligands expressed in the olfactory bulb and the mechanism through which they coordinate the formation of the sensory map.

Author Contributions

CL wrote the commentary.

ORCID iD

Claudia Lodovichi  <https://orcid.org/0000-0002-0490-4846>

REFERENCES

1. Miyamichi K, Serizawa S, Kimura HM, Sakano H. Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelium determine the dorsal/ventral positioning of glomeruli in the olfactory bulb. *J Neurosci.* 2005;25:3586-3592. doi:10.1523/JNEUROSCI.0324-05.2005.
2. Mombaerts P, Wang F, Dulac C, et al. Visualizing an olfactory sensory map. *Cell.* 1996;87:675-686. doi:10.1016/s0092-8674(00)81387-2.
3. Wang F, Nemes A, Mendelsohn M, Axel R. Odorant receptors govern the formation of a precise topographic map. *Cell.* 1998;93:47-60. doi:10.1016/s0092-8674(00)81145-9.
4. Barnea G, O'Donnell S, Mancina F, et al. Odorant receptors on axon termini in the brain. *Science.* 2004;304:1468. doi:10.1126/science.1096146.
5. Dubacq C, Jamet S, Trembleau A. Evidence for developmentally regulated local translation of odorant receptor mRNAs in the axons of olfactory sensory neurons. *J Neurosci.* 2009;29:10184-10190. doi:10.1523/JNEUROSCI.2443-09.2009.
6. Holt CE, Martin KC, Schuman EM. Local translation in neurons: visualization and function. *Nat Struct Mol Biol.* 2019;26:557-566. doi:10.1038/s41594-019-0263-5.
7. Maritan M, Monaco G, Zamparo I, Zaccolo M, Pozzan T, Lodovichi C. Odorant receptors at the growth cone are coupled to localized cAMP and Ca²⁺ increases. *Proc Natl Acad Sci U S A.* 2009;106:3537-3542. doi:10.1073/pnas.0813224106.
8. Imai T, Suzuki M, Sakano H. Odorant receptor-derived cAMP signals direct axonal targeting. *Science.* 2006;314:657-661. doi:10.1126/science.1131794.
9. Zamparo I, Francia S, Franchi SA, et al. Axonal odorant receptors mediate axon targeting. *Cell Rep.* 2019;29:4334-4348. doi:10.1016/j.celrep.2019.11.099.
10. Theroux S, Pereira M, Casten KS, et al. Raf kinase inhibitory protein knockout mice: expression in the brain and olfaction deficit. *Brain Res Bull.* 2007;71:559-567. doi:10.1016/j.brainresbull.2006.11.010.
11. Assens A, Dal Col JA, Njoku A, et al. Alteration of Nrp1 signaling at different stages of olfactory neuron maturation promotes glomerular shifts along distinct axes in the olfactory bulb. *Development.* 2016;143:3817-3825. doi:10.1242/dev.138941.
12. Cutforth T, Moring L, Mendelsohn M, et al. Axonal ephrin-As and odorant receptors: coordinate determination of the olfactory sensory map. *Cell.* 2003;114:311-322. doi:10.1016/s0092-8674(03)00568-3.
13. Lorenzon P, Redolfi N, Podolsky MJ, et al. Circuit formation and function in the olfactory bulb of mice with reduced spontaneous afferent activity. *J Neurosci.* 2015;35:146-160. doi:10.1523/JNEUROSCI.0613-14.2015.
14. Connelly T, Savigner A, Ma M. Spontaneous and sensory-evoked activity in mouse olfactory sensory neurons with defined odorant receptors. *J Neurophysiol.* 2013;110:55-62. doi:10.1152/jn.00910.2012.
15. Nakashima A, Takeuchi H, Imai T, et al. Agonist-independent GPCR activity regulates anterior-posterior targeting of olfactory sensory neurons. *Cell.* 2013;154:1314-1325. doi:10.1016/j.cell.2013.08.033.