Published in final edited form as:

Dev Neurobiol. 2014 March ; 74(3): 259–268. doi:10.1002/dneu.22122.

Mini-review: Making scent of the presence and local translation of odorant receptor mRNAs in olfactory axons

Caroline Dubacq^{1,2}, Coralie Fouquet^{1,2}, and Alain Trembleau^{1,2}

¹Université Pierre et Marie Curie (UPMC), Unité Mixte de Recherche (UMR) 7102, F-75005 Paris, France

²Centre National de la Recherche Scientifique, UMR 7102, F-75005 Paris, France

Abstract

Rodents contain in their genome more than 1,000 functional odorant receptor genes, which are specifically expressed by the olfactory sensory neurons projecting from the olfactory epithelium to the olfactory bulb. Strong evidence for the presence and local translation of odorant receptor mRNAs in the axon of olfactory sensory neurons was obtained, but no function has been assigned to these axonal mRNAs yet. The aim of this review is to discuss the evidence for the presence and local translation of odorant receptor mRNAs in olfactory sensory axons, and to speculate on their possible function in the wiring of the mouse olfactory sensory projections.

The cloning of Odorant Receptor (OR) -encoding genes in rodents led to the identification, in the early 1990s, of the largest family of G-Protein Coupled Receptors (GPCRs) in mammals (Buck and Axel, 1991), with more than 1,000 functional genes in the mouse genome, distributed into 2 broad classes, “fish-like” Class I genes, and Class II genes (Zhang and Firestein, 2002; Zhang et al., 2004). Soon after this discovery, strong evidence was obtained for the presence of *OR* mRNAs in the axons of olfactory sensory neurons (OSNs) (Ressler et al., 1994; Vassar et al., 1994). The latter observations had a critical influence on understanding the wiring diagram of the mammalian olfactory system, by providing strong evidence for the sorting and convergence of axons expressing the same OR into small subsets of glomeruli within the olfactory bulb (see Fig. 1 for a brief presentation of key features of the olfactory sensory projections in rodents). However, while evidence accumulated for the local translation of *OR* mRNAs in OSN axons (Dubacq et al., 2009), whether *OR* mRNAs have any function in OSN axons remained controversial. Meanwhile, strong evidence was compiled for a critical role of mRNA local translation in axon guidance elsewhere in the CNS (reviewed in Jung et al., 2012). Thus, it now appears legitimate to return to the question of axonal mRNAs, their local translation in OSN axons, and their physiological significance. The aim of this mini-review is to discuss the evidence for the presence of *OR* mRNAs in olfactory sensory axons and for their local translation, and to speculate on their possible function in the wiring of the mouse olfactory sensory projections.

Early evidence for the presence of *OR* mRNAs in olfactory sensory axons

The first evidence of *OR* mRNAs in OSN axons was published in 1994 by two different groups (Ressler et al., 1994; Vassar et al., 1994). Importantly, this observation allowed the authors to decipher in molecular terms the topographic organization of olfactory sensory projections in rodents, and the way olfactory information may be encoded within the olfactory bulb (OB). Their basic observations were: 1) *OR* mRNAs are detectable, using in situ hybridization, at the target sites of OSN axons in the OB, called glomeruli, 2) specific *OR* probes label small and distinct subsets of glomeruli, 3) the position of these specific glomeruli is constant in different individuals within a species (Ressler et al., 1994; Vassar et al., 1994). This led to the view that all OSNs expressing the same *OR* converge their axons onto a small number of glomeruli in the OB (Fig. 1), and to a model in which exposure to a particular odorant could activate a specific pattern of stereotyped topographically organized glomeruli within the OB. The existence of such an *OR*-based topographical map in the OB was thereafter confirmed using an elegant genetic approach (Mombaerts et al., 1996), and it has been established that each glomerulus in the adult mouse is homogeneously innervated by OSNs that all have the same identity in terms of *OR* expression (Treloar et al., 2002 and Fig. 1). It has been shown later on that building such a wiring diagram involves distinct sets of mechanisms ensuring on the one hand the *positioning* of glomeruli along the antero-posterior and dorso-ventral axes, and on the other hand the *fine sorting* of OSN axons and their coalescence to form glomeruli (for reviews see Mombaerts, 2006; Imai and Sakano, 2007; Zou et al., 2009; Lodovichi and Belluscio, 2012; Mori and Sakano, 2011).

Glomeruli are specialized structures of about 55 micrometers diameter in mice (Richard et al., 2010) that are devoid of cell bodies and are innervated by the OSN axons that arborize within the glomerular neuropil. Also found in the glomeruli are the dendritic processes of OB interneurons and second order projection neurons, both of which receive OSN synapses. Finally, astrocytic processes distribute within the glomerulus and help to define subglomerular compartments. Given this organization and the known expression of *OR* genes in OSNs, it was likely that the *OR* mRNAs retrieved in glomeruli were compartmentalized in OSN axons. However, they could also originate from the expression of *OR* genes in either OB neurons or astrocytes extending their dendrites/processes into glomeruli. However, three sets of arguments unambiguously showed that *OR* mRNAs detected in glomeruli were of OSN axonal origin (Ressler et al., 1994; Vassar et al., 1994): 1) no significant expression of *OR* genes was detected in bulbar neurons or astrocytes, 2) an additional and significant signal was also observed in the outermost layer of the OB (olfactory nerve layer -ONL-), which includes essentially OSN axon shafts and glial cells but does not contain OB neuronal dendritic processes, and 3), most importantly, the in situ hybridization signal in glomeruli was greatly decreased after axonal deafferentation following chemical lesion of OSNs (Vassar et al., 1994).

Fifteen years later, the levels of *OR* mRNAs could be rigorously compared using RT-qPCR between the axonal and the non-axonal compartments of OSNs (measured from OB or olfactory epithelium -OE- extracts, respectively). For each *OR* gene tested, the ratio of axonal over non-axonal *OR* mRNAs appeared significantly higher in early postnatal mice (postnatal day 4, P4 – in the range of 0.9 to 3%, depending on the *OR* mRNA studied) than

in adult mice (in the range of 0.1 to 0.3%) (Dubacq et al., 2009). This parallels the ratio between growing and mature OSN axons (higher at P4 than in adult mice), highly suggesting that the axonal localization of *OR* mRNAs is more prominent in developing than in mature OSN axons.

It is of interest to note that, despite the clear evidence for the presence of *OR* mRNAs in OSN axons in 1994, the fact that these may have some function in this compartment was not really envisaged or discussed. Such a hypothesis was probably not viewed seriously at that time for several reasons. First, until the end of the nineties, it was believed that vertebrate axonal proteins were synthesized in the neuronal cell bodies and thereafter transported in the axons, and that axons were devoid of the protein synthesis machinery (Droz and Leblond, 1962). There was also poor indication in rodents for the axonal localization of mRNAs, except in a few systems like the hypothalamo-neurohypophyseal system (Jirikowski et al., 1990; Mohr et al., 1991; Trembleau et al., 1994; Trembleau et al., 1995). Strong evidence for roles of axonal mRNAs in developmental processes or plasticity would not be documented until years later (Campbell and Holt, 2001; Zheng et al., 2001; Zhang and Poo, 2002; Wu et al., 2005; Hanz et al., 2003; Yao et al., 2006; Leung et al., 2006; Andreassi et al., 2010; Tcherkezian et al., 2010; Hillefors et al., 2007). Last but not least, although the idea that *OR* might “somehow be involved in axonal guidance or synaptic target recognition in the bulb” was already proposed in one of the two seminal papers (Ressler et al., 1994), there was no evidence at that time for the presence of *OR* proteins within axons, making it difficult to conceive that *OR* mRNAs may be locally translated in axons.

Evidence for the presence of functional *OR* proteins in olfactory sensory axons

Odorant receptor proteins were unambiguously localized in situ at the level of OSN axons in 2004, thanks to the production by several laboratories of specific antibodies against a number of *ORs*, which allowed the reliable detection of these proteins on tissue sections (Barnea et al., 2004; Strotmann et al., 2004). Using several *OR*-specific antibodies, it turned out that *OR* immunoreactivity was detected in glomeruli and incoming bundles of axons on OB sections, demonstrating the presence of the corresponding *ORs* in the distal part of OSN axons. For at least some of the antibodies used in these studies, labeling specificity was demonstrated by the colocalization, in individual glomeruli, of *OR* immunoreactivity and GFP, in genetically-engineered mice where an *ires-tauGFP* cassette was inserted downstream of the *OR* coding sequence (Barnea et al., 2004).

Additional evidence for the presence of *OR* proteins on OSN axons was also obtained using alternative approaches. Through homologous recombination, Feinstein et al. genetically-engineered mice with GFP fused to the C-terminal extremity of the M71 *OR*. They observed GFP in glomeruli, demonstrating the presence of M71 in axons (Feinstein et al., 2004). Second, using imaging techniques on hemi-head preparations, the Lodovichi laboratory showed that applying odorants onto glomeruli activated calcium signalling in OSN axons. This demonstrated both that *OR* proteins were present at the surface of OSN axons and that they were functional in this compartment (Maritan et al., 2009).

The two papers that first described the axonal localization of ORs made a further interesting observation: whereas very high OR immunoreactivity was detected on OSN axons within the glomeruli and their immediately adjacent fascicles, very little or no OR immunoreactivity was observed in more proximal shafts of OSN axons, between the OE and the OB (Barnea et al., 2004; Strotmann et al., 2004). As discussed by Strotman et al., this may be due to the fact that ORs are inserted into the plasma membrane (hence becoming more accessible to antibodies) only after reaching the OB (Strotmann et al., 2004). However, it may also reflect a quantitatively defined differential abundance of OR protein along OSN axons, with relatively high concentrations in the distal axonal segments, and lower concentrations in the proximal and intermediate regions. Such compartmentalization may be the result of the differential targeting/accumulation of ORs produced in OSN cell bodies and subsequently transported along the axons. Alternatively, given the presence of *OR* mRNAs in OSN axons, a spatio-temporal regulation of their translation along OSN axons may also participate in this differential distribution of axonal ORs. The question then is: are *OR* mRNAs translated within OSN axons and, if yes, is this translation regulated in a spatio-temporal manner ?

Evidence for the local translation of *OR* mRNAs in olfactory sensory axons

The OB contains the distal segments and terminals of OSN axons but not the OSN cell bodies, and there is no detectable expression of *OR* genes by cells located within the OB (see above). Taking advantage of these properties, we developed a biochemical assay with the objective of determining if *OR* mRNAs are translated within OSN axons in vivo (Dubacq et al., 2009). We showed that a significant fraction of axonal *OR* mRNAs extracted from adult OBs were associated with polysomes. Very interestingly, the levels of axonal *OR* mRNAs associated with polysomes were higher in P4 compared to adult mice, as well as in adult mice during the period of massive regeneration of OSN axons that follows a chemical OE lesion (Dubacq et al., 2009). The latter sets of data suggests the local translation of *OR* mRNAs in OSN axons, and further indicate that this process might be more prominent in immature and growing axons than in mature axons. Interestingly, another mRNA, *Olfactory Marker Protein* (*OMP*), known to be restricted to the axons of mature OSNs but not encoding an OR, did not show such regulation: it appeared translated at similarly low levels in OSN axons at all developmental/regeneration stages examined. Overall, these data suggest that local translation of mRNAs is probably more prominent in developing/growing axons than in mature axons, and/or that *OR* mRNAs are specifically subject to a developmental translational regulation in OSN axons, with higher translational activity in growing axons than in mature ones. In the near future, it will be critical to obtain definitive evidence for the neosynthesis in axons of full length OR proteins (i.e. through local metabolic labeling performed in vivo, or in compartmentalized culture systems), and to demonstrate that they are properly addressed to the plasma membrane.

OR proteins and the wiring of olfactory sensory projections in the olfactory bulb

The critical role played by OR proteins in the wiring of olfactory sensory projections has been strongly established. Evidence for this unusual function of sensory receptors was

already reviewed and discussed in detail (Mombaerts, 2006; Imai and Sakano, 2007; Zou et al., 2009; Lodovichi and Belluscio, 2012; Mori and Sakano, 2011), and we will focus here on only two critical aspects: the role of ORs in the sorting of OSN axons through homotypic fasciculation, and the mechanisms by which ORs may play this role.

As they exit the OE, OSN axons assemble into tightly packed bundles of hundreds to thousands axons (Li et al., 2005). Hence, along its migration from the OE toward its final targets within an OB glomerulus, each individual OSN axon is likely apposed within these bundles to other OSN axons that express a different OR and are not targeted to the same glomerulus. It has been shown that the sorting of OSN axons occurs in at least two independent steps. In the first step, called OSN axon pre-target sorting, subpopulations of OSN axons segregate from each other in the olfactory nerve through regulations involving classical guidance cues (Imai et al., 2009). Interestingly, it was shown that axons expressing Class I ORs are segregated from axons expressing Class II OR in the olfactory nerve, and that this process does not involve OR proteins, as was demonstrated by swapping Class I and Class II coding sequences using homologous recombination in mice (Bozza et al., 2009). Following this pre-target sorting step, an additional sorting of OSN axons allows the axons having the same OR identity to fasciculate together, ending up with the coalescence of the axons in uniquely innervated glomeruli. This homotypic fasciculation of OSN axons occurs in the ONL and clearly depends on OR proteins, as shown by genetic manipulations in mouse (reviewed in Mombaerts, 2006).

There is a consensus in the field that the precise sorting and homotypic fasciculation of OSN axons, leading to their convergence onto glomeruli, depends on OR proteins. In contrast, the mechanisms by which ORs control this process is still a matter of debate. On the one hand, it has been proposed that the OR-dependent activation of a G-protein/adenylate cyclase cascade leads to cAMP-dependent differential expression of guidance and/or adhesion molecules, which secondarily controls axon sorting (Imai et al., 2006; Serizawa et al., 2006; Imai and Sakano, 2007). In strong support of this hypothesis, manipulating cAMP levels in OSNs disrupts the glomerular organization (Imai et al., 2006; Col et al., 2007; Zou et al., 2007; Chesler et al., 2007), and the cAMP pathway controls expression of several adhesion/guidance molecules (Imai et al., 2006; Serizawa et al., 2006). It is not clear, however, where this OR-dependent activation of the cAMP cascade would occur in OSNs, whether in the cell body or in axons. In addition, it remains unknown what activates ORs in this developmental process initiated before birth, even though odorant-evoked activity in OSNs has recently been documented as early as embryonic day 16 for 2 ORs (Lam and Mombaerts, 2013). Besides these important questions, it remains to be shown how the 1,000 different OSN populations defined by their specific OR identity would have specific cAMP activation patterns (Zou et al., 2009).

Alternatively, in a contextual model for self-sorting of OSN axons into glomeruli, Feinstein and Mombaerts proposed that the OR-dependent sorting of OSN axons may rely on interactions between multimolecular complexes containing ORs (Feinstein et al., 2004; Feinstein and Mombaerts, 2004; Mombaerts, 2006). According to this hypothesis, recognition of axons of the same OR identity depends on specific 3-D structures of ORs or OR-derived peptides, associated to co-factors. As they dynamically interact with each other,

growth cones may sample neighboring axons for OR similarity. If maximum adhesion occurs between axons of the same OR identity, such dynamic interactions may mediate the sorting of OSN axons according to their identity, as was recently simulated in a simple model (Chaudhuri et al., 2011). The beauty of this view relies in its parsimony: it does not necessitate fine tuning through OR activation of specific cAMP-dependent transcription of unique sets of adhesion/guidance molecules in each OSN axon population. However, since it is not known whether OR-containing complexes are able to mediate axon-axon interactions, this model remains speculative.

Regardless of the mechanism involved in the OR-dependent sorting of OSN axons, this process is clearly controlled in space: it occurs only when OSN axons cross the olfactory nerve layer (ONL), and not during earlier steps of axon migration from the OE towards the OB (Treloar et al., 2002; Miller et al., 2010). What could be the mechanism controlling the initiation of OR-dependent sorting of axons only when growing axons have reached the ONL?

Speculation: the local translation of *OR* mRNAs as a means to spatially control the OR-dependent sorting of OSN axons

In a variety of neural systems, the local translation of mRNAs in axons is a very efficient means to control in space and time critical developmental processes such as cue-dependent turning, axon collapse, neuronal survival and axon regeneration (Zheng et al., 2001; Zhang and Poo, 2002; Wu et al., 2005; Hanz et al., 2003; Yao et al., 2006; Leung et al., 2006; Andreassi et al., 2010; Tcherkezian et al., 2010; Hillefors et al., 2007). In many cases, unique extracellular cues trigger the translation of specific mRNAs within growth cones, through the activation of a specific transduction pathway, ending up with the neosynthesis of proteins endowing the growth cone with new properties (reviewed in Andreassi and Riccio, 2009; Jung et al., 2012).

We propose that a regulated local translation of *OR* mRNAs in axons may allow controlling the distribution of ORs along OSN axons, which secondarily may trigger the space-dependent sorting of OSN axons (Fig. 2). According to our model, OSN axons extending from the OE toward the OB may contain *OR* mRNAs but no or little OR proteins (Fig. 2A). This view is in line with previous observations reporting no or little detectable OR immunoreactivity along proximal and intermediate segments of OSN axons, between the OE and the OB (Barnea et al., 2004; Strotmann et al., 2004). During this step of development, classical guidance cues (i.e. neuropilins, semaphorins, robo, slits, etc) probably ensure the targeting of each individual axon to a small area of the OB along the antero-posterior and dorso-ventral axes (for review see Mori and Sakano, 2011). Arriving in the ONL, axons may be exposed to extracellular signals activating *OR* mRNA translation within OSN axons (Fig. 2B). Such signals may be produced by neuronal populations of the OB (i.e. mitral cells) or by olfactory ensheathing cell populations located in the ONL. It should be stressed here that if similar cis-regulatory elements controlling this translational activation are conserved in the OR family, a single or small number of molecules could ensure the translational activation of *OR* mRNAs within the 1,000 populations of OSN axons expressing different ORs.

Given the established functions of ORs in the wiring of the olfactory axons, we propose two non-exclusive models:

Model I

Once ORs are expressed by growth cones, they may trans-interact directly or indirectly within multimolecular complexes (Fig. 2C), as proposed by Feinstein and Mombaerts (2004), hence controlling the self-sorting of axons through dynamic interactions. This model supposes that OR-containing complexes provide differential adhesiveness, with maximum adhesion mediated by trans-interactions between identical ORs, and lower adhesion mediated by heterotypic interactions (Fig. 2C).

Model II

Functional expression of ORs on axons, triggered by their local translation, leads to differential activation of the cAMP pathway in growth cones, each axon population being characterized by a specific activation pattern/level of cAMP (Fig. 2D). Such an OR-dependent cAMP activation may be due to ligand-independent constitutive activity (Rosenbaum et al., 2009). cAMP differentially activates the expression of adhesion/guidance molecules in different populations of OSN axons (Fig. 2E). This process involves a retrograde signalling towards the nucleus, transcriptional activation of genes encoding adhesion/guidance cues, and their functional expression on axons. Finally, trans-interactions between these adhesion/guidance molecules expressed by OSN axons (i.e. Kirrel2, kirrel3, EphA, eprinA, for review see Mori and Sakano, 2011) end up with their self-sorting.

In a third model combining Model I and Model II, direct or indirect interactions between neosynthesized ORs (Model I) may trigger the activation of the Gs/cAMP cascade, controlling the differential synthesis of *bona fide* adhesion molecules in the different OSN populations (Model II).

In all cases, the regulated local translation of *OR* mRNAs may ensure that the OR-dependent sorting of OSN axons does not begin *before* OSN axons reach the ONL. What could be the reason for such a delayed OR-dependent sorting? A possible answer to this question is that the full sorting of OSN axons is a multi-step and hierarchical process, in which OSN axons probably need to go through sequential sorting to be properly guided to a restricted area of the OB, along its antero-posterior and dorso-ventral axes (Nedelec et al., 2005; Miller et al., 2010; Bozza et al., 2009). As an example, there is a pre-target sorting that segregates Class I from Class II OR-expressing axons in two distinct areas of the OB (Bozza et al., 2009; Imai et al., 2009). Such a hierarchical process may allow the enrichment of axon populations that will ultimately project to closely-located areas in unique territories, hence increasing the probability of interactions and homotypic fasciculation of axons having the same OR identity before the final OR-dependent sorting step. Determining if the sorting of axons is impaired in experimental conditions in which the presence of OR proteins on axons is induced prematurely may allow to test this hypothesis.

Conclusions

It remains to be determined whether the local translation of *OR* mRNAs in the ONL is indeed a mechanism allowing the spatial control of OR-dependent sorting of OSN axons. The interest of such a model, however, is that it enables us to make predictions that can be tested experimentally.

It will be of particular interest to determine where and when *OR* mRNAs are translated along developing OSN axons, and to assess the trafficking of neosynthesized OR proteins at the plasma membrane. Particular attention should be paid to the ONL, to determine if neosynthesized ORs are functional in this area. The mechanisms regulating the trafficking of ORs to the olfactory cilia in the epithelium are also unknown. It might prove to be interesting to ask if the mechanisms of dendritic/cilia targeting share any properties or mechanisms with axonal compartmentalization.

From a functional point of view, it will be critical to determine whether the local translation of ORs in axons is necessary for the proper OR-dependent sorting of OSN axons. To this aim, it will be necessary to interfere with the transport and/or local translation of *OR* mRNAs, to determine if blocking the neosynthesis of ORs in axons impairs their sorting and homotypic fasciculation. As a prerequisite, it is needed to identify the mechanisms controlling *OR* mRNA transport in axons, as well as their translation, which typically involve cis-acting elements located in the mRNA sequence, and trans-acting factors interacting with these elements (Sotelo-Silveira et al., 2006; Andreassi and Riccio, 2009; Jung et al., 2012). Finally, the possible role of axonal ORs as local regulators of differential adhesiveness between OSN axons still has to be explored.

Acknowledgments

The authors are grateful to L. Bally-Cuif, I. Dusart and C.A. Greer for their comments on the manuscript. The team “Development and Plasticity of neural Networks” is member of the Paris School of Neuroscience and of the Labex Bio-Psy networks, and supported by UMPC, CNRS, ANR-2010-BLAN-1401-01 and NIH 1RO1DC012441 grants.

References

- Andreassi C, Riccio A. To localize or not to localize: mRNA fate is in 3'UTR ends. *Trends Cell Biol.* 2009; 19:465–474. [PubMed: 19716303]
- Andreassi C, Zimmermann C, Mitter R, Fusco S, De Vita S, Saiardi A, Riccio A. An NGF-responsive element targets myo-inositol monophosphatase-1 mRNA to sympathetic neuron axons. *Nat Neurosci.* 2010; 13:291–301. [PubMed: 20118926]
- Barnea G, O'Donnell S, Mancia F, Sun X, Nemes A, Mendelsohn M, Axel R. Odorant receptors on axon termini in the brain. *Science.* 2004; 304:1468. [PubMed: 15178793]
- Bozza T, Vassalli A, Fuss S, Zhang JJ, Weiland B, Pacifico R, Feinstein P, Mombaerts P. Mapping of class I and class II odorant receptors to glomerular domains by two distinct types of olfactory sensory neurons in the mouse. *Neuron.* 2009; 61:220–233. [PubMed: 19186165]
- Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell.* 1991; 65:175–187. [PubMed: 1840504]
- Campbell DS, Holt CE. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron.* 2001; 32:1013–1026. [PubMed: 11754834]
- Chaudhuri D, Borowski P, Zapotocky M. Model of fasciculation and sorting in mixed populations of axons. *Phys Rev E Stat Nonlin Soft Matter Phys.* 2011; 84:021908. [PubMed: 21929021]

- Chesler AT, Zou DJ, Le Pichon CE, Peterlin ZA, Matthews GA, Pei X, Miller MC, Firestein S. A G protein/cAMP signal cascade is required for axonal convergence into olfactory glomeruli. *Proc Natl Acad Sci U S A*. 2007; 104:1039–1044. [PubMed: 17215378]
- Col JA, Matsuo T, Storm DR, Rodriguez I. Adenylyl cyclase-dependent axonal targeting in the olfactory system. *Development*. 2007; 134:2481–2489. [PubMed: 17537788]
- Droz B, Leblond CP. Migration of proteins along the axons of the sciatic nerve. *Science*. 1962; 137:1047–1048. [PubMed: 13887927]
- 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. [PubMed: 19692593]
- Feinstein P, Mombaerts P. A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell*. 2004; 117:817–831. [PubMed: 15186781]
- Feinstein P, Bozza T, Rodriguez I, Vassalli A, Mombaerts P. Axon guidance of mouse olfactory sensory neurons by odorant receptors and the beta2 adrenergic receptor. *Cell*. 2004; 117:833–846. [PubMed: 15186782]
- Hanz S, Perlson E, Willis D, Zheng JQ, Massarwa R, Huerta JJ, Koltzenburg M, Kohler M, van-Minnen J, Twiss JL, Fainzilber M. Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron*. 2003; 40:1095–1104. [PubMed: 14687545]
- Hillefors M, Gioio AE, Mameza MG, Kaplan BB. Axon viability and mitochondrial function are dependent on local protein synthesis in sympathetic neurons. *Cell Mol Neurobiol*. 2007; 27:701–716. [PubMed: 17619140]
- Imai T, Sakano H. Roles of odorant receptors in projecting axons in the mouse olfactory system. *Curr Opin Neurobiol*. 2007; 17:507–515. [PubMed: 17935969]
- Imai T, Suzuki M, Sakano H. Odorant receptor-derived cAMP signals direct axonal targeting. *Science*. 2006; 314:657–661. [PubMed: 16990513]
- Imai T, Yamazaki T, Kobayakawa R, Kobayakawa K, Abe T, Suzuki M, Sakano H. Pre-target axon sorting establishes the neural map topography. *Science*. 2009; 325:585–590. [PubMed: 19589963]
- Jirikowski GF, Sanna PP, Bloom FE. mRNA coding for oxytocin is present in axons of the hypothalamo-neurohypophysial tract. *Proc Natl Acad Sci U S A*. 1990; 87:7400–7404. [PubMed: 2268384]
- Jung H, Yoon BC, Holt CE. Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. *Nat Rev Neurosci*. 2012; 13:308–324. [PubMed: 22498899]
- Lam RS, Mombaerts P. Odorant responsiveness of embryonic mouse olfactory sensory neurons expressing the odorant receptors S1 or MOR23. *Eur J Neurosci*. 2013
- Leung KM, van Horck FP, Lin AC, Allison R, Standart N, Holt CE. Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci*. 2006; 9:1247–1256. [PubMed: 16980963]
- Li Y, Field PM, Raisman G. Olfactory ensheathing cells and olfactory nerve fibroblasts maintain continuous open channels for regrowth of olfactory nerve fibres. *Glia*. 2005; 52:245–251. [PubMed: 15968636]
- Lodovichi C, Belluscio L. Odorant receptors in the formation of the olfactory bulb circuitry. *Physiology (Bethesda)*. 2012; 27:200–212. [PubMed: 22875451]
- 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. [PubMed: 19218439]
- Miller AM, Maurer LR, Zou DJ, Firestein S, Greer CA. Axon fasciculation in the developing olfactory nerve. *Neural Dev*. 2010; 5:20. [PubMed: 20723208]
- Mohr E, Fehr S, Richter D. Axonal transport of neuropeptide encoding mRNAs within the hypothalamo-hypophyseal tract of rats. *EMBO J*. 1991; 10:2419–2424. [PubMed: 1868830]
- Mombaerts P. Axonal wiring in the mouse olfactory system. *Annu Rev Cell Dev Biol*. 2006; 22:713–737. [PubMed: 17029582]
- Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R. Visualizing an olfactory sensory map. *Cell*. 1996; 87:675–686. [PubMed: 8929536]

- Mori K, Sakano H. How is the olfactory map formed and interpreted in the mammalian brain ? *Annu Rev Neurosci.* 2011; 34:467–499. [PubMed: 21469960]
- Nedelec S, Dubacq C, Trembleau A. Morphological and molecular features of the mammalian olfactory sensory neuron axons: What makes these axons so special? *J Neurocytol.* 2005; 34:49–64. [PubMed: 16374709]
- Ressler KJ, Sullivan SL, Buck LB. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell.* 1994; 79:1245–1255. [PubMed: 7528109]
- Richard MB, Taylor SR, Greer CA. Age-induced disruption of selective olfactory bulb synaptic circuits. *Proc Natl Acad Sci U S A.* 2010; 107:15613–15618. [PubMed: 20679234]
- Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature.* 2009; 459:356–363. [PubMed: 19458711]
- Serizawa S, Miyamichi K, Takeuchi H, Yamagishi Y, Suzuki M, Sakano H. A neuronal identity code for the odorant receptor-specific and activity-dependent axon sorting. *Cell.* 2006; 127:1057–1069. [PubMed: 17129788]
- Sotelo-Silveira JR, Calliari A, Kun A, Koenig E, Sotelo JR. RNA trafficking in axons. *Traffic.* 2006; 7:508–515. [PubMed: 16643274]
- Strotmann J, Levai O, Fleischer J, Schwarzenbacher K, Breer H. Olfactory receptor proteins in axonal processes of chemosensory neurons. *J Neurosci.* 2004; 24:7754–7761. [PubMed: 15342743]
- Tcherkezian J, Brittis PA, Thomas F, Roux PP, Flanagan JG. Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell.* 2010; 141:632–644. [PubMed: 20434207]
- Treloar HB, Feinstein P, Mombaerts P, Greer CA. Specificity of glomerular targeting by olfactory sensory axons. *J Neurosci.* 2002; 22:2469–2477. [PubMed: 11923411]
- Trembleau A, Morales M, Bloom FE. Aggregation of vasopressin mRNA in a subset of axonal swellings of the median eminence and posterior pituitary: light and electron microscopic evidence. *J Neurosci.* 1994; 14:39–53. [PubMed: 8283246]
- Trembleau A, Melia KR, Bloom FE. BC1 RNA and vasopressin mRNA in rat neurohypophysis: axonal compartmentalization and differential regulation during dehydration and rehydration. *Eur J Neurosci.* 1995; 7:2249–2260. [PubMed: 8563974]
- Vassar R, Chao SK, Sitcheran R, Nunez JM, Vossell LB, Axel R. Topographic organization of sensory projections to the olfactory bulb. *Cell.* 1994; 79:981–991. [PubMed: 8001145]
- Wu KY, Hengst U, Cox LJ, Macosko EZ, Jeromin A, Urquhart ER, Jaffrey SR. Local translation of RhoA regulates growth cone collapse. *Nature.* 2005; 436:1020–1024. [PubMed: 16107849]
- Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ. An essential role for beta-actin mRNA localization and translation in Ca²⁺-dependent growth cone guidance. *Nat Neurosci.* 2006; 9:1265–1273. [PubMed: 16980965]
- Zhang X, Poo MM. Localized synaptic potentiation by BDNF requires local protein synthesis in the developing axon. *Neuron.* 2002; 36:675–688. [PubMed: 12441056]
- Zhang X, Firestein S. The olfactory receptor gene superfamily of the mouse. *Nat Neurosci.* 2002; 5:124–133. [PubMed: 11802173]
- Zhang X, Rodriguez I, Mombaerts P, Firestein S. Odorant and vomeronasal receptor genes in two mouse genome assemblies. *Genomics.* 2004; 83:802–811. [PubMed: 15081110]
- Zheng JQ, Kelly TK, Chang B, Ryazantsev S, Rajasekaran AK, Martin KC, Twiss JL. A functional role for intra-axonal protein synthesis during axonal regeneration from adult sensory neurons. *J Neurosci.* 2001; 21:9291–9303. [PubMed: 11717363]
- Zou DJ, Chesler A, Firestein S. How the olfactory bulb got its glomeruli: a just so story? *Nat Rev Neurosci.* 2009; 10:611–618. [PubMed: 19584894]
- Zou DJ, Chesler AT, Le Pichon CE, Kuznetsov A, Pei X, Hwang EL, Firestein S. Absence of adenylyl cyclase 3 perturbs peripheral olfactory projections in mice. *J Neurosci.* 2007; 27:6675–6683. [PubMed: 17581954]

Figure 1.

Lower right drawing schematizes the organization of the rodent olfactory system, displayed here on a sagittal section of the head, with the olfactory epithelium (OE, represented in blue) lying in the nasal cavity, and the olfactory bulb (OB, represented in red) located at the anterior extremity of the brain. Inset representing a small area of the system (upper left part of the figure) illustrates some important features of the anatomical organization of the olfactory sensory projections from the OE to the OB. Olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) are represented with the same color. Only 5 different populations are represented here but the mouse olfactory system actually comprises about 1,000 OSN populations, each expressing a specific OR. OSNs expressing the same OR are scattered in large areas of the OE. Axons emerging from the OE assemble in tightly packed heterotypic bundles and form branches of the olfactory nerve (Olf. nerve). Although a pre-target sorting does occur in the Olf. nerve (not represented here), this sorting step is OR-independent. The OR-dependent sorting of OSN axons, leading to the formation of homotypic bundles and their coalescence in homogeneously innervated glomeruli only takes place in the outer layer of the OB, called olfactory nerve layer (ONL). Specific odorant receptor mRNAs were detected, using *in situ* hybridization, in subsets of OSNs, in the ONL and in a few glomeruli. Selected OR proteins were detected, using immunofluorescence, in subsets of OSNs, in a few glomeruli and their incoming axon bundles within the ONL. No significant OR immunoreactivity was documented in the olfactory nerve. Thick broken line

represents the cribriform plate ; thin broken line delineates the limit of the olfactory nerve layer.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

As they extend from the OE to the OB, growing OSN axons form heterotypic fascicles, where we propose that OSN growth cones contain *OR* mRNAs but little or no OR proteins (A). As they penetrate into the OB and cross its most superficial layer (ONL), they may encounter an extracellular signal activating *OR* mRNA translation and the subsequent trafficking of OR proteins to the plasma membrane (B). We postulate that neosynthesized ORs may then trigger the sorting and homotypic fasciculation of axons. Two non-exclusive models are envisaged. In **Model I**, direct or indirect trans-interactions between ORs (or

peptides derived from ORs) in multimolecular complexes, which can be heterotypic or homotypic, may mediate differential adhesion between axons (**C**). If homotypic interactions lead to maximal adhesion, then dynamic interactions between axons may lead to their sorting (**F**). In **Model II**, neosynthesized axonal ORs are activated and trigger a Gs-cAMP-dependent retrograde signaling (**D**), which in turns activates the transcription of guidance/adhesion molecules (**E**). If axons expressing different ORs differentially activate different transcriptional programs, then each OSN population of a given OR identity will specifically express the same guidance/adhesion molecules, allowing the sorting and homotypic fasciculation of axons (**F**). A third Model combining Model I and Model II, in which the Gs-cAMP cascade is activated by trans-interactions between complexes containing ORs, may also be proposed.