Spatio-temporal specification of olfactory bulb interneurons.

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Serena Bovetti - Paolo Peretto - Aldo Fasolo - Silvia De Marchis

Abstract Olfactory bulb (OB) interneurons are continuously generated throughout development and in adulthood, and are derived from different progenitor zones. Once integrated in the OB circuits, interneurons play essential roles in olfactory information processing by modulating the activity of major output neurons. These functions are performed by multiple classes of neurons that differ in their spatial distribution, morphology, neurochemical and synaptic properties. This diversity, and the continuous neurogenesis make the understanding of the specification mechanisms in the OB a challenging task. New studies suggest that both intrinsic and extrinsic cues are involved in fate determination of OB interneurons. In both development and adulthood the expression of specific transcription factors not only defines different progenitor regions but also precise interneuronal phenotypes. Here we discuss recent findings on the molecular mechanisms regulating production and diversity of OB interneurons with respect to the spatial and temporal parameters.

Keywords Olfactory bulb - Specification - Interneuron Neurogenesis - SVZ - LGE

Introduction

The olfactory bulb (OB) is one of the two regions in the mammalian forebrain in which neurogenesis persists throughout life (Alvarez-Buylla and Lim 2004). In both embryos and adults, OB interneuronal precursors migrate tangentially from their production site (the lateral ganglionic eminence, LGE, or the subventricular zone, SVZ, respectively) to reach the bulb where they integrate and differentiate into different types of interneurons, within the granule (GCL) and glomerular (GL) layers (Luskin 1993; Lois and Alvarez-Buylla 1994). Although the molecular mechanisms governing precursors migration and olfactory interneurons differentiation are starting to be elucidated, many aspects remain unresolved. One of the relevant open questions is to which extent position and time control specific aspects of interneuron identity. In particular, it remains to be elucidated whether different OB interneurons are generated by a unique type of multipotent progenitor cells in the LGE/SVZ or if they are generated by distinct progenitors present at different locations or at different developmental stages. Several recent studies focused on the importance of the intrinsic determination of cellular commitment. These findings suggest that cells have a predetermined commitment that makes them ‘‘partially’’ specified to a phenotype very early in their differentiation pathway. However, the intrinsic specification alone is not sufficient to guarantee a specific cell fate, and the extrinsic factors play a fundamental role in this process. Possibly, OB progenitors could be lineage-restricted before they leave the LGE/SVZ, or they could become restricted at different positions along

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the migration pathway, or, still, cells could remain multipotent until they reach the OB and differentiate into specific interneuronal types according to local environmental cues. Another important issue is related to the developmental potential of progenitors at different time: are selective populations of OB interneurons generated within specific embryonic and postnatal developmental windows?
The spatio-temporal mechanisms regulating the specification of the OB interneurons are starting to be elucidated and are becoming one of the most debated items of the last few years. In the following paragraphs we discuss recent progress in understanding OB interneuron specification.

Origins of the OB interneurons

Previous birth dating studies suggest that most granule and periglomerular cells in the mouse OB are generated between E18 and P5 (Hinds 1968), although recent studies report the initial production of OB interneurons before E18 (Wichterle et al. 2001 ; Stenman et al. 2003 ; Tucker et al. 2006). During embryonic development, in contrast to the local origin of the major excitatory output (mitral and tufted) neurons, OB interneurons arise primarily from the LGE. Fate mapping studies clearly show that precursors in the LGE starting from E14.5 produce progeny that migrate into the presumptiveOB via a distinct pathway, and differentiate intoOBinterneurons (Wichterle et al. 2001 ; Stenman et al. 2003 ; Tucker et al. 2006). Nevertheless, there is significant evidence that, besides the LGE, interneurons can be generated locally from endogenous precursor cells within the embryonic OB (Vergano-Vera et al. 2006).
In the early postnatal (Luskin 1993) and adult rodent (Lois and Alvarez-Buylla 1994) OB interneuron precursors are mainly generated in the SVZ of the lateral ventricle that develops from residual progenitors of the embryonic LGE and maintains neurogenic activity throughout life. Similarly to the embryos, a small proportion of early newlyborn postnatal OB interneurons are produced locally within the OB (Lemasson et al. 2005). From their site of origin in the SVZ, interneuron precursors migrate tangentially along the rostral migratory stream (RMS) to reach the OB where they complete their differentiation (Alvarez-Buylla and Garcia-Verdugo 2002). Neural stem cells with the characteristics of astrocytes persist in the adult SVZ (Doetsch et al. 1999) and to a lesser extent along the RMS up to the core of the OB (Gritti et al. 2002). Thus, in a process that begins in the embryo and continues postnatally and into adult life, the vast majority of OB interneurons derive from precursors that have migrated tangentially from the basal forebrain. Accordingly, the astroglial-like stem cells of the SVZ have been demonstrated to derive from ventral (striatal) radial glia (Merkle et al. 2004), although a recent study indicates a dorsal radial glial source for SVZ astrocytes of the dorsolateral corner of the lateral ventricle (Ventura and Goldman 2007).

Heterogeneity of the OB interneurons

OB interneurons consist of different subtypes which are variably defined by their spatial distribution, morphological, molecular and functional characteristics (Shipley et al. 2004 ; Kosaka et al. 1998 ; Kosaka and Kosaka 2005a ; Parrish-Aungst et al. 2007 ; Puopolo and Belluzzi 1998). Major classes of OB interneurons are located in the GCL and GL. The large majority of interneurons in the GCL are GABAergic inhibitory granule cells (GCs; Shipley et

al. 2004 ; Parrish-Aungst et al. 2007). The GCL also contains a subpopulation of calretinin (CR) expressing GCs which only partially colocalize with the GABAergic phenotype (Parrish-Aungst et al. 2007). In contrast to the limited number of neurochemically distinct GC types, periglomerular (PG) cells are highly heterogeneous in neurochemical content. A recent quantitative analysis performed in the mouse OB shows that about 50% of all cells in the GL are GABAergic (Parrish-Aungst et al. 2007). The GABAergic population comprises a subpopulation expressing dopamine or its synthesizing key enzyme tyrosine hydroxylase (TH) that account for about 10–13% of all glomerular neurons (Kosaka and Kosaka 2005b). At least three further non-overlapping GL neurochemical classes can be identified by the expression of different calciumbinding protein, namely CR, calbindin (CB) and neurocalcin (NC) (Kosaka et al. 1998 ; Kosaka and Kosaka 2005b). Similarly to the TH population also CB expressing cells are largely GABAergic, whereas CR and NC show only low colocalization with GAD (Parrish-Aungst et al. 2007).
Recently, it is becoming more and more accepted that a certain degree of heterogeneity already exists in OB interneuron precursors migrating along the RMS. According to this, the phenotypic differentiation of a subset of migrating neuroblasts into dopaminergic cells has been demonstrated (Baker et al. 2001). Moreover, a subpopulation of SVZ precursors en route to the OB has been shown to acquire GABAergic phenotype before reaching their final target (De Marchis et al. 2004). These data indicate that migrating neuroblasts are not undifferentiated cells but are partially preselected into specific interneuron class lineages. Their final fate likely reflects the balance between intrinsic determinants, represented by the expression of specific transcription factors, and external cues encountered at different levels during the migration pathway.

Spatial specification of LGE progenitors

During the embryonic period, the LGE is known to give rise to OB interneurons as well as striatal projection neurons (Stenman et al. 2003). Concurrent with the production of these cell types in the LGE, precursors in the adjacent medial (MGE) and caudal (CGE) ganglionic eminences generate cortical, striatal and hippocampal interneurons. Numerous studies have started to unravel the molecular mechanisms that determine how these regions are specified to generate specific classes of neurons. In a recent work, Tucker and collaborators asked whether autonomous factors or local signals within the LGE distinguish OB interneuron precursors from precursors destined to other forebrain regions and influence their subsequent migration and differentiation (Tucker et al. 2006). Using an organotypic culture assay that preserves the geometry of the developing telencephalon, they show that homotypically transplanted E14.5 LGE cells migrate to the OB rudiment, while MGE cells transplanted into the LGE almost completely avoid the OB and undergo a robust tangential migration into the cortex. The counterpart experiment, transplanting homotypically E14.5 MGE or ectopically LGE into MGE, shows that while MGE cells behave normally migrating to the cortex, LGE cells are not able to reach either OB or cortex. Similar results were previously obtained using homo- and heterotypical transplantation of E13.5 MGE and LGE precursors, providing insight into the derivation and differentiation of a broad range of forebrain interneurons, including periglomerular and granule cells in the OB (Wichterle et al. 2001). According to these studies, OB precursors autonomously acquire, between E12.5 and E14.5, a distinct identity and migratory specificity based by their

position in the LGE. Local LGE or MGE cues are thus insufficient to reprogram the migratory fate of heterologous precursors; nevertheless, local signals are required for significant migration of LGE cells into the OB rudiment.
The LGE displays a considerable degree of determination. Besides the OB interneurons, the LGE gives rise also to striatal projection neurons (Stenman et al. 2003). Patterning mechanisms reflecting the combinatorial expression of different transcription factors establish distinct domains in the ventral forebrain and confer to the different LGE precursors the unique ability to migrate to the OB or to the striatum and acquire distinctive characteristics associated with their final targets (Tucker et al. 2006). The different progenitors are localized in specific zones of the LGE confirming the evidences of a position-dependent commitment obtained by transplantation studies (Wichterle et al. 2001). DLX/Gsh2/Er81-expressing precursor cells in the dorso-lateral ganglionic eminence (dLGE), are the mainly source of OB interneurons, while the DLX/ISL1-positive precursor cells located in the most ventral part of the LGE SVZ, define cells that will differentiate into striatal projection neurons (Stenman et al. 2003) (Fig. 1).
The dLGE selectively express also other transcription factors like Pax6 and Sp8 (Stenman et al. 2003 ; Waclaw et al. 2006). Doublestaining for these two markers at E13.5, reveals a little colocalization with a majority of Sp8-only cells and a minority of Pax6-only cells (Waclaw et al. 2006), suggesting a further regionalization within the dLGE, which represents an heterogeneous compartment of molecular different precursors possibly already committed to a specific phenotype.
Other genes such as Nkx2.1, and Gsh1 and Gsh2 have been shown to cooperate to define the boundaries between the MGE and LGE. Nkx2.1, for instance, is expressed exclusively in the MGE and its loss of function results in the transformation of much of the MGE into an LGE-like progenitor zone (Puelles et al. 2000 ; Sussel et al. 1999). Gsh1 is expressed in a ventrodorsal gradient with its highest expression in the MGE while Gsh2 is expressed in a dorsoventral gradient with its highest expression in the ventricular zone (VZ) of the dLGE (Toresson et al. 2000 ; Corbin et al. 2000 ; Yun et al. 2003). Gsh2 is coexpressed with another marker of LGE progenitors called Mash1 and partially with Pax6 in a boundary zone at the pallial/subpallial limit (Yun et al. 2003). Loss of Gsh2 function results in a ventral extension of expression of different dorsal regulators including Pax6, and a consequent respecification of much of the dLGE into ventral pallium. Dorsalization of the LGE is at early stages, by birth the striatum appears normal and this ‘‘recovery’’ correlates with increased levels of Gsh1 expression that compensates the Gsh2 loss (Toresson and Campbell 2001).
MGE and LGE derived cells engrafted into adult SVZ behave similar to what is reported in the homocronically transplantation assays (Wichterle et al. 1999 , 2001 ; Tucker et al. 2006). Large numbers of LGE precursors migrate to the OB where they differentiate into periglomerular and granule cells whereas MGE cells engrafted in adult SVZ, disperse in the striatum cortex and septum without reaching the OB (Wichterle et al. 1999). The local cues are insufficient to reprogram the migratory fate of MGE precursors whereas LGE cells selectively migrate along the pathway to the OB. This potential is very similar to that of the postnatal and adult SVZ (Lois and Alvarez-Buylla 1994 ; Luskin 1993) supporting the derivation of SVZ from LGE. The behavioral similarity of embryonic, postnatal and adult precursors suggests that an analogous mechanism might be used in adulthood. According to this, Er81, Pax6 and Sp8

expression is not restricted to the embryonic phase of development, but has been detected in cells of the postnatal/adult SVZ, as well as in the RMS and in the GCL and GL of the OB, suggesting that a regional specification of neuronal subtypes might also apply to adult neurogenesis (Stenman et al. 2003 ; Waclaw et al. 2006).

Specification of the different OB interneuronal phenotypes

Major categories of local OB interneurons are PG and Gcs respectively located in the GL and GCL. PG and GCs appear to derive primarily from separate progenitors. Indeed, using a retroviral lineage method, Reid and collaborators (1999) show that while half of all PG cells labeled at E14 and E15 share a common progenitor with GCs, later labeling (E17) results in 100% single cell types clones suggesting a progressive restriction of cell fate as development proceeds (Reid et al. 1999). Moreover, a recent work suggested a new concept of spatial separation of granule and periglomerular neurons specification. Retroviral lineage tracing showed that SVZ precursors generate few PG cells and more Gcs, whereas RMS precursors give rise to a larger population of PG neurons suggesting that in adult neurogenesis different neuronal subtypes are specified at different positions (Hack et al. 2005). This regionalization appears to be at least partially mediated by Pax6 expression. Indeed, Pax6 expression in RMS precursor cells is required to progress towards a PG neuron subtype and its target deletion strongly reduces the proportion of PG cells normally generated (Hack et al. 2005). Moreover, Pax6 governs the specific differentiation of dopaminergic periglomerular and superficial granule cells in the adultOB(Hack et al. 2005 ; Kohwi et al. 2005). Pax6 is expressed in most proliferating SVZ progenitors but only in a subpopulation of migrating neuroblasts, further indicating that migrating neuroblasts are heterogeneous. Transplantation of GFP-positive dLGE precursors from Pax6 knock-out mice into wild-type adult SVZ environment produce neuroblasts capable of migrating into the OB but fail to generate dopaminergic periglomerular and superficial granule cells (Kohwi et al. 2005). Moreover, Pax6 overexpression in both SVZ and RMS is sufficient to induce differentiation of PG cells toward dopaminergic phenotypes (Hack et al. 2005).
According to the Hack study (2005), adult SVZ precursors proceed from a less-committed neurogenic state into the SVZ to a more-committed state closer to the OB, with many PG neurons precursors become fate-restricted at much more rostral position than granule neuron precursors.
Consistent with this idea of progressive neuronal fate specification, Hack and collaborators show that adult SVZ cells commit to both neuronal and glial lineages whereas precursors in the RMS already show a restricted fate commitment. The acquisition of a specific phenotype seems to be at least partially mediated by the transcription factor Olig2 which is expressed only by the transit amplifying cells in the SVZ and opposes the neurogenic role of Pax6 promoting oligodendrogenesis (Hack et al. 2005).
Dopaminergic and GABAergic phenotypes differentiation is controlled by the expression of the homeodomaincontaining transcription factors Dlx1, Dlx2, Dlx5 and Dlx6, that are expressed in progenitors of OB local circuit neurons and in postmitotic periglomerular and granule cells (Liu et al. 1997 ; Stuhmer et al. 2002 ; Long et al. 2003). Target deletion of Dlx2 induces a great reduction of TH^+ PG cells and Dlx1 and Dlx2 double knock-out show a decrease in both TH and GABAergic PG and GCs (Qiu et al. 1995 ; Bulfone et al. 1998). Similarly to

Dlx2 mutant, Dlx5$^{-/-}$ have a disruption defect in OB local circuit neurons phenotypes and the mutation appears to preferentially affect granule cells that downregulate GAD67, GAD65 and TH expression (Long et al. 2003 ; Levi et al. 2003). Among the intrinsic factors controlling dopaminergic differentiation of PG interneurons the transcription factor Er81 is a possible candidate, since it has been demonstrated to be expressed in the OB GL and outer GCL with a pattern similar to that of Pax6 (Stenman et al. 2003). Accordingly, a recent study shows a strong coexpression between Er81 and TH (Saino-Saito et al. 2007). This transcription factor may function as positive regulator of dopaminergic differentiation although its expression is not sufficient for dopaminergic neuron differentiation as demonstrated by a paradigm of odor deprivation resulting in severe TH downregulation and only partial Er81 decline (Saino-Saito et al. 2007).

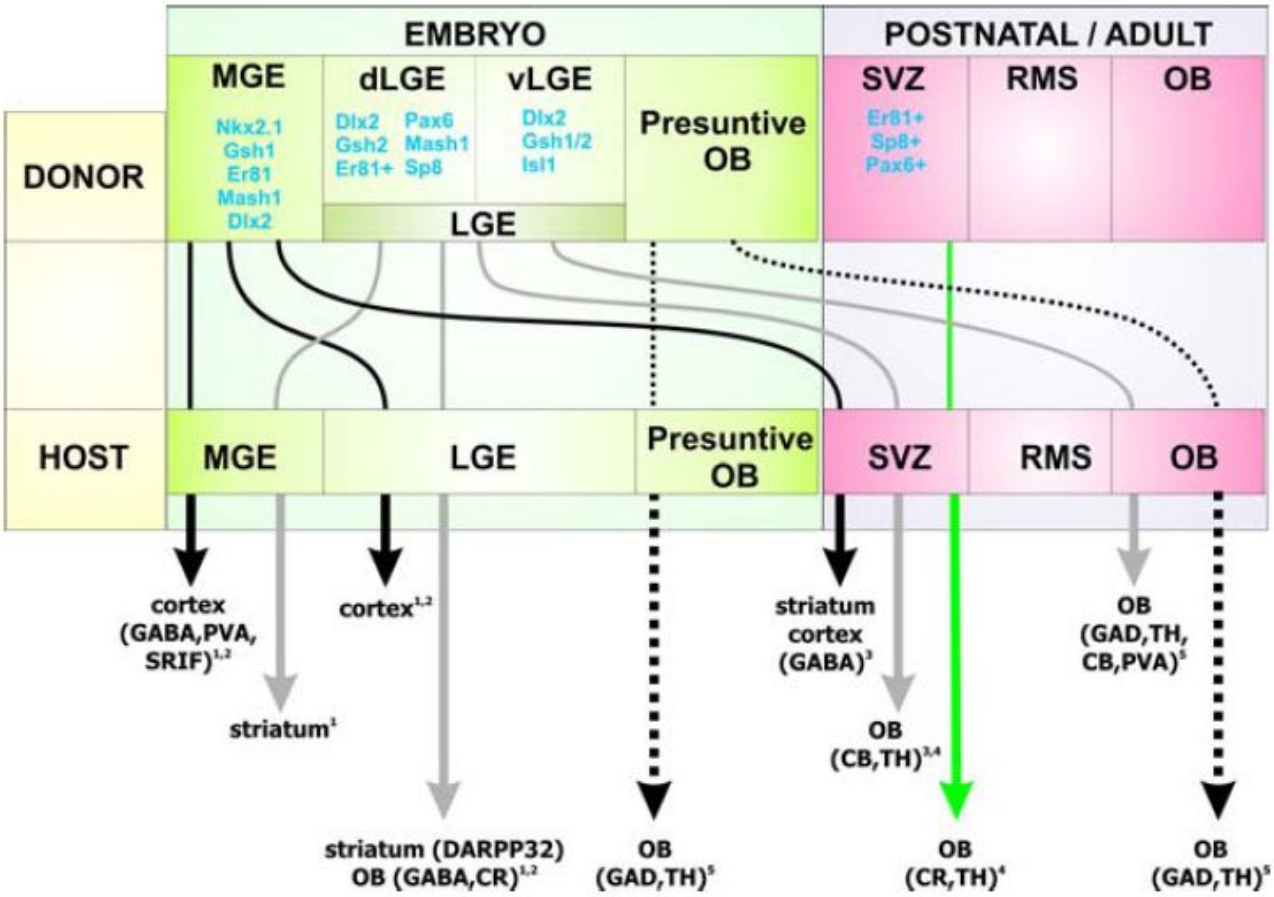

Fig.1 Major factors governing cellular specification in the ganglionic eminences and SVZ, and phenotypes originated after homo- and heterotypical transplants in both embryos and adults. MGE: medial ganglionic eminence; dLGE: dorsolateral ganglionic eminence; vLGE: ventrolateral ganglionic eminence; SVZ: subventricular zone; RMS: rostral migratory stream; OB: olfactory bulb.

[1]Wichterle et al. (1999),
[2]Wichterle et al. (2001),
[3]Tucker et al. (2006),
[4]De Marchis et al. (2007),
[5]Vergano-Vera et al. (2006)

The emerging model of a molecular specification of distinct olfactory interneuron subtypes has been recently reinforced by the discovery of Sp8 involvement in the specification of the calretinin and GABAergic/non-dopaminergic populations of PG cells (Waclaw et al. 2006). Sp8 is a member of the Sp1 zinc finger transcription factor gene family, expressed in the embryonic dLGE as well as in the adult SVZ, RMS and OB. While most of the migrating neuroblasts appear to express Sp8 in the RMS, only the calretinin and GABAergic/non-dopaminergic populations express it in the GL, suggesting that a restriction in fate determination occurs somewhere in the final part of the migration pathway (Waclaw et al. 2006). Conditional Sp8 mutants show an increase in cell death of calretinin and GABAergic/non-dopaminergic populations within the dLGE at late embryonic stages as well as in the postnatal RMS and OB of the conditional mutants. In addition to the increased cell death, calretinin cells displayed abnormal migration patterns, resulting in a severe reduction in both calretinin and GABAergic/non-dopaminergic subtypes. Sp8 is thus important for these cell types to fully differentiate, migrate and/or survive.

Temporal differences in the generation of OB interneuron subtypes

Olfactory interneurons are generated during the whole life of the organism (Luskin 1993 ; Lois and Alvarez-Buylla 1994). Recent findings suggest that the potentiality of SVZ progenitors is modulated in time and that specific subtypes of PG and GC interneurons are generated at different ages (Lemasson et al. 2005 ; De Marchis et al. 2007). Two spatio/temporally distinct populations of newborn neurons integrate into the OB GCL. Indeed, early born granule cells (P3–P7) constitute a larger population, predominantly target to the external GCL, whereas newly generated cells in older mice were positioned deeper in the GCL (Lemasson et al. 2005).
We recently showed that the potentiality of SVZ progenitors is modulated in time and specific neurochemical classes of PG interneurons are generated at different ages (De Marchis et al. 2007). In vivo cell labelling in neonate and adult, together with embryonic and neonatal transplants in hetero- and homochronic environments, demonstrate that SVZ progenitors preferentially adopt the CB phenotype during embryonic life, whereas acquisition of the same identity gradually becomes less frequent during postnatal development and adulthood. In contrast, the production of CR- and TH-expressing PG cells follows an opposite trend with a progressive increase with age (De Marchis et al. 2007). According to previous studies (Hack et al. 2005 ; Kohwi et al. 2005 ; Waclaw et al. 2006) this work suggests that the population of SVZ progenitors with a PG fate is composed of a heterogeneous pool of neurochemically specified elements, and indicates that the relative contribution of these different subsets changes with time. Therefore, neurogenesis in the OB does not reflect a simple turnover of the whole interneuron population of the GL, but it may serve to finely modulate the relative amounts of distinct OB interneuron subpopulations.

The role of the environment: instructive or selective?

The continual integration of specific types of newly generated neurons in the OB might have a profound effect on olfactory system function. Olfactory discrimination, learning and memory have been attributed to changes at

reciprocal dendro–dendritic synapses between mitral/tufted cells and interneurons. The continual addition of interneurons, which modulate the coding of olfactory information through lateral inhibition and synchronization of firing of the mitral/tufted cells, might provide a novel substrate for adapting to complex, changing environments (Laurent 2002 ; Yokoi et al. 1995). Cell death, which is also prominent in the adult OB (Corotto et al. 1994 ; Petreanu and Alvarez-Buylla 2002), may also effect rapid changes in neural circuits. According to this, modeling studies have suggested that activity-dependent survival of adult-generated neurons can redistribute the representation of odorants to maximize olfactory discrimination (Cecchi et al. 2001). Moreover, recent works demonstrated the involvement of the sensory input in regulating the survival of newborn neurons (Rochefort et al. 2002 ; Saghatelyan et al. 2004). Experience shapes the maturation of newborn neurons in the OB but it is yet unknown whether it affects specific interneuron phenotypes generation, survival and/or differentiation. Previous works showed that olfactory deprivation by naris closure or the chemical lesion of the olfactory epithelium, induce a strong decrease in PG cells TH expression, suggesting that functional activity can specifically regulate PG cells phenotypes (Baker et al. 1983 , 1993 , 2001) affecting already integrated OB interneurons. An unanswered question is whether the olfactory function might also regulate the phenotype of newly integrated cells. We can hypothesize that changes in the extrinsic cues at different levels of the SVZ-OB pathway act to modulate the fate of partially precommitted precursors or alternatively, and more likely, the occurrence of selective mechanisms in the OB favoring the survival of specific committed precursors.

Concluding remarks

In the last decades new emphasis in the study of the OB has been driven by the discovery of adult neurogenesis in this region. The sites involved in the genesis of the OB interneurons have been clearly established both during development and adulthood. Progress has been made in the understanding of the molecular mechanisms involved in the spatio-temporal specification of OB progenitors. Moreover, according to recent findings the OB interneuron lineage appears to be more complex than expected. The emerging view is that different OB interneuron phenotypes are generated at different ages and derive from distinct sets of progenitors early committed toward a specific fate. The transcription factors involved in the specification of different interneuron subtypes are starting to be unraveled. Nevertheless, the complete genetic programs controlling OB interneuron lineages and the relative contribution of cell autonomous mechanisms and environmental signaling to cell specification and integration in this system need to be further characterized. Considering the relevance of adult neural stem cells and their potential use in cell replacement therapy for brain repair, a deep understanding of these processes is of crucial importance.

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