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Review

The olfactory bulb as an independent developmental domain

L López-Mascaraque*[,1,3] and F de Castro[2,3]

[1] Instituto Cajal-C.S.I.C., Madrid, Spain
[2] Hospital Ramón y Cajal, Madrid, Spain
[3] Both authors contributed equally to this work
* Corresponding author: L López-Mascaraque, Instituto Cajal, CSIC, Avenida del Doctor Arce 37, 28002 Madrid, Spain. Tel: 915854708; Fax: 915854754; E-mail: mascaraque@cajal.csic.es

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Abstract

The olfactory system is a good model to study the mechanisms underlying guidance of growing axons to their appropriate targets. The formation of the olfactory bulb involves differentiation of several populations of cells and the initiation of the central projections, all under the temporal and spatial patterns of gene expression. Moreover, the nature of interactions between the olfactory epithelium, olfactory bulb and olfactory cortex at early developmental stages is currently of great interest. To explore these questions more fully, the present review aims to correlate recent data from different developmental studies, to gain insight into the mechanisms involved in the specification and development of the olfactory system. From our studies in the *pax6* mutant mice (*Sey^{Neu}/Sey^{Neu}*), it was concluded that the initial establishment of the olfactory bulb central projections is able to proceed independently of the olfactory sensory axons from the olfactory epithelium. The challenge that now remains is to consider the validity of the olfactory bulb as an independent development domain. In the course of evaluating these ideas, we will review the orchestra of molecular cues involved in the formation of the projection from the OB to the olfactory cortex.

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Keywords: axonal guidance; neurogenesis; olfactory epithelium; mutants; development; piriform cortex

Abbreviations: CP, cortical plate; GE, ganglionic eminence; LOT, lateral olfactory tract; OB, olfactory bulb; OBLS, olfactory bulb like structure; OC, olfactory cortex; OE, olfactory epithelium; ON, olfactory nerve; OR, olfactory receptor; OSN, olfactory sensory neuron

Introduction

The effect that the arrival of afferent fibers to their appropriate targets has on CNS development has not yet been fully established. Does it awake the developmental program of the cells at the site being innervated or, does their arrival simply serve to refine the later steps of the developmental program? In order to address this question, much attention has been focused on the sophisticated development of the mammalian cerebral cortex where two different theories have been proposed to explain the mechanisms underlying its formation. In the 'protomap' model, cortical regions are patterned prior to the migration of the newborn neurons (intrinsic control),[1] an event presumably specified by important molecular determinants.[2] In this model, the arrival of innervating axons would merely serve to modify and refine the protomap (an important facet of maintenance). In the second model, the 'protocortex' theory, the newborn cortical neurons are a homogeneous cell population, that later on in corticogenesis are patterned into the distinct areas by the specific cues supplied by axons growing in from the thalamus (extrinsic control).[3]

The fact that one or both of these hypotheses may also operate during the development of the olfactory system is currently of great interest. The nature of the interactions between the olfactory epithelium (OE), olfactory bulb (OB) and olfactory cortex at early developmental stages is currently providing us with much food for thought.[4,5] In order to gain a better insight into the mechanisms involved in the specification and development of the olfactory system, this review aims to explore recent data from different developmental studies. Our studies in the *pax-6* mutant mouse led us to conclude that the initial establishment of the OB central projections can proceed independently of the olfactory sensory axons from the OE.[6] This raises the question as to whether we can consider the OB as an independent developmental domain, which in turn would offer support to the protomap hypothesis. In the course of evaluating these ideas, we will review the orchestra of molecular cues involved in forming the projections from the OB to the olfactory cortex.

A brief overview of the olfactory system

The establishment of the basic circuitry in the mammalian olfactory system commences with the olfactory sensory neurons (OSN) in the OE, a structure that develops during embryogenesis from the olfactory placode. Axons from OSN project to the OB, where they form synapses on the dendrites of mitral/tufted cells to form the OB glomeruli.[7,8] Each of the approximately 5 million OSNs in a rodent nose expresses only one of the 1000–1300 different olfactory receptor genes.[9,10] The OSNs that express the same odorant receptor gene are randomly distributed within the nasal cavity but their axons selectively project into only 1–4 of the 2000 glomeruli in the OB (for a review, see Mombaerts).[11] The primary axons of the projection neurons from the OB, mitral/tufted cells, preferen-

tially grow into a very narrow region of the ventro-lateral part of the telencephalon, adjacent to the pial surface. Here they form the lateral olfactory tract (LOT).[7,12–17] The axons of the LOT, send off collateral branches that target areas in the olfactory cortex, anterior olfactory nucleus, piriform cortex, olfactory tubercle, entorhinal cortex, and several amygdaloid nuclei.[14,15,18–20] Recent studies have indicated that a stereotypic map exists of OB afferents to the olfactory cortex. Inputs from OSNs that express the same odor receptor map to precise clusters of target neurons in the olfactory cortex. This arrangement may permit each unique odor receptor code to be integrated, both to generate diverse complex odor sensations and to subsequently transmit such information to other cortical areas.[21] The final layering of the OB will depend on the arrival of tangential migrating cells from the subventricular zone, the prospective interneurons of the OB (reviewed by Goldman and Luskin).[22]

Given this brief introduction to the olfactory system, the two development schemes cited above would suggest very different developmental routes. While the protomap theory predicts a simultaneous and independent initial organization of OE, OB, and the olfactory cortex, the protocortex theory predicts that the development of the OB would be a secondary process, dependent upon the arrival of the OSN fibers from the OE. In this model, the subsequent arrival of the LOT fibers to the cortex would be necessary to induce differentiation at that level.

Mutual influences between OE and OB

The influence of the OE in triggering OB development has been well established in amphibians[23–25] (for a review, see, Brunjes and Fraizer).[26] However, this relationship has not been so intensely studied in mammals, although it has been shown that the arrival of olfactory axons does appear to have a dramatic influence on cell kinetics and the rate of precursor differentiation in the OB-anlage.[27] It has been also suggested that inductive interactions from the frontonasal-mesenchyme are essential for the morphogenesis of both the OE and the OB.[5,28] However, such work has not been extended to the very early stages of the olfactory placode formation.[5]

At the early stages of development, an area of the rostral telencephalon of the mammalian brain is already specified as an OB primordium.[16] The arrival of OSN axons induces the macroscopic evagination of the OB and its prenatal layering, but not the differentiation of the mitral cells themselves.[29,30] In fact, the differentiation of mitral cells commences earlier.[31] The OSNs start to express the olfactory marker protein at E16 in the rat (around E14–14.5 in the mouse), well after the establishment of the first connections from the OE to the OB.[32–35] However, the inductive role of the OB in this type of maturation remains unclear. In the homozygous *pax-6* mutant, a deficient OB (named OBLS, olfactory bulb like structure) starts to develop in the telencephalic vesicle in the total absence of an olfactory nerve.[6] Mitral-like cells from the OBLS extend axons that form a bundle reminiscent of the LOT (see more details below). These observations suggest that the protomap hypothesis might be applicable to early developmental events in the olfactory system (i.e., mitral/tufted cells neurogenesis in the OB primordium, and OSN neurogenesis in the OP, with their respective efferent projections). However, if this were the case, multiple tissue-tissue inductive interactions[5] might also be important for events later in development (i.e., the macroscopical evagination and layer formation of the OB). This suggestion is supported by the fact that neurogenesis is delayed in both the OB and OE, it is initiated at E10-10.5 in the OB (mouse, mitral cells) and by E9.5 in the OE (mouse, OSNs) (mouse;[6,35,36] rat;[29–32] see Figure 1). In the different areas of the olfactory cortex, neurogenesis occurs in an antero-posterior succession, starting well before the arrival of innervating OB axons[18,37] (Figure 1). Recently, it has been suggested that supernumerary glomeruli in the OB do not stabilize because the number of afferent axons is too small to support stable glomerular formation. This event has been named the 'interdependence phenomenon' among OSN axons.[38] A summary of the time scale of generation and axon extension in the rat and mouse for the three primary components of the olfactory system is shown in Figure 1. In mice, the first OSNs are generated by E9 and the first OSN axons differentiate by E9.5-10.5. These axons do not arrive in the telencephalic vesicle/OB primordium before E11, 0.5 days after the first mitral cells are generated (E10.5). The first mitral cell axons leave the OB at E11.5 and begin to innervate the olfactory cortex at E15, long after the initiation of neurogenesis in the piriform cortex (E9.5-11).

In accordance with the protocortex hypothesis, we could imagine that the OE and the OB develop independently? The protocortex hypothesis is based on sequential events, which correspond to a kind of hierarchy. Within this hierarchy, the OE should prevail over the OB, i.e.: the OE should dictate OB development from the first moment, as has been suggested by some authors.[26,27,39] However, none of these authors have proposed that a similar relationship might exist between the OB and the olfactory cortex, perhaps because of the well known studies regarding the triggering of neurogenesis in the cortex.[18,31,37] Shouldn't these studies be discussed in the context of the development of the olfactory system or when arguing in favor of a sequential series of events between the OE and OB? One of the strongest points in support of the protocortex hypothesis is the fact that mitral cell neurogenesis starts at E11 in the mouse[35] and has been estimated to commence at E14 in the rat.[27] On the basis of the timescale represented in Figure 1, one would expect mitral cell neurogenesis to commence at E12.5 in the rat and not at E14. This implies that at the time that these authors consider that the first OSN axons are entering the telencephalic vesicle, neurogenesis has already started in the OB-anlage. Thus, the sequence of events (OE–OB) proposed by these authors is only maintained due to the differences in determining the embryonic ages. Exactly the same independence is observed in the olfactory cortex (Figure 1) where cortical neurons are generated well before the arrival of mitral cell axons.

Now, let us turn our attention to results from different experimental models in which the OB or the OE has been altered. Projection neurons of the OB strongly express the mammalian *brachyury* homologue gene *Tbr1*. In *Tbr1*

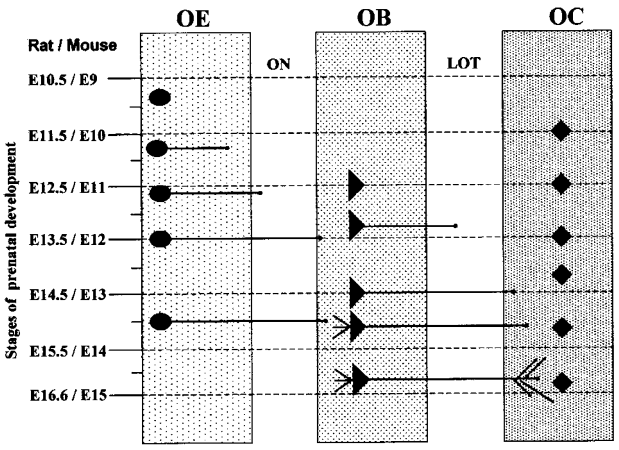

Figure 1 Schematic representation of neurogenesis and axonogenesis in the olfactory system. Neurogenesis of olfactory sensory neurons (OSN, ovals) has started by E9-9.5, of mitral cells (triangles) by E11 and in the olfactory cortex by E10 (rhombus). Axogenesis of the mitral cells (E11.5) is triggered before the arrival of the olfactory nerve (ON) axons to the telencephalic surface (E12). Mitral cells display dendrites at the time that the first ON axons enter into the OB (E13.5). Lateral olfactory tract axons collateralize to invade their cortical targets by E14.5-15. Cell generation in the three compartments of the olfactory system start independently, presumably due to intrinsic control mechanisms (protomap hypothesis). Embryonic developmental stages correspond to mouse (see rat equivalent in the left of the scheme). In the figure we have summarized data from different authors (see below) on a rat/mouse scale in which the date of detection of the vaginal plug in the mother is E0. (Studies summarized in this scheme are from references: 6, 16, 27, 29–37)

knockout mice, a few cells with a mitral cell phenotype survive, but they do not project to the olfactory cortex (see below). Nevertheless, despite the absence of their target (mitral cell dendrites), OSN axons converge normally in the OB forming glomerular-like spheres[40] (Table 1). Similarly, parallel studies in mice that are null-mutants for the mammalian homeobox genes *Dlx-1* and *-2*, have demonstrated the absence of interneurons[40] (Table 1). Since in these mice axons converge to form glomerular spheres in the absence of OB interneurons it seems that neither do these cells contribute significantly to the targeting of OSN axons[40] (Table 1). Conversely, in mutations of the homeobox gene, *Emx2* (but not the *Emx1*), the olfactory nerve fails to make contact with the OB. As a result, the mitral cell layer is disorganized, although an apparently normal LOT does form[41] (Table 1). The data from these *Tbr-1*, *Dlx-1*, *Dlx-2* and *Emx2* mutants argue in favour of the establishment of a topographic map between the OE and presumptive OB independent of the projection neurons and the interneurons of the OB, and the putative cues provided by them. However, the data from the Emx2 mutants does suggest that the OSN axons may help to ensure the orderly arrangement of mitral cells, although the functional significance of this is unclear. One singular case is the low affininity low growth factor receptor, p75NTR, which indirectly influences the growth of olfactory axons (in the early postnatal period there is an exuberant growth of some OSN axons), but the convergence into the OB is normal, and they originated a transient abnormal lamination in the dorsocaudal OB.[42]

What happens after the genetic or surgical elimination of the OE? Odorant receptor gene expression by OSNs is completely independent of the OB, either in the absence of an OB during embryogenesis[43] or during regeneration after OSN lesion.[44,45] Neither total nor partial sensory olfactory deprivation (closure of the nostril by electrocauterization) in newborns has any apparent effect on the number of OSNs in the OE[46] (Table 1). Similarly, although olfactory deprivation results in a 25% size reduction of the OB, the cellular populations remain correctly laminated and their numbers appear to be normal[46–51] (Table 1). However, Meisaimi and Safari[52] reported a larger reduction in the number of tufted cells than of mitral cells following olfactory deprivation. Neither the number nor the size of the glomeruli were noticeably different after olfactory deprivation, although an increase in the ratio of mitral/granule cells was observed.[46] Moreover, the pattern of reinnervation established by newly generated OSNs after global chemical deafferentation resembles the original topography.[53] In summary, naris closure appears to have its major effects early postnatally, though such procedure would be difficult to carry out prenatally and therefore this has not been pursued in the embryo.[26] In general, deprivation commenced in postnatal/adult animals, when all the connections between the OE and the OB have already been established. Nevertheless, similar changes have been observed when odor-evoked signaling was genetically eliminated, before connections are established, for example in mice lacking either functional olfactory cyclic nucleotide-gated channels[54] (Table 1), $G_{(olf)}$, the major G protein α subunit in olfactory receptors[55] or OMP, the olfactory marker protein, which takes part in odor perception[56] (Table 1). Furthermore, some peripheral olfactory projections are affected in mice deficient for a cyclic nucleotide-gated channel subunit, suggesting that the pathfinding of these axons is in part influenced by odorant-dependent activity.[57] This is especially relevant for the mitral cells, whose only response to naris closure relates to the perikaryal size and the reciprocal synapses between mitral and granule cells, while the LOT cross-sectional area remains completely normal.[46] No hypertrophic changes in non-deprived laminae of the OBs were observed, suggesting that no compensatory changes occur after olfactory deprivation.[46]

Interestingly, blocking odor transduction by either naris closure or by genetically engineering the loss of receptors or channels, does not affect the convergence of OSN axons in the OB[54,55] (Table 1). Moreover, the restoration of the projection from the OE after genetic ablation suggests that the positional cues involved in the formation of the olfactory projection might persist in the OB throughout the entire life of the animal.[58] Thus, a putative 'pioneer' mechanism for the rest of the olfactory fibers seems unlikely, although higher resolution studies are called for to address this specific issue.[58,59] Together, this could explain the fact that continuously-generated OSNs establish the proper connections within the OB throughout the animals lifetime, and the map of olfactory projections remains stable, despite the continual renewal of the OSN population.

Table 1 Results from different experimental models in which the olfactory bulb or the olfactory epithelium has been altered

	Animal	OSN	ON	Convergence ON into OB	Mitral/Tufted cells	OB interneurons	LOT
Axotomy and chemical destruction							
OP ablation[24]	*Xenopus* embryos	∅	∅	∅	∅	∅	∅
ON section partial[24]	*Xenopus* embryos	L (50%)	L (50%)	?	L (50%)	?	?
OB ablation[44]	Adult rat	N	∅	∅	N	N	N
OB ablation[45]	Juvenile catfish	N (regenerate)	∅ (regenerate)	∅	∅	∅	∅
Total/partial postnatal olfactory deprivation							
Naris occlusion[48]	Postnatal rat	—	—	—	N (Small OB)	N (Small OB)	N
Nostril closure by electrocauterization[46]	Neonatal mice	N	N	N	N (Small OB)	N	N
Stimulus-deprived Closure of one nostril	P25 rats	—	—	—	L (40%) (Small OB)	?	?
Surgical closure of an external naris[50]	P1–P30 rats	—	—	—	N (Small OB)	I (Small OB)	N
Focal denervation[49]	P10 and P20 rats				L		N
Chemical deafferentation[53]	Adult *H-OMP-lacZ-6* transgenic mice	N (recovery)	N (recovery)	N (recovery)	N	N	?
Functional deprivation							
$G_{OLF}^{-/-}$[55]	Neonatal mice	N*	N	N	N	?	?
O_{CNG}-*channel*$^{-/-}$[54]	Mice	N*	N	N	N (Small OB)	N	N
Targeted mutagenesis of the OCNC1 gene[57]	Mice with unresponsive OSNs	N*	N	N	?	?	?
Mutant animals							
Pax-6$^{-/-}$[6]	Mouse *Sey* embryos	∅	∅	∅	Mitral like cells in OBLS	Disorganized	N N
EMx2$^{-/-}$[41]	Mouse embryos	N?	N?	∅	Disorganized	N	N
Xt$^{J-/-}$[6]	Mouse embryos (OB agenesis)	N	N	∅	∅	∅	∅
Tbr1$^{-/-}$[40]	Mouse embryos	N	N	N	∅	Disorganized	∅
Dlx1$^{-/-}$, *Dlx2*$^{-/-}$[40]	Mouse embryos	N	N	N	N	∅	?
NCAM-180$^{-/-}$[34]	Mouse embryos	N	N	N	N (Small OB)	N (Small OB)	N
OMP$^{-/-}$[56]	Postnatal mice	N	N	N	N	N	?
p75NTR$^{-/-}$[42]	Embryos and postnatal mice	N	N	N	N (OB protruded	N (OB protruded)	N

N = Normal phenotype/structure; N* = Normal phenotype/structure but non responsive to odors; ∅ = Not formed/disappeared; I = Increased number of cells; L = Low number of cells; ? = not described. Abbreviations: LOT, lateral olfactory tract; OB, olfactory bulb; OBLS, olfactory bulb-like structure; ON, olfactory nerve; OSN, olfactory sensory neuron.

It has become widely accepted that different mutations for the *pax-6* gene, including the spontaneous mutation *Small Eye*,[60,61] produce different alterations. These alterations include the absence of eyes and of nasal structures such as the OE and the OB. Using a panel of region and field specific molecular markers, careful study of these animals revealed the presence of a prospective OB in the rostrolateral part of the telencephalic vesicle, named the olfactory bulb-like structure[6] (OBLS; Table 1). Our findings suggest that the *pax-6* mutation disturbs anatomical development, while early OB differentiation and the beginning of the central projections are not affected. To date, we have been unable to identify an olfactory nerve that enters the OBLS in these mutants, although in some cases we have observed immature structures suggesting that a remnant of the OE is present in the *Small Eye* mutants.[6] Unfortunately, the PCD mutant (spontaneous Purkinje cell-deficient mouse), in which a complete loss of mitral cells has been demonstrated, does not provide a complementary model because mitral cell loss occurs postnatally (at 3–4 months, the tufted cells remaining normal). Moreover, the number of cells in the OB is normal in the embryo and young adult PCD mice.[62] However, the thickness of the LOT in the postnatal animals does become significantly reduced after the loss of mitral cells.

In summary, these studies demonstrate that OB cells can survive without the OE and can even begin to differentiate without the arrival of its primary afferent fibers. Furthermore, OSNs are able to survive and are capable of completing their continuous replacement and axonal regeneration even in the absence of OB, although the rate of replacement may be affected. Nevertheless, both structures seem to be independent of one another for their respective survival. These observations do not preclude the

conclusions drawn by some authors that the OSN axons influence later stages of OB development.[16,23,27,34,51]

Molecular signals guiding the formation of the LOT

If the formation of the distinct olfactory structures and axons initially progresses independently, identifying developmental cues that control these processes might shed light on some of the more controversial points reviewed here. It is possible that axons from the AOB projection neurons, located deep within the LOT, could serve as pioneer axons to subsequently guide OB axons. The timing of the formation of the LOT, and of the arrival of mitral cell axons to their final targets in the olfactory cortex supports this idea.[15,16,31] However, in the absence of AOB axons, the OB mitral cells enter and form a LOT.[36] This observation seems to rule out the notion that AOB projection neurons provide pioneer axons and rather, suggests that intrinsic signals from the telencephalon are more likely to govern the formation of the LOT.[6] Below, we shall analyze two of the candidate mechanisms.

1-Contact-mediated mechanisms Cell adhesion molecules are good candidates to act as contact mediated signals in the formation of the LOT, particularly molecules such as NCAM-H,[63] OCAM,[64] and Nr-CAM. The latter molecules may act together or in combination with TAG-1/axonin-1[65] although to date, they have not been directly implicated in the formation of the LOT. Other important contact-molecules like reelin or the ephrins/Eph signaling system do not appear to participate in the formation/targeting of the LOT.[66,67] The existence of guide-post cells for mitral/tufted cell axons has also been proposed.[16] Such cells have been identified by the mAb lot1 as a subset of early-generated neurons formed exclusively in the neocortex, which migrate to reach their position in the basal telencephalon, thereby enclosing the LOT area.[68,69] These lot1-positive cells may produce factors that either attract or support axonal outgrowth. Indeed, lot1-positive cells seem to form a border in most areas of the olfactory cortex, beyond which LOT axons do not grow.[68] However, the addition of the mAB lot1 does not alter the formation of the LOT in organotypic cultures.[68] Given the poor degree of heterogeneity expected in the LOT projection when compared to the olfactory nerve, it seems more likely that such contact-mediated mechanisms contribute less to the formation of the LOT than in the case of the olfactory nerve.[59,70]

2-Secreted cues It seems highly probable that secreted cues are involved in the formation of the LOT.[71] The first one to be identified, Slit-2, is putatively secreted by the septum and acts through its functional receptor Robo-1.[72,73] However, Slit-2 may not play a simple role in this process.[36,69] Recently, the group of Pini suggested that a concurrent cue may also exist that is responsible for the repellent activity of the septum and that which may be different from Slit-2 (see Figure 2).[74] LOT axons also respond selectively to the secreted semaphorins. Sema 3F repels LOT axons, preventing them from invading the cortical plate and the ganglionic eminence, while Sema 3B attracts LOT axons, forcing them to remain at the surface of the telencephalon (see Figure 2).[17] Meanwhile, other secreted semaphorins, e.g. Sema 3A, do not appear to influence the outgrowth of OB axons.[17,72] Interestingly, within the OB, some cells and their axons express Neuropilin-1 and -2,[6,36,75] functional receptors of the semaphorin family. However, the only two knockout mice so far analyzed, carrying a null mutation for the secreted Sema 3A and for its receptor Neuropilin-1, do not show major alterations in the developing LOT.[76] Functional receptors for secreted molecules of the Netrin family, such as DCC or Unc5H3 are strongly expressed by developing mitral cells of the OB.[77,78] However, neither secreted Netrin-1 nor a secreted form of the Netrin-G1 (anchored to plasmalemma membrane) exert any effect on OB axonal outgrowth.[17,72,79]

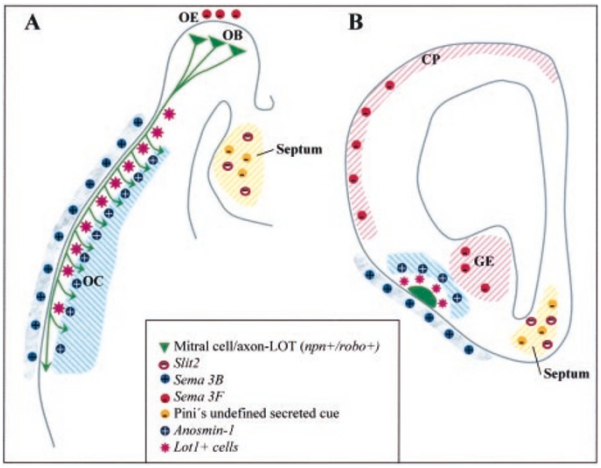

Figure 2 Cues guiding axonal outgrowth from projection neurons of the olfactory bulb. Horizontal (A) and coronal (B) sections of the head showing olfactory structures and annexes. Mitral cells and their axons (LOT) are represented in green. Different chemorepellents prevent LOT axons from entering different structures (septum, ganglionic eminence, cortical plate) and force them to occupy a position at the surface of the telencephalic vesicle. This is reinforced by lot1$^+$-cells (violet stars) and two chemoattractants, Sema 3B (secreted by progenitors of the skull bones) and anosmin-1 (produced by the olfactory cortex). The latter is critical for the formation of collaterals from the LOT axons (green arrows in A) that will invade their targets (see text for details). Abbreviations: CP: cortical plate; GE: ganglionic eminence; OB: olfactory bulb; OC: olfactory epithelium; OE: olfactory epithelium

It has been suggested that a stereotypic map of mitral cell termination in the olfactory cortex might be established by odor receptors providing an input to those mitral cells.[21] Thus, the OSNs expressing the odor receptor I7 terminate on mitral cells that innervate the I7 glomerulus and, in turn, the axons from those mitral cells would sort to form synaptic clusters in piriform cortex. Axonal collaterals bud from the primary axons of the LOT. This phenomenon is a widespread strategy used by growing axons to invade target areas in the developing nervous system,[80] and the only way for mitral and tufted cells from the OB to invade olfactory target areas in the cortex *in vivo*.[81] It is widely accepted that the same cues involved in guiding axonal pathfinding should be the ones responsible for axonal collateralization. One example is that of Slit-2, a well-known chemorepellent for axonal guidance that seems to be a positive regulator of sensory axonal branching.[69,82] On the other hand, other studies suggest that different cues may

be used to induce collateral axon branching than for axonal pathfinding.[81] The lot1-positive cells, which do not seem to be involved in the collateral branching of the OB mitral cells could provide an example of this phenomenon.[68] Anosmin-1, a protein defective in Kallmann syndrome (syndrome: anosmia plus hypogonadotropic hypogonadism) seems to be directly involved in the formation of mitral cell axon collaterals during the developmental period in which the olfactory cortex is colonized by LOT collaterals.[83] After a protracted period of waiting, possible to facilitate neutralization or overriding of inhibitors of collateral sprouting as suggested by Fujisawa's group,[68] Anosmin-1 enhances axonal sprouting but does not affect the growth of primary OB axons. At earlier developmental stages (rat E15), Anosmin-1 seems to contribute to the guidance of primary OB axons, but it does not trigger sprouting.[83] In contrast, expression patterns do not suggest a role for slit molecules in the collateralization of LOT axons that invade olfactory cortex.[84]

Taken together, it would appear that the cues which guide OB axonal outgrowth act in a cooperative way that includes both attractive and repulsive signals, perhaps in a hierarchy as suggested elsewhere.[85] It is noteworthy that some of the cues which do not seem to be useful for guiding OB mitral cell axons (e.g. Sema 3A, Netrin-1), do appear to be important for the growth of OSN axons.[86,87] Therefore, the possible roles played by molecules in the development of the olfactory pathway may be diverse and indeed, the possibility still exists that cues that attract OSN axons but repel mitral cell axons might remain to be identified. Finally, we must also remember that the interaction of secreted molecules with non-diffusible components of the extracellular matrix may have important functional implications for the establishment of the proper synaptic connectivity during development.[36,83]

As has been recently described, it seems that the cues that guide OSN axons to form proper synapses within the OB are preserved throughout the life of the animal.[58,59] It is also remarkable that the cues involved in the formation of the LOT are present and expressed in the correct structures in the *Small Eye* mutant mouse.[6] This might explain how a LOT-like projection forms in these mutants from the ectopic and malformed OBLS.[6] Both these studies emphasize the idea of an independent OB and therefore, support the existence of a protomap during the initial development of the olfactory system. However, we must not forget that, among all the signals that we have reviewed, both for the neurogenesis of mitral cells in the OB and for the initiation of the LOT formation, *Tbr-1* may play a critical role.[40] (Table 1).

Conclusions

From the data discussed above, we conclude that the protomap hypothesis provides a more plausible format than the protocortex theory for the development of the olfactory pathway. Indeed, relatively little data seems to support the protocortex model for the initial developmental events. The OB organizes independently of afferent projections from OSNs or other external influences. At later stages, signals provided by OSN inputs, and eventually other tissue-derived cues, will contribute to confer the mature appearance of OB, as recognized in adults. In the same sense, our studies of the mouse mutant *pax-6*, strongly suggest that the initial establishment of the central OB projections proceeds independently of the OSN axons that are arriving from the olfactory epithelium. This work opened up the possibility of identifying the intrinsic molecular guidance cues that are required for the patterning of the olfactory system. Thus, although it still remains poorly studied, we hypothesize that the independent development of the olfactory cortex follows the predicted protomap hypothesis. Indeed, studies performed elsewhere in the brain strongly support the idea that a protomap governs the development of the piriform cortices. For example, in the *Gbx-2* null-mutant mice, thalamic differentiation is disrupted and thalamic axons do not innervate the cortex. However, the patterns of several region-specific markers in the cortex develop normally, suggesting that factors intrinsic to the neocortex are responsible for the development of the cortex, or, at least, for the expression of these markers.[88,89] Furthermore, if the strong morphogenetic agent FGF8 is ectopically expressed in the posterior telencephalon before the arrival of thalamic axons, the somatosensory cortex is partially duplicated, and an additional population of thalamic axons develops to innervate these extra barrels.[2]

Factors extrinsic to the developing cortex (the arrival of thalamo-cortical fibers) have also been shown to control the specification of the different cortical areas.[90] An extension to the *protomap* hypothesis leads us to consider the regional expression in other telencephalic structures in the absence of a patterned afferent input. In this context, it has been generally accepted that the development and differentiation of the olfactory bulb depends on the arrival of olfactory sensory axons. Homozygous *small eye* mutant mice lack nasal structures including the olfactory epithelium and, an emergent olfactory bulb. Therefore, the initial development of the OB is not dependent of the OSN input

These studies suggest that a more profound analysis of protomap formation in the different structures that compose the olfactory cortex would be extremely interesting. It will be particularly important, to determine the molecular basis that governs the differentiation of the olfactory cortex into the LOT specific synaptic clusters as recently identified by Buck's group.[21] It would also be useful to be able to analyze the relevance of the arrival of sensory afferents in refining the development of the OB. Finally, a model with an OB but lacking the LOT could be also determinant in clarifying the true influence that this projection has on the development of cortical structures.

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