Published in final edited form as:

Neuroscientist. 2008 June ; 14(3): 251–263. doi:10.1177/1073858408315026.

Charting Plasticity in the Regenerating Maps of the Mammalian Olfactory Bulb

DIANA M. CUMMINGS and LEONARDO BELLUSCIO

Developmental Neural Plasticity Unit, National Institute of Neurological Disorders and Stroke,
National Institutes of Health, Bethesda, Maryland

Abstract

The anatomical organization of a neural system can offer a glimpse into its functional logic. The basic premise is that by understanding how something is put together one can figure out how it works. Unfortunately, organization is not always represented purely at an anatomical level and is sometimes best revealed through molecular or functional studies. The mammalian olfactory system exhibits organizational features at all these levels including 1) anatomically distinct structural layers in the olfactory bulb, 2) molecular maps based upon odorant receptor expression, and 3) functional local circuits giving rise to odor columns that provide a contextual logic for an intrabulbar map. In addition, various forms of cellular plasticity have been shown to play an integral role in shaping the structural properties of most neural systems and must be considered when assessing each system's anatomical organization. Interestingly, the olfactory system invokes an added level of complexity for understanding organization in that it regenerates both at the peripheral and the central levels. Thus, olfaction offers a rare opportunity to study both the structural and the functional properties of a regenerating sensory system in direct response to environmental stimuli. In this review, we discuss neural organization in the form of maps and explore the relationship between regeneration and plasticity.

Keywords

Olfactory system; Map; Sensory neurons; Olfactory bulb; Intrabulbar map

Sensory Systems and Maps

Sensory systems serve to translate the external environment into neural representations and signals that allow organisms to survive. In each sensory modality the outside world is transposed onto the central nervous system via a series of maps that generally serve to refine information as it travels from a peripheral receptive field to the cortex. In the visual system specialized light-sensing neurons, the rods and cones, reside in the retina and are capable of detecting a single photon of light that is converted to electrical signals that are relayed through the thalamus and then transmitted to the primary visual cortex. Within the retina these rods and cones are arranged in a two-dimensional array where nearest neighbor relations are critical to faithfully transpose images of the external world so that they are accurately deciphered by the brain. The resulting map generated by the retina is then reproduced several times, first by retinal projections to the lateral geniculate nucleus (LGN) and then through LGN projections to the visual cortex. Similarly, in the auditory system tonotopic maps exist that transpose the

frequency map present in the cochlea to the auditory cortex. In the rodent somatosensory system, the whisker pads display a characteristic and stereotyped organization that is spatially transposed from the periphery to the brainstem. Information is transferred from vibrissae forming barrelettes in the trigeminal nucleus, to the bar-relloids in the thalamus, and finally to whisker barrels in the somatosensory cortex. In each case the organization of axonal projections preserves the spatial relationship of the peripheral whisker pads.

The visual and somatosensory systems serve to transfer information from an organism's physical space and self onto the nervous system in a way that preserves the spatial relationships of the stimuli. As chemosensation is typically used to detect volatile and/or solubilized chemical cues from the environment, one might not expect to find maps at the periphery of these sensory systems. Indeed, gustation and olfaction do not appear to exhibit stereotypic spatial maps at the periphery. One widely held model of taste coding has purported that taste receptors are broadly tuned across five modalities (bitter, sour, sweet, salty, and umami) that are organized into exclusive regions of the tongue to form a "tongue map." However, recent evidence suggests that although specific taste receptor cells do mediate single taste qualities in agreement with a "labeled line model," there is no strict "tongue map" because each modality is present in all taste regions of the tongue and palate (reviewed in Chandrashekar and others 2006). Similarly, the olfactory system presents a rudimentary form of organization at its periphery by broadly placing sensory neurons in a series of spatially distinct circumscribed zones. However, no evidence of a true epithelial map exists. In this review, we will examine the organization of the mammalian olfactory system and, in particular, the series of maps that arise from peripheral projections to the olfactory bulbs and within each bulb. We will also discuss the functional implications of these representations and explore how the olfactory system maintains its highly organized state within the context of ongoing neural regeneration and plasticity at multiple levels.

General Organization of the Olfactory System

The mammalian olfactory system is bilaterally symmetric with two independent nasal cavities each containing a sensory epithelium that sends information in an ipsilateral fashion directly to the olfactory bulbs (Fig. 1). Within each nasal cavity olfactory sensory neurons (OSNs) residing in the epithelial sheet send a ciliated dendritic knob into the nasal mucosa to detect odors. Odorant receptors are found within the ciliary membrane and, when activated by odorant stimuli, cause G-proteins to stimulate the production of cAMP via adenylate cyclase activity (reviewed in Pifferi and others 2006). Increased levels of cAMP open cyclic nucleotide-gated (CNG) channels in the cell's membrane and lead to nerve firing. OSN axons carry these impulses to the olfactory bulb (OB) where they form synapses within specialized regions of neuropil called glomeruli. Interestingly, the olfactory epithelium is a regenerating structure that continuously produces new OSNs throughout the life of an organism. This property is advantageous in that OSNs directly exposed to environmental insults can be replaced to maintain olfactory function. However, ongoing turnover in the receptor epithelium also creates an interesting organizational challenge because order must be preserved as old neurons are lost and replaced by new neurons.

OR Expression and Organization in the Epithelium

The cloning of odorant receptors (ORs) by Buck and Axel in the early 1990s provided a molecular logic to the organization and function of the mammalian olfactory system. These landmark studies demonstrated that a given OR is only expressed in a subset of OSNs typically confined to one of four broad zones in the epithelium (Buck and Axel 1991). Within each zone the expression of a given OR is essentially stochastic. Each OSN expresses only one of the ~1300 OR genes and the gene selected defines its function in response to odorants. Because

OSNs expressing the same OR are scattered within a zone, the organization of the olfactory system at its periphery appears to be loosely tied to a reproducible spatial pattern.

The Glomerular Map

The organization of OSN projections to the OB in relation to olfactory coding has captivated researchers for decades. Early electrophysiological studies (Adrian 1950) initially suggested that an “odotopy” exists on the surface of the bulb whereby different olfactory stimuli excite different regions. A more precise and functional glomerular map emerged with the advent of the 2-deoxyglucose (2-DG) uptake method (Sharp and others 1975; Stewart and others 1979). Studies using 2-DG added support to the concept of an odotopy on the OB and implicated glomeruli as functional units that respond to odorants in overlapping, but unique, combinations. Recently, the chemotopic organization of the OB has been thoroughly reviewed and analyzed (Johnson and Leon 2007), stressing the importance of glomerular activity patterns in odorant coding. Current techniques, including fMRI imaging, intrinsic signal imaging, and calcium-sensitive dyes generally confirm the results of earlier 2-DG studies.

The cloning of ORs introduced a new set of molecular tools for investigating olfactory system organization. Initial in situ hybridization studies demonstrated that OSNs scattered within a zone but expressing the same receptor project axons to only a few glomeruli within the same bulb (Ressler and others 1994; Vassar and others 1994). With access to OR sequences, gene targeting was used to label all of the OSNs that expressed the same OR (Mombaerts and others 1996). Researchers were then able to follow the axonal projections to the olfactory bulb and a very precise molecular map of glomeruli became clear. All OSNs expressing the same OR typically project to a set of four glomeruli with one on the medial and one on the lateral side of each bulb in a stereotyped fashion (Fig. 1). Thus, each pair of glomeruli has a molecular identity that is determined by the OR expressed in the enervating OSNs, and the ensemble of glomerular pairs forms two mirror-symmetric maps on the surface of each bulb.

How does the glomerular map form during development? Researchers have created transgenic mice in which ORs drive the expression of reporter genes (e.g. β -galactosidase [β -gal] or green-fluorescent protein [GFP]) in combination with standard immunohistochemical approaches to show that glomerular development occurs gradually. Protoglomeruli are first detected in the bulb during midgestation and continue to form postnatally with glomeruli increasing both in size and number progressively along an anterior to posterior gradient (Pomeroy and others 1990; Bailey and others 1999). It is interesting that OR gene expression appears to follow a very similar developmental time course. Some ORs, such as P2, are first expressed as early as embryonic day 13 (E13), whereas others do not appear until the postnatal period, for example, M71 and M72. Does the time of emergence of a given OR determine the position of its target glomerulus? In other words, do ORs expressed by OSNs that terminate in ventral glomeruli appear earlier in development than those that target dorsal glomeruli? If so, then the time course of OR appearance might contribute to the anterior-ventral to posterior-dorsal gradient of OB development. Based upon the developmental profiles of only a limited number of ORs and the axonal projections of their associated OSNs, it appears that developmental timing may indeed be playing a role in glomerular location. However, a comprehensive study is necessary to truly address this possibility.

In addition to the developmental timing of OR expression, many factors appear to play a role in the formation and/or maintenance of the glomerular map. OSNs express cell surface glycoproteins and carbohydrates, for example, that are known to interact with lectins and other possible receptors (reviewed in St. John and others 2002). Because broad regions of the MOE and OB express glycoproteins, these molecules are not likely to mediate the targeting of OSN axons to specific glomeruli. However, axons expressing the same glycoproteins appear to sort into bundles within the nerve layer (Key and Akeson 1993) and may be involved in the

fasciculation of OSN axons that express a given OR. Cell surface adhesion molecules are a second group implicated in the broad organization of OSN to OB projections. Recent studies suggest that certain cell surface molecules facilitate zone-to-zone topography rather than the specific targeting of a given glomerulus. For example, a member of the neural cell adhesion molecule (NCAM) family, OCAM, is expressed by OSNs in zones II, III, and IV and the corresponding zones of the OB, but not in zone I or the dorsomedial bulb (Mori and others 1985; Schwob and Gottlieb 1986; Yoshihara and others 1997).

Although certain adhesion molecules may underlie the zonal organization of the olfactory system, other families of molecules have been implicated in the global patterning of the OB itself. Experiments that examined the role of ephrins in the glomerular layer, for example, found that altering the expression levels of ephrin A gene subtypes can shift glomeruli along the anterior-posterior axis (Cutforth and others 2003). Interestingly, another set of molecules and their receptors, semaphorins and neuropilins, may be involved in patterning the dorsal-ventral axis of the glomerular layer (Schwartz and others 2000; Taniguchi and others 2003). A very recent investigation using mutant mice showed that the Slit family of axon guidance molecules and receptor, Robo-2, are important in the dorsal-ventral targeting of OSN axons from zone I (Cho and others 2007). This study revealed that these guidance cues are expressed in a dorsal-ventral gradient in the olfactory bulb and that deletion of any one of them results in a glomerular shift toward a more ventral position.

Although guidance molecules are popular candidates for leading axons to their targets, an unexpected hypothesis suggests that the OR itself plays an instructive role in determining the OSN axonal targets. Genetic studies used homologous recombination to substitute the coding region of one receptor for another in a given locus and examined the effect this had on axonal targeting (Mombaerts and others 1996; Wang and others 1998). Prior glomerular mapping studies had clearly demonstrated that the location of a given glomerulus is fairly invariant in control animals. Yet, when the OR coding region was swapped, there was a dramatic shift in the location of the resulting glomeruli to ectopic locations. Moreover, replacing the OR coding sequence with that of a simple marker such as β -gal completely abolished glomerular convergence within those neurons. Thus, the intriguing role of the OR, along with the above-mentioned guidance factors, suggests that an intricate interplay exists between multiple processes that result in “correct” glomerular targeting by OSN axons. This complex interaction makes it difficult to assign specific aspects of glomerular convergence to individual molecules.

The Odor Column

The glomerulus is the primary organizing center for processing odor information in the olfactory bulb and functions as a local communication hub for the multiple cell types involved in regulating information flow (Fig. 2A). OSNs send axons carrying olfactory information from the periphery to the glomerulus where they synapse directly with the output neurons of the bulb, the mitral and tufted cells. In addition, each glomerulus sends an intrabulbar projection to its partner on the opposite side of the bulb via external tufted cells (Fig. 3). A diverse population of interneurons, known as juxtaglomerular cells, surrounds each glomerulus and extends processes into the glomerulus forming synaptic connections with both the input and output neurons. Similarly, granule cells in the deep layers of the bulb further modulate information flow through synaptic connections with the lateral dendrites of the output neurons. Although the highly organized, laminar organization of the bulb is readily apparent in histological sections, more recent studies using pseudorabies viral tracing have confirmed the presence of a columnar organization that runs perpendicular to the surface of the bulb (Willhite and others 2006; see also Fig. 2B). Therefore, an odor column is composed of a glomerulus at its apex with its output and associated regulatory circuitry lying directly beneath and extending

through the granule cell layer at its base. This anatomical circuitry is at the center of many studies investigating plasticity and regeneration within the olfactory system.

The Olfactory Intrabulbar Map

Recent studies, along with earlier tract-tracing experiments, have determined that the mirror symmetric maps present on an olfactory bulb are linked to one another through a set of intrabulbar projections (Liu and Shipley 1994; Marks and others 2006; Schoenfeld and others 1985). These projections are mediated by external tufted cells (ETCs), which reside just beneath the glomerular layer and send axonal projections to the internal plexiform layer (IPL) beneath the isofunctional glomerulus on the opposite side of the bulb (Belluscio and others 2002; Lodovichi and others 2003). The result is a reciprocal connection between glomerular pairs that receive input from OSNs expressing the same OR (Fig. 3). Thus, these projections establish an intrabulbar map that links the two mirror symmetric glomerular representations to one another in a point-to-point fashion and may exist to allow the two halves of the bulb to coordinate their response to odorant stimuli.

How is the intrabulbar map established? The molecular basis behind the precise patterning of the intrabulbar map is currently unexplored. However, it is enticing to consider that the same factors that are responsible for organizing the glomerular map are extended through the odor column to help establish and fine-tune the specificity of intrabulbar projections. Such a mechanism would facilitate accurate formation of the entire glomerular circuitry by enabling appropriate presynaptic and postsynaptic components of a glomerulus to be drawn to the same spatial region of the bulb. This would enable the whole bulb to be patterned at once in a coordinated and synchronous manner with the odor column rather than the glomerulus serving as its broader functional unit.

The Role of Activity in Olfactory Map Formation

Glomerular Map: Odorant-Induced versus Spontaneous Activity

What role does activity play in olfactory map formation? Numerous studies have addressed this question with respect to the glomerular map. As mentioned above, a mature glomerular map consists of stereotypically arranged glomerular pairs that are homogeneously enervated by axons of a single OR type (see Fig. 1). Several studies have shown that heterogeneous and ectopic glomeruli are present in an immature map (Treloar and others 2002; Zou and others 2004; Kerr and Belluscio 2006). Furthermore, odorant-induced activity is involved in the fine-tuning of OSN axonal projections. In one study, olfactory deprivation during development via unilateral naris closure resulted in a prolonged persistence of heterogeneous glomeruli suggesting a lack of refinement (Zou and others 2004). Alternatively, enhancing levels of odorant stimulation with behavioral conditioning right after birth was shown to accelerate the refinement of glomeruli (Kerr and Belluscio 2006). Together, these results indicate that odorant-induced activity is critical for precise refinement of OSN axons to their glomerular targets (Fig. 4).

Other investigations exploring the role of odorant-induced activity in glomerular map formation have taken the strategy of manipulating OR signal transduction. Gene targeting was used to disrupt prominent olfactory signaling transduction components including: 1) the olfactory cyclic nucleotide gated channel (OCNC1), 2) the G-protein G_{olf} , and 3) adenylyl cyclase 3 (AC3) (Zheng and others 2000; Belluscio and others 1998; Col and others 2007; Zou and others 2007; Chesler and others 2007). Each of these mutations clearly resulted in anosmic mice. Axon targeting was also examined by determining the ability of OSNs expressing a given receptor (usually P2) to accurately form their characteristic pair of glomeruli in the bulb. Although these mutations often caused broad, general changes to the olfactory system (i.e.,

anosmia, reduced bulb size), the specific phenotype of the glomerular maps varied. Mutating the OCNC1 channel, for example, affects axonal convergence of glomeruli along the dorsal surface of the OB but not necessarily along the ventral aspect (Zheng and others 2000). Manipulating G_{olf} does not affect map formation, but preliminary evidence suggests that the maintenance of the glomerular map is altered (Belluscio and others 1998; Belluscio L, unpublished data, 1998). Interestingly, mutating AC3 seems to have the most severe effect on glomerular map formation, suggesting that it may be playing a broader role than transducing odorant-induced signals. This may not be so surprising, because AC3 can alter the levels of a broad second messenger such as cAMP, which is likely to influence other pathways including those responsible for axon guidance. Indeed, one recent study suggests that cAMP signaling is controlled by Gs activity in the axon terminals (Imai and others 2006), and that patterning of the glomerular map is based upon varying levels of cAMP, determined by the activity profile associated with each OR (Imai and others 2006). Still another laboratory has linked AC3 function to the expression of neuropilin, a more classical form of guidance molecule also found in primary OSNs (Col and others 2007). Thus, loss of odorant-induced activity resulting from these mutations has led to variable effects on the formation and maintenance of the glomerular map, and the way in which they interact is still being sorted out (see Table 1). Ongoing research is focused on the connections between components of the signal transduction pathway and additional downstream pathways.

What is the role of spontaneous activity on glomerular map formation? One group used gene targeting to impede all spontaneous activity in OSNs through two different approaches. First, they targeted the expression of the tetanus toxin light chain (TeTxLC) to the axon terminals of mature OSNs, resulting in the inhibition of both evoked and spontaneous vesicular release (Yu and others 2004). In this case there was no alteration in the glomerular map, because labeled P2 and MOR28 axons targeted their glomeruli appropriately. However, when release was selectively blocked in only a subset of neurons expressing a given OR, leaving all others functionally intact, the result was quite different. Indeed, glomerular convergence was disrupted in the silenced axons, suggesting that a competition-based mechanism may be involved in OSN axon targeting (see below). Using a second approach, this same group selectively increased the expression of an inward rectifying potassium channel (Kir2.1) in mature OSNs, severely diminishing their excitability. Interestingly, this approach to electrically silence mature OSNs by impeding their ability to fire action potentials resulted in a broad disruption of the glomerular map. This result indicated that at least a minimal level of electrical response throughout the OSN population is necessary to accurately establish a map (Yu and others 2004). Thus, although both the TeTxLC- and the Kir2.1-based strategies support an essential role for spontaneous activity in glomerular map formation, the more severe phenotype associated with reduced neural firing suggests that electrical competence may be playing a broader role in OSN development than simple synaptic communication.

What happens to the glomerular map when spontaneous activity is increased rather than reduced? A line of mice was created with enhanced olfactory activity through the targeted disruption of a different potassium channel (Kv1.3), expressed in mitral cells. The resulting mice exhibited a 10,000-fold lower detection threshold for odorants than control mice, and thus were termed “super-smellers” (Fadool and others 2004). These mice showed an increase in the total number of glomeruli, although typically smaller in size compared with control animals, and a normal convergence of P2 neurons. However, a recent and more thorough analysis of these mice has revealed that there is a differential effect on glomerular map organization such that refinement of some glomeruli is more severely affected than others (Biju and others 2008). Interestingly, the super-smellers typically exhibited fewer OSNs that expressed a given OR, whereas the density of OR proteins was sometimes greater.

Competition and Forming the Glomerular Map

What features of activity are important in the patterning of the bulb? Recent studies suggest that activity-dependent competition is much more critical in OB organization than the mere presence or absence of activity at a uniform level. In one study investigators used OCNC1 knockout (KO) mice similar to the ones previously used for anosmic studies. In these experiments, however, they took advantage of the fact that the OCNC1 gene is located on the X chromosome, creating a mosaic knockout in the epithelium of female mice (Zhao and Reed 2001). This enabled them to assess the effect of activity deprivation in a subpopulation of OSNs, although others maintained their ability to respond to odorants. The results clearly demonstrated that a competition mechanism was at play in the epithelium, because OSNs that were unable to respond to odorants decreased in number over time and were displaced by OSNs with the functional CNG channel. Interestingly, if naris occlusion was introduced in these animals, OSNs expressing either wild-type or mutant copies of the OCNC1 gene survived in equal numbers on the occluded side. This further suggested that the OCNC1 mutation was able to affect OSN survival through an activity-dependent mechanism.

Is there a molecular basis for activity-based competition associated with OSN axon targeting? OSN axons face at least two challenges upon reaching the bulb. First, they must sort and join bundles of axons expressing the same OR and penetrate the inner nerve layer. Second, they must form and reinforce synapses within appropriate glomeruli and eliminate those that have targeted inappropriately. Although it is still unclear how this process occurs, several recent studies have pointed to candidate factors that appear to be involved. One study identified a set of adhesion molecules known as Kirrels that are expressed in a varied pattern throughout the epithelium such that expression of a given OR can be associated with a given Kirrel protein (Serizawa and others 2006). Interestingly, odorant-induced activity is shown to directly influence expression levels of these Kirrel proteins, suggesting that they may be involved in activity-dependent OSN axon sorting. A more recent study explored the effect of activity-based competition on the pruning of OSN axonal branches within glomeruli and found a direct association between odorant-induced activity and synaptic remodeling (Cao and others 2007). This study revealed that the process that eliminates OSN axonal branches is directly dependent upon competition for brain-derived neurotrophic factor (BDNF) such that a decrease in BDNF rescues inactive arbors from elimination. Furthermore, this process is mediated through a p75 signaling mechanism. Each of these studies suggests that OSNs may follow two main dogmas of activity-dependent wiring of neural systems: 1) that “neurons that fire together wire together,” supported by a number of studies in the visual system; and 2) that competition for a limiting supply of trophic factor can be used to selectively refine connections such as occurs in the neuromuscular junction. Thus, the interplay between activity and cellular signaling in a competitive environment is probably critical to glomerular map formation.

Intrabulbar Map: A Greater Activity Dependence

The accuracy of intrabulbar projections is directly dependent upon odorant-induced activity. One study exploring the formation of the intrabulbar map has clearly shown that activity plays a much greater role in its formation and maintenance than it does for the glomerular map (Marks and others 2006). During the first postnatal week the intrabulbar axons target the opposite side of the bulb in a spatially broad manner (Fig. 4). As the bulb matures, the intrabulbar projections gradually refine to glomerular precision by postnatal week 7. Interestingly, this refinement is activity dependent not only during formation, but also for maintenance such that loss of odorant-induced activity not only prevents refinement but causes a broadening of axonal projections that had previously refined (Fig. 4). Moreover, activity can either increase or decrease the rate of intrabulbar map refinement, because exposure to odorants accelerates refinement of those projections associated with the activated glomeruli. Unlike most sensory systems, this activity-dependent plasticity is not limited to a critical period but

continues into adulthood, requiring activity for anatomical maintenance of this map. These data suggest that the intrabulbar and glomerular maps may be intimately linked both anatomically and functionally such that their emergent organization is interdependent.

Regeneration within the Odor Column

The SVZ: A Source of New Neurons for the Olfactory Bulb

During late embryonic and early postnatal development there is a tremendous level of neural proliferation that occurs both within the peripheral olfactory epithelium and in the subventricular zone (SVZ), the central source of new neurons for the OB. Recently, great strides have been made concerning the SVZ and the process of neuronal replenishment that takes place throughout life in the olfactory bulb. Although the SVZ was identified as a source of dividing cells that migrate into the adult OB as early as the 1960s (Altman 1969; Kaplan and Hinds 1977), and subsequent studies have demonstrated the capacity of these cells to differentiate and integrate into bulb's neural circuitry as interneurons (Lois and Alvarez-Buylla 1994; Lois and others 1996; Luskin 1993), the mechanisms that govern this process still remain unclear (Fig. 5A, B).

A recent focus of investigation concerns the susceptibility of these young neurons to changes in levels of afferent activity. Studies have shown that a reduction in odorant-induced activity via naris closure causes a substantial decrease in the survival of interneuron populations (reviewed in Brunjes 1994). Further work has demonstrated that activity is critical during weeks 2 to 8 of an emerging olfactory bulb interneuron and that 50% of newborn neurons die during this critical time (Petreanu and Alvarez-Buylla 2002; Winner and others 2002; Yamaguchi and Mori 2005). Surprisingly, however, a recent study showed that enhancing the olfactory stimulation through an olfactory discrimination task resulted in greater turnover of new neurons in regions of the bulb that were not stimulated by the olfactory cues associated with the learning task (Alonso and others 2006). Perhaps a "use it or lose it" mechanism of survival exists among new olfactory bulb interneurons where stimulation of particular regions provides trophic support for neural cells already participating in the circuitry that results in less turnover in these active areas.

Cell Turnover within the Odor Column

New neurons entering the bulb from the rostral migratory stream (RMS) are not inserted randomly as interneurons into the glomerular or granule cell layers; rather they are carefully integrated into the organized circuitry of an odor column. Most (~90%) newly generated cells in the adult OB become granule cells, the most numerous interneuron cell type in the bulb (Hack and others 2005). Periglomerular (PG) cells make up the second population of interneurons and include a diverse population of GABAergic (~50%), calretinin-containing (~30%), dopamine-containing (13%), and calbindin-containing (~10%) neurons (Parrish-Aungst and others 2007; Whitman and Greer 2007). When researchers examined PG cell turnover using bromodeoxyuridine (BrdU)-based assays, they found that the different PG cell types were added in generally the same proportions that exist in the overall population of the bulb (Whitman and Greer 2007; see also Fig. 5C-F).

Neural activity has clearly been shown to affect survival of various cell types within the layers of the olfactory bulb (Brunjes 1994). The most dramatic effects associated with changes in odorant-induced activity occur in the granule cell population. However, there are clear effects within the PG cell population as well. The tyrosine hydroxylase (TH)-positive PG cells are perhaps the most extensively characterized PG cell type regarding activity-dependent modulation. Early studies have shown TH expression in these cells to be highly dependent and

directly correlated with odorant-induced activity (Baker and others 1983). However, because PG cells turn over, it is unclear whether TH neurons die or simply turn off the TH gene.

Does odorant-induced activity play an instructive role on neurons as they are migrating through the RMS to take residence in the OB? Because the bulb receives direct sensory input from OSNs, perhaps this environmental stimulation influences the migration and fate of new neurons through the activation of distinct odor columns. This notion is consistent with the activity patterns observed in functional studies that show that individual odorants typically activate only certain regions of the bulb and, consequently, distinct odor columns. Thus, only the circuitry within those regions would be affected by a given stimulus, enabling reorganization within specific odor columns through selective odor experiences.

Regeneration and Plasticity Working Together

The olfactory system has two unique attributes: 1) unusual regenerative properties with neural stem cells incorporated into both the sensory sheet and the olfactory bulb throughout adulthood, and 2) continual plasticity of its neural circuitry related to alterations in the stimulus environment. We propose that the convergence of these two properties is not a coincidence but rather that they coevolved to work together in a coordinated manner to produce a neural network with such extraordinary plasticity. Furthermore, we suggest that regeneration itself creates a molecular environment in the bulb that promotes plasticity and sensitizes the neural circuitry to activity-dependent alterations.

Sensory systems like the visual and somatosensory systems are all defined by a maturation period of heightened plasticity referred to as a “critical period.” Critical periods are transient windows of time that usually follow a stage of initial patterning during which imprecise neural connections are refined in an activity-dependent manner. For most sensory systems there is little evidence for anatomical reorganization after these critical windows close. By contrast, in the olfactory system several lines of research suggest a lack of a critical period in establishing its organization and indicate that ongoing plasticity prevails in maintaining the circuitry of the olfactory bulb (Cummings and others 1997; Marks and others 2006).

Why does the olfactory system possess such high levels of regeneration? Mammals are born with a repertoire of receptors capable of recognizing thousands of different odorants, including those that may never be encountered and those that are crucial for survival. Thus, the olfactory system is faced with a formidable challenge in determining how to maintain a system that is both broad in its detection spectrum and highly sensitive at the same time. Perhaps chemosensation adapted to such a challenge by using the natural interplay between plasticity and regeneration that is present in most mammals. In the epithelium an obvious explanation is that sensory neurons are directly exposed to the environment and thus are highly prone to damage so they must be replaced to maintain sensitivity to odorants. This explanation, however, does not directly account for the regeneration observed in the olfactory bulb. One possibility is that a certain level of regeneration or plasticity must be maintained within the bulb simply to compensate or adjust to the ongoing turnover of OSN axons within each glomerulus that would necessitate continuous synaptic reorganization. A second possibility is that given the importance of the olfactory system for survival in many species, regeneration imparts a certain level of inherent flexibility by enabling broad alterations in the wiring of the system. As a result a species could quickly adapt and adjust their sensitivity to new odorants. This would allow the olfactory system the potential to expand or contract different populations of cells as well as their connections in response to environmental conditions and needs.

Similar forms of plasticity and regeneration may be present in other systems. Because neural cell regeneration and replacement has only recently become generally accepted in the olfactory system, perhaps prolonged plasticity at some level also exists in other sensory systems as well.

For example, recent studies in the visual system revealed that certain features, for example, orientation selectivity and contrast sensitivity, can fully recover in dark-reared ferrets once visual experience was initiated (Li and others 2006). Furthermore, in contrast to the irreversible effects of monocular deprivation on ocular dominance during the critical period, binocular deprivation through dark exposure in adult rats reinstates ocular dominance plasticity (He and others 2007). Other instances of adult brain plasticity have been shown using high-resolution two-photon microscopy to examine structural remodeling of dendritic spines in the mouse barrel cortex both in response to sensory-induced activity and deprivation (Trachtenberg and others 2002; Zuo and others 2005). Taken together, these examples suggest that adult plasticity does exist in other sensory systems, at least to a certain degree. Perhaps, through the use of new live-imaging technologies combined with advances in cell-labeling and tracking techniques, investigators may reveal other areas of CNS plasticity that were previously assumed to be hard-wired following development.

Conclusion

We have discussed the mammalian olfactory system in light of its organization as a series of spatial maps that are generally believed to be the basis for olfactory coding. Although the mechanisms that underlie their precise formation and maintenance are still unclear, a growing body of data suggests that they emerge from a complex interplay of molecular and functional factors. One prominent and rather unique feature of this system is its ability to regenerate both at the peripheral and the central level, which we believe greatly facilitates its ability to reorganize itself. Thus, it is this rare set of attributes combined with its role in communicating with the outside world that make the olfactory system ideal for studying the interaction between development, sensory input, and regeneration.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institutes of Health—National Institute of Neurological Disorders and Stroke. We thank Beth Belluscio, Josh Bagley, and Rory McQuinston for helpful discussions and comments on the manuscript.

References

- Adrian ED. The electrical activity of the mammalian olfactory bulb. *Electroencephalogr Clin Neurophysiol* 1950;2(4):377–88. [PubMed: 14793507]
- Alonso M, Viollet C, Gabellec MM, Meas-Yedid V, Olivo-Marin JC, Lledo PM. Olfactory discrimination learning increases the survival of adult-born neurons in the olfactory bulb. *J Neurosci* 2006;26(41):10508–13. [PubMed: 17035535]
- Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol* 1969;137(4):433–57. [PubMed: 5361244]
- Bailey MS, Puche AC, Shipley MT. Development of the olfactory bulb: evidence for glia-neuron interactions in glomerular formation. *J Comp Neurol* 1999;415(4):423–48. [PubMed: 10570454]
- Baker H, Cummings DM, Munger SD, Margolis JW, Franzen L, Reed RR, et al. Targeted deletion of a cyclic nucleotide-gated channel subunit (OCNC1): biochemical and morphological consequences in adult mice. *J Neurosci* 1999;19(21):9313–21. [PubMed: 10531436]
- Baker H, Kawano T, Margolis FL, Joh TH. Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat. *J Neurosci* 1983;3(1):69–78. [PubMed: 6130133]
- Belluscio L, Gold GH, Nemes A, Axel R. Mice deficient in G(olf) are anosmic. *Neuron* 1998;20(1):69–81. [PubMed: 9459443]
- Belluscio L, Lodovichi C, Feinstein P, Mombaerts P, Katz LC. Odorant receptors instruct functional circuitry in the mouse olfactory bulb. *Nature* 2002;419(6904):296–300. [PubMed: 12239567]

- Biju KC, Marks DR, Mast TG, Fadool DA. Deletion of voltage-gated channel affects glomerular refinement and odorant receptor expression in the mouse olfactory system. *J Comp Neurol* 2008;506(2):161–79. [PubMed: 18022950]
- Brunjes PC. Unilateral naris closure and olfactory system development. *Brain Res Brain Res Rev* 1994;19(1):146–60. [PubMed: 8167658]
- Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 1991;65(1):175–87. [PubMed: 1840504]
- Cao L, Dhilla A, Mukai J, Blazeski R, Lodovichi C, Mason CA, et al. Genetic modulation of BDNF signaling affects the outcome of axonal competition in vivo. *Curr Biol* 2007;17(11):911–21. [PubMed: 17493809]
- Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. *Nature* 2006;444(7117):288–94. [PubMed: 17108952]
- Chesler AT, Zou DJ, Le Pichon CE, Peterlin ZA, Matthews GA, Pei X, et al. A G protein/cAMP signal cascade is required for axonal convergence into olfactory glomeruli. *Proc Natl Acad Sci U S A* 2007;104(3):1039–44. [PubMed: 17215378]
- Cho JH, Lepine M, Andrews W, Parnavelas J, Cloutier JF. Requirement for Slit-1 and Robo-2 in zonal segregation of olfactory sensory neuron axons in the main olfactory bulb. *J Neurosci* 2007;27(34):9094–104. [PubMed: 17715346]
- Col JA, Matsuo T, Storm DR, Rodriguez I. Adenylyl cyclase-dependent axonal targeting in the olfactory system. *Development* 2007;134(13):2481–9. [PubMed: 17537788]
- Cummings DM, Henning HE, Brunjes PC. Olfactory bulb recovery after early sensory deprivation. *J Neurosci* 1997;17(19):7433–40. [PubMed: 9295389]
- Cutforth T, Moring L, Mendelsohn M, Nemes A, Shah NM, Kim MM, et al. Axonal ephrin-As and odorant receptors: coordinate determination of the olfactory sensory map. *Cell* 2003;114(3):311–22. [PubMed: 12914696]
- Fadool DA, Tucker K, Perkins R, Fasciani G, Thompson RN, Parsons AD, et al. Kv1.3 channel gene-targeted deletion produces “super-smeller mice” with altered glomeruli, interacting scaffolding proteins, and biophysics. *Neuron* 2004;41(3):389–404. [PubMed: 14766178]
- Farbman AI, Brunjes PC, Rentfro L, Michas J, Ritz S. The effect of unilateral naris occlusion on cell dynamics in the developing rat olfactory epithelium. *J Neurosci* 1988;8(9):3290–5. [PubMed: 2459323]
- Hack MA, Saghatelian A, de Chevigny A, Pfeifer A, Ashery-Padan R, Lledo PM, et al. Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci* 2005;8(7):865–72. [PubMed: 15951811]
- He HY, Ray B, Dennis K, Quinlan EM. Experience-dependent recovery of vision following chronic deprivation amblyopia. *Nat Neurosci* 2007;10(9):1134–6. [PubMed: 17694050]
- Imai T, Suzuki M, Sakano H. Odorant receptor-derived cAMP signals direct axonal targeting. *Science* 2006;314(5799):657–61. [PubMed: 16990513]
- Johnson BA, Leon M. Chemotopic odorant coding in a mammalian olfactory system. *J Comp Neurol* 2007;503(1):1–34. [PubMed: 17480025]
- Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* 1977;197(4308):1092–4. [PubMed: 887941]
- Kerr MA, Belluscio L. Olfactory experience accelerates glomerular refinement in the mammalian olfactory bulb. *Nat Neurosci* 2006;9(4):484–6. [PubMed: 16547509]
- Key B, Akeson RA. Distinct subsets of sensory olfactory neurons in mouse: possible role in the formation of the mosaic olfactory projection. *J Comp Neurol* 1993;335(3):355–68. [PubMed: 8227525]
- Li Y, Fitzpatrick D, White LE. The development of direction selectivity in ferret visual cortex requires early visual experience. *Nat Neurosci* 2006;9(5):676–81. [PubMed: 16604068]
- Liu WL, Shipley MT. Intrabulbar associational system in the rat olfactory bulb comprises cholecystokinin-containing tufted cells that synapse onto the dendrites of GABAergic granule cells. *J Comp Neurol* 1994;346(4):541–58. [PubMed: 7983243]
- Lodovichi C, Belluscio L, Katz LC. Functional topography of connections linking mirror-symmetric maps in the mouse olfactory bulb. *Neuron* 2003;38(2):265–76. [PubMed: 12718860]

- Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. *Science* 1994;264(5162):1145–8. [PubMed: 8178174]
- Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. *Science* 1996;271(5251):978–81. [PubMed: 8584933]
- Luskin MB. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* 1993;11(1):173–89. [PubMed: 8338665]
- Marks CA, Cheng K, Cummings DM, Belluscio L. Activity-dependent plasticity in the olfactory intrabulbar map. *J Neurosci* 2006;26(44):11257–66. [PubMed: 17079653]
- Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, et al. Visualizing an olfactory sensory map. *Cell* 1996;87(4):675–86. [PubMed: 8929536]
- Mori K, Fujita SC, Imamura K, Obata K. Immunohistochemical study of subclasses of olfactory nerve fibers and their projections to the olfactory bulb in the rabbit. *J Comp Neurol* 1985;242(2):214–29. [PubMed: 2418070]
- Parrish-Aungst S, Shipley MT, Erdelyi F, Szabo G, Puche AC. Quantitative analysis of neuronal diversity in the mouse olfactory bulb. *J Comp Neurol* 2007;501(6):825–36. [PubMed: 17311323]
- Petreanu L, Alvarez-Buylla A. Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J Neurosci* 2002;22(14):6106–13. [PubMed: 12122071]
- Pifferi S, Boccaccio A, Menini A. Cyclic nucleotide-gated ion channels in sensory transduction. *FEBS Lett* 2006;580(12):2853–9. [PubMed: 16631748]
- Pomeroy SL, LaMantia AS, Purves D. Postnatal construction of neural circuitry in the mouse olfactory bulb. *J Neurosci* 1990;10(6):1952–66. [PubMed: 2355260]
- 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(7):1245–55. [PubMed: 7528109]
- Schoenfeld TA, Marchand JE, Macrides F. Topographic organization of tufted cell axonal projections in the hamster main olfactory bulb: an intrabulbar associational system. *J Comp Neurol* 1985;235(4):503–18. [PubMed: 2582006]
- Schwartz GA, Kostek C, Ahmad N, Dibble C, Pays L, Puschel AW. Semaphorin 3A is required for guidance of olfactory axons in mice. *J Neurosci* 2000;20(20):7691–7. [PubMed: 11027230]
- Schwob JE, Gottlieb DI. The primary olfactory projection has two chemically distinct zones. *J Neurosci* 1986;6(11):3393–404. [PubMed: 3772438]
- Serizawa T, Sawada T, Kitayama T. Peptide motifs that recognize differences in polymer-film surfaces. *Angew Chem Int Ed Engl* 2006;46(5):723–726. [PubMed: 17154206]
- Sharp FR, Kauer JS, Shepherd GM. Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. *Brain Res* 1975;98(3):596–600. [PubMed: 1182541]
- St John JA, Clarris HJ, Key B. Multiple axon guidance cues establish the olfactory topographic map: how do these cues interact? *Int J Dev Biol* 2002;46(4):639–47. [PubMed: 12141452]
- Stewart WB, Kauer JS, Shepherd GM. Functional organization of rat olfactory bulb analysed by the 2-deoxyglucose method. *J Comp Neurol* 1979;185(4):715–34. [PubMed: 447878]
- Taniguchi M, Nagao H, Takahashi YK, Yamaguchi M, Mitsui S, Yagi T, et al. Distorted odor maps in the olfactory bulb of semaphorin 3A-deficient mice. *J Neurosci* 2003;23(4):1390–7. [PubMed: 12598627]
- Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 2002;420(6917):788–94. [PubMed: 12490942]
- Treloar HB, Feinstein P, Mombaerts P, Greer CA. Specificity of glomerular targeting by olfactory sensory axons. *J Neurosci* 2002;22(7):2469–77. [PubMed: 11923411]
- Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R. Topographic organization of sensory projections to the olfactory bulb. *Cell* 1994;79(6):981–91. [PubMed: 8001145]
- Wang F, Nemes A, Mendelsohn M, Axel R. Odorant receptors govern the formation of a precise topographic map. *Cell* 1998;93(1):47–60. [PubMed: 9546391]
- Whitman MC, Greer CA. Adult-generated neurons exhibit diverse developmental fates. *Dev Neurobiol* 2007;67(8):1079–93. [PubMed: 17565001]

- Willhite DC, Nguyen KT, Masurkar AV, Greer CA, Shepherd GM, Chen WR. Viral tracing identifies distributed columnar organization in the olfactory bulb. *Proc Natl Acad Sci U S A* 2006;103(33):12592–7. [PubMed: 16895993]
- Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG. Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur J Neurosci* 2002;16(9):1681–9. [PubMed: 12431220]
- Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, Gaggar A, et al. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* 2000;27(3):487–97. [PubMed: 11055432]
- Yamaguchi M, Mori K. Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. *Proc Natl Acad Sci U S A* 2005;102(27):9697–702. [PubMed: 15976032]
- Yoshihara Y, Kawasaki M, Tamada A, Fujita H, Hayashi H, Kagamiyama H, et al. OCAM: a new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons. *J Neurosci* 1997;17(15):5830–42. [PubMed: 9221781]
- Yu CR, Power J, Barnea G, O'Donnell S, Brown HE, Osborne J, et al. Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. *Neuron* 2004;42(4):553–66. [PubMed: 15157418]
- Zhao H, Reed RR. X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system. *Cell* 2001;104(5):651–60. [PubMed: 11257220]
- Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P. Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. *Neuron* 2000;26(1):81–91. [PubMed: 10798394]
- Zou DJ, Chesler AT, Le Pichon CE, Kuznetsov A, Pei X, Hwang EL, et al. Absence of adenylyl cyclase 3 perturbs peripheral olfactory projections in mice. *J Neurosci* 2007;27(25):6675–83. [PubMed: 17581954]
- Zou DJ, Feinstein P, Rivers AL, Mathews GA, Kim A, Greer CA, et al. Postnatal refinement of peripheral olfactory projections. *Science* 2004;304(5679):1976–9. [PubMed: 15178749]
- Zuo Y, Yang G, Kwon E, Gan WB. Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex. *Nature* 2005;436(7048):261–5. [PubMed: 16015331]

Fig. 1.

Organization of the mammalian olfactory system. (A) Schematic shows a frontal view of a section through the nasal cavity which is roughly subdivided into four zones. Olfactory sensory neurons (OSNs) expressing a given odorant receptor (OR) are generally found within a zone and project axons to one of two glomeruli in the corresponding zone of the olfactory bulb (OB). Typically, one isofunctional glomerulus is located on the medial side and the other is positioned on the lateral side of the same OB. Thus, splitting the OB along the sagittal axis would effectively divide the bulb into two mirror-image representations of the glomerular map. (B) A flattened representation of the bulb shows a bilaterally symmetric glomerular map at the surface mediated by OSN axons within the context of the OB layers. GL = glomerular layer; EPL = external plexiform layer; MCL = mitral cell layer; IPL = internal plexiform layer; GCL = granule cell layer. (C) Schematic of the organization and cells within the main olfactory epithelium (MOE). Within the basal cell layer horizontal and globose basal cells serve as stem cells that divide and differentiate into immature OSNs. Once these immature OSN axons form synaptic contacts within a glomerulus and send a ciliated, dendritic knob into the nasal cavity, they are characterized as mature OSNs. Odorants bind to ORs on cilia that extend from these knobs and initiate a G-protein–coupled signaling cascade. Sustentacular cells form the most superficial layer in the MOE and provide support for the OSNs.

Fig. 2.

The odor column. (A) Schematic illustrates the three broad cell types that make up the odor column. Input from axons of olfactory sensory neurons (OSNs) that express the same odorant receptor (OR; green) synapse onto mitral and tufted cell dendrites (red and yellow, respectively) within the same glomerulus. These projection neurons carry information out of the odor column. Interneurons (blue) consist of two main categories: 1) periglomerular cells that modulate information transfer in the glomerular layer (GL), and 2) granule cells that are positioned in the granule cell layer (GCL) and send dendrites apically into the external plexiform layer (EPL) to form synaptic contacts with the lateral dendrites of mitral cells (red). One subtype of projection neurons, tufted cells (yellow), connect “isofunctional columns” by projecting their axons across the olfactory bulb (OB) to the internal plexiform layer (IPL) below the isofunctional glomerulus. (B) One way to visualize the columnar organization of the OB is to inject a small amount of fluorescent tracer into the superficial layers of the bulb. This confocal image of a coronal section through the OB illustrates cells that took up the dye (tetramethylrhodamine) after it was iontophoretically injected into the superficial EPL.

Fig. 3.

The intrabulbar map. (A) A cartoon of the olfactory bulb (OB) depicting reciprocal intrabulbar projections that link the two symmetric halves of the glomerular map in a spatially specific manner. (B) A schematic of the cellular circuitry that connects isofunctional odor columns within the same bulb to produce the intrabulbar map. Tufted cells that send dendrites to a glomerulus on the medial side project axons across the OB to terminate in the internal plexiform layer (IPL) below the lateral isofunctional glomerulus.

Fig. 4. Plasticity in the olfactory bulb maps. (A) Schematic illustrates that in the newborn olfactory system, olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) (green) send axons to multiple, nonhomogeneous glomeruli on both the medial and the lateral aspects of the olfactory bulb (OB). Tufted cells (red) that extend dendrites to these glomeruli send axons across the OB and terminate in a broad region of the internal plexiform layer (IPL). The nonhomogeneous innervation of glomeruli by OSN axons and broad termination pattern of tufted cell projections are characteristic of immature glomerular and intrabulbar maps. (B) In the adult OB, OSN axons that express a given OR converge onto approximately two homogeneous glomeruli, forming a mature glomerular map. The intrabulbar map follows this developmental refinement such that tufted cell axons terminate in a restricted region of the IPL below isofunctional glomeruli. (C) Odor deprivation for extended periods beginning during development prevents the glomerular map from refining and nonhomogeneous glomeruli persist during adulthood. The intrabulbar map also changes in that tufted cell projections broaden in response to reduced activity following naris closure. Interestingly, this broadening

does not only occur during a development critical period, but also takes place in adulthood after the intrabulbar map had already refined itself.

Fig. 5.

Centrally derived regeneration of olfactory bulb neurons. (A) This schematic highlights the migration path of subventricular zone (SVZ) neurons through the rostral migratory stream (RMS) to the olfactory bulb (OB). GL = glomerular layer; MCL = mitral cell layer; GCL = granule cell layer; AOB = anterior olfactory bulb. (B) Inset panel: New neurons join the mature olfactory bulb as either periglomerular or granule cells. (C–F) Confocal images showing examples of new cells added to the adult OB as visualized through dual immunofluorescence for bromodeoxyuridine (BrdU; green) and one of several markers for classes of periglomerular (PG) neurons (red). Arrows point to double-labeled cells in sections processed for BrdU and tyrosine hydroxylase (TH; C), calbindin (D), or calretinin (E). (F) Cells in the granule cell layer are also dual-labeled for BrdU and calretinin. Scale bar = 50 μ m.

Table 1

Effects of Activity Manipulations on Olfactory Maps

Manipulation	Epithelium	Glomerular Map	Intrabulbar Map
Naris occlusion	Reduces thickness ^a	Prevents refinement ^b	Prevents refinement ^c
Odor enrichment	No known effect	Accelerates refinement ^d	Accelerates refinement ^c
OCNC1-KO	Reduces thickness ^e	Varied effects on formation ^f	Prevents refinement ^c
G _{olf} -KO	Mild effects ^g	Affects maintenance ^g	N/A
AC3-KO	Mild effects ^h	Varied effects on formation ^{i,j,k}	N/A
Kir2.1 mutation	Effects OR expression ^l	Affects formation and maintenance ^l	N/A
Kv1.3 mutation	Reduces OSN number ^m	Affects formation ^{m,n}	N/A

OCNC1 = olfactory cyclic nucleotide gated channel subunit; KO = knockout.

^aFarbman and others 1988;

^bZou and others 2004;

^cMarks and others 2006;

^dKerr and Belluscio 2006;

^eBaker and others 1999;

^fZheng and others 2000;

^gBelluscio and others 1998;

^hWong and others 2000;

ⁱChesler and others 2007;

^jCol and others 2007;

^kZou and others 2007;

^lYu and others 2004;

^mBiju and others 2008; and

ⁿFadool and others 2004.