Odor perception and olfactory bulb plasticity in adult mammals

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Abstract

The adult mammalian olfactory bulb is unique in that olfactory sensory neurons project directly, without prior thalamic relay, to the olfactory bulb. This review discusses evidence for the direct involvement of the olfactory bulb in odor perception and its modulation by olfactory experience. We first discuss recent data showing that the olfactory bulb exhibits a high level of plasticity in response to olfactory experience including exposure, enrichment and learning. We next review evidence showing that in return, experimental manipulation of the olfactory bulb neural network changes how odorants are processed and perceived. We finally review in more detail a few experiments showing a tight correlation between the modulation of olfactory bulb neural processing and odor perception. We argue that the olfactory bulb has evolved to be an adapting network, allowing animals to adjust olfactory computations to changing environments.
1- Introduction

Sensory processing involves a hierarchy of interconnected sensory, cortical and sub-cortical areas. When discussing these hierarchies and interactions, olfactory processing is often set apart due to the lack of direct thalamic pathways between sensory and cortical processing areas. Indeed, olfactory signals, after being received and transformed by sensory neurons, are projected directly to an anatomically well studied cortical structure, the olfactory bulb, with projections to the thalamus established further downstream of the pathway. A recent review has compared the olfactory bulb to the thalamus in its functional signal processing properties (Kay and Sherman 2007) and discussed how both structures act as early processing stages in which cortical and modulatory feedback can substantially shape sensory representations. Here, we review recent evidence showing that indeed, the olfactory bulb is more than a relay station or a feedforward filter but rather actively shapes, and is actively shaped by, olfactory perception, a notion introduced more than 20 years ago by Freeman and colleagues (Freeman and Schneider, 1982). A crucial component of this function are the central projections to the olfactory bulb, including cortical, sub-cortical and modulatory projections (reviewed in Shipley and Ennis 1996); (Figure 1). We discuss data showing how olfactory experience changes the olfactory bulb network and how manipulations of the olfactory bulb network change odor perception on several time scales. We finally review in more detail a few experiments showing a tight correlation between the modulation of olfactory bulb neural activity and odor perception. Literature pertaining to neonatal olfactory learning has been intensely and clearly reviewed elsewhere (McLean and
Harley 2004; Sullivan and Dryer 1996), hence this review will focus on adult odor perception.

2- Olfactory experience modulates the olfactory bulb neural network

The olfactory bulb, which is the first cortical relay of the olfactory pathway, presents a high level of plasticity in response to olfactory experience including short term exposure, enrichment and associative learning (Figure 2A). Indeed, a simple manipulation of olfactory experience, such as exposure to odorants, can modify the bulbar neural network and more specifically the odor response patterns of bulbar output neurons (Buonviso and Chaput 2000). For example, a short-term (20 minute) exposure of adult rats to an odorant, in the absence of any paired reinforcement, reduced the subsequently recorded proportion of mitral cells responding to odorants with increased firing rates, while increasing the proportion of mitral cells that were inhibited in response to odors (Buonviso and Chaput 2000). Surprisingly, the proportion of excitatory responses to odors was decreased not only to the exposure odor but also to other novel odors. Moreover, it has been shown that even in the anesthetized rat, less than a minute exposure to an odorant can fine-tune the receptive field of mitral cells (Fletcher and Wilson 2003) and that a simple odor exposure modifies olfactory bulb circuit and mitral cell single-unit responses to subsequent odor presentation (Kay and Laurent 1999; Spors and Grinvald 2002). In response to associative learning, it has been observed that after giving birth, female sheep develop a selective recognition for their lamb accompanied by an increase of the number of mitral cells in the olfactory bulb that respond to lamb odor (Kendrick et al. 1992). Early work by Freeman and colleagues showed that the dynamics of bulbar odor responses are modulated by associative learning and that partially
distributed olfactory bulb activity correlates with odor specific behavioral responding (see for example Freeman and Schneider, 1982, Grajski and Freeman, 1989). Aversive conditioning of odor stimuli in adult rabbits induced changes in their bulbar electroencephalogram map (Gray et al. 1986), and in rats, odor-reward conditioning modulates LFP oscillations (Beshel et al. 2007; Kay et al. 1996; Martin et al. 2004; Martin et al. 2006). Modifications of the bulbar network following exposure or experience can also be observed using immediate early gene (IEG) like c-fos, arg 3.1 or zif268 (expression modulated by sensory input and plasticity) mapping. Odor exposure induces a specific increase in c-fos and arg 3.1 expressions in some particular olfactory bulb quadrants. Previous familiarization with the test odor results in a decreased expression of both IEGs in these quadrants, leading to the alteration of the odor-specific pattern of c-fos and arg 3.1 expression (Montag-Sallaz and Buonviso 2002), whereas enrichment during ten days induces an increase in the number of Zif268-positive cells in response to an odorant in the granule cell layer as well as modulates activation patterns in the glomerular cell layer (Mandairon et al. 2008a; Woo et al. 2007).

The inhibitory neurons in the olfactory bulb, which regulate the activity of mitral cells, are continuously generated in adulthood. Precursors proliferate in the sub-ventricular zone of lateral ventricles and then newly generated cells migrate along the rostral migratory stream to reach the olfactory bulb (Temple and Alvarez-Buylla 1999). Once in the olfactory bulb, they migrate radially towards more external layers and acquire characteristics of mature granule and periglomerular interneurons (Lois and Alvarez-Buylla 1994; Petreanu and Alvarez-Buylla 2002). These newborn cells integrate into the neuronal network and modulate olfactory processing (Carleton et al. 2003). Current experiments show that sensory deprivation decreases the survival of newborn cells in the olfactory bulb (Mandairon et al.
2003; Mandairon et al. 2006c), whereas odor enrichment enhances the survival of newborn cells (Rochefort et al. 2002) and modifies the responses of adult-born neurons to odorants (Magavi et al. 2005). Similarly, the acquisition of an olfactory discrimination task also modulates the survival of newborn granule cells in an odor and task specific manner ((Alonso et al. 2006; Mandairon et al. 2006b); Figure 2B).

In summary, most recent data on olfactory bulb network changes due to olfactory experience suggest that experience modulates bulbar networks by modulating the effect of inhibition. This modulation of inhibition is evidenced by changes in mitral cell responses to odorants (Buonviso and Chaput 2000), activation patterns of inhibitory interneurons in response to odor stimulation (Mandairon et al. 2008a), changes in inhibitory neuron survival in the glomerular and granule cell layers (Alonso et al. 2006; Mandairon et al. 2006b; Mouret et al. 2008) as well as changes in oscillatory dynamics (Beshel et al. 2007; Martin et al. 2004).

3- **Manipulations of the olfactory bulb neural network modulate odor perception**

A crucial function of the olfactory bulb is to integrate afferent information conveyed by olfactory sensory neurons with centrifugal neuromodulatory inputs (in particular the cholinergic, noradrenergic and serotonergic systems) as well as other central (e.g., olfactory cortical) inputs (Halasz 1990; Luskin and Price 1982; Shipley et al. 1996). Both ascending (sensory) and centrifugal (modulatory and cortical) inputs can shape the bulbar network on various timescales and consequently modulate olfactory perception (Figure 2A). For example, perceptual effects of ascending inputs can be mimicked by direct activation of the olfactory bulb network via local NMDA injections, clarifying that local bulbar mechanisms
are involved in perceptual changes due to sensory enrichment ((Mandairon et al. 2006d) (Figure 2C).

Centrifugal inputs include, for example, cholinergic projections from the nucleus of the horizontal limb of the diagonal band of Broca (Luskin and Price 1982; Macrides et al. 1981; Zaborszky et al. 1986). Manipulations of the bulbar network by changing cholinergic function have been shown to affect olfactory perception by changing rodents’ ability to differentiate between perceptually similar odorants (Linster et al. 2001; Mandairon et al. 2006a), and in the case of muscarinic receptor modulation only, to affect the time span for olfactory short term memory (Ravel et al. 1994).

The olfactory bulb receives significant input from the noradrenergic pontine nucleus locus coeruleus (McLean et al. 1989; Shipley et al. 1996). Manipulation of the bulbar network by experimental interference with noradrenergic activity in the olfactory bulb has resulted in changes in olfactory perception and memory formation. Doucette and collaborators found that local blockade of noradrenergic receptors in the olfactory bulb decreases the ability of mice to learn a two-odor discrimination task in a go/no-go testing paradigm (Doucette et al. 2007). The decreased ability to learn the discrimination is evident when chemically and perceptually highly similar odorant mixtures are presented as choice odors. Moreover, spontaneous discrimination between chemically related odorants is decreased when noradrenergic receptors, and in particular α1 receptors, are blocked; reward-motivated discrimination learning is not impaired, but is slowed in rats in which both α and β receptors are blocked (Mandairon et al. 2008b). Bulbar noradrenaline has also been implicated in the formation of olfactory habituation memory (Guérin et al. 2008 ) and has been shown to be important for the acquisition and / or formation of conditioned odor
preferences or odor-specific memories (Kaba and Keverne 1988; Kendrick et al. 1992; McLean and Harley 2004; Sullivan and Dryer 1996).

In addition to neuromodulatory inputs, the olfactory bulb receives projections in particular from pyramidal cells in secondary olfactory cortical, frontal cortex, and hippocampal structures (Luskin and Price 1982; Macrides et al. 1981); these inputs have been shown to affect bulbar processing (Figure 2A). Reversible blockade of central inputs to the olfactory bulb in awake behaving rabbits showed that both bulbar EEG dynamics and unit firing are modulated by central feedback (Gray and Skinner, 1988). Using electrical lesions of the olfactory peduncle, sparing output from the olfactory bulb while decreasing feedback inputs to the olfactory bulb, Kiselycznyk and collaborators have demonstrated that manipulation of the bulbar efferent inputs change the formation of odor-reward associations, but not primary bulbar odor representations (Kiselycznyk et al. 2006 ). A separate study by Martin and collaborators showed that decreased efferent inputs to the OB result in dramatic changes of bulbar dynamics, presumably affecting odor-reward association learning by desynchronizing bulbar and cortical networks (Martin et al. 2004; Martin et al. 2006 ).

In summary, experimental manipulations of the bulbar network, by changes in receptor function, efferent input or neuromodulation, always result in perceptual changes evidenced by changes in discrimination, odor-reward association learning and memory formation. These data clearly implicate the olfactory bulb in shaping olfactory representations, perception and learning.

4- Correlation between odor perception and neural network changes

Previous experiments by our group and others have provided evidence for a predictive relationship between olfactory bulb neural activity and odor perception (Cleland et al. 2007;
Kay and Laurent 1999; Youngentob et al. 2006). Recently, this predictive relationship has been further tested by manipulations affecting both bulbar network computations and odor perception, which have demonstrated that changes in bulbar computation are predictive of changes in perception.

As described above, olfactory experience changes odor perception in animals exposed to odors in a daily fashion: these animals subsequently tend to discriminate between chemically similar odorants more easily (Escanilla et al. 2008; Mandairon et al. 2006d; 2006e) (Figure 3A). We have recently shown that the perceptual changes are accompanied by an increase in inhibitory neuron responsiveness in the olfactory bulb (Mandairon et al. 2008a), suggesting an increase in inhibitory processing (Figure 3B&C). Computational modeling clearly showed that the increase in inhibitory neuron responsiveness is predictive of the behaviorally observed, relative odor non-specific, increase in perceptual discrimination ((Mandairon et al. 2006d); Figure 3Di&Dii).

In recent experiments, Beshel et al. (2007) showed that modulations of olfactory bulb dynamics can be correlated to manipulations of task difficulty when rats are asked to discriminate chemically very similar odorants in order to receive a food reward. Higher power gamma oscillations, presumably reflecting higher synchrony among bulbar neurons, are observed for more difficult discrimination tasks (Beshel et al. 2007). Computational modeling has previously shown that better synchronization among neurons responding to sensory stimuli leads to higher discrimination ability within an olfactory network (Cleland and Linster 2002; Linster and Cleland 2001). These data strongly suggest an active modulation of bulbar dynamics as a function of task demands.
At the neural level, several recent experiments have shown a tight correlation between mitral cell odor responses and olfactory perception (Figure 4A). An exhaustive study by Doucette et al. (2008), following up on earlier studies (Kay and Laurent 1999), showed that mitral cell responses to odorants associated with reward or absence of reward diverge as the animal learns to associate one but not the other with the reward (Doucette and Restrepo 2008). Chaudhury et al. (2009) showed that manipulations of cholinergic activity in the olfactory bulb lead to changes in mitral cell responsiveness to odorants which are predictive of the changes observed in perceptual discriminations in response to the same manipulations ((Chaudhury et al. 2009) (Figure 4B,C&D).

In summary, recent experiments clarify that odor representations are shaped in the olfactory bulb in a manner predictive of perceptual odor qualities. While not all types of behavioral tasks are equally affected, the results from a variety of experiments clarify that indeed, olfactory experience shapes bulbar computations and in return, bulbar computations shape olfactory perception.

5 Conclusions

The data and experiments reviewed above illustrate how perceptual events and olfactory bulb processing influence each other in a reciprocal manner. Olfactory experience, both passive and active, leads to changes in the olfactory bulb neural network, which in turn changes how odors are processed and experienced. Observed behavioral and processing changes can be short-lasting or long-lasting, depending on the exact manipulations and behavioral demands used. While it is clear that much olfactory processing, in particular the associations of olfactory stimuli, is located in downstream brain areas such as piriform cortex, orbitofrontal cortex, hippocampus and amygdala, the representations conveyed to
these areas are highly plastic and experience dependent and depend on the exact state of bulbar processing. Hence, the state and plasticity of bulbar processing needs to be taken into account when olfactory stimuli are used to study learning and memory.
References


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**Figure 1.** Schematic depiction of bulbar neural network. Olfactory sensory neurons (OSN) project axons to individual glomeruli in which they activate periglomerular (PG), external tufted (ET) and mitral/tufted (Mi) cells. The high convergence between OSN axons and bulbar neurons provides high signal-to-noise ratio for incoming signals. The glomerular neural circuits have been proposed to compute contrast-enhancement and normalization functions (Cleland et al. 2007) on the input signal from the OSNs. The outgoing activity of the OB, provided by mitral/tufted cell (Mi) projections is further modulated by interactions with inhibitory granule cells (G), thought to create the dynamics necessary for synchronization, plasticity and further modulation of contrast. Central inputs including cholinergic (ACh), noradrenergic (NA) and cortical projections further modify bulbar output signals.

**Figure 2.** A. Schematic illustration of network modulation by experience (left side) and perceptual modulation by network manipulations (right side). See text for details and citations. B. Modulation of newborn cell survival is an example for network modulations induced by olfactory experience. The graphs show a color coded map of BrdU-positive cell density in the olfactory bulb in pseudo-conditioned group exposed to +/- limonene (left) and conditioned group to +/- limonene (right). BrdU-positive cells were counted in the granule cell layer on every fifth section of the olfactory bulb (14 μm thickness, sampling interval = 70 μm). The granule cell layer was divided into 36 sectors of 10° with the reference axis drawn on the ventral aspect of the subependymal layer of the olfactory bulb. The volume of the granule cell layer was represented by an array where each bin has the value of BrdU-
positive cells density in one 10° sector, and each column corresponds to one section (For details, see Mandairon et al. 2006b)).

C. Manipulation of the bulbar network via daily injections of NMDA modulates behavioral perception. The two enantiomers of limonene and terpinene were not discriminated before NMDA infusion (left). After the 10 days of NMDA administration directly in the olfactory bulbs, animals were able to discriminate between +/- limonene and +/- terpinene (For details, see Mandairon et al. 2006d)).

**Figure 3.** A. Correlated network processing and perceptual changes after daily exposure to odorants. B. Rats’ ability to discriminate between the two enantiomers of limonene was first tested using a habituation/cross-habituation test. Rats were then divided into four experimental groups, and submitted to daily enrichment with odorants by introducing a teaball containing swabs saturated with pure odorant into their cages for 1 h daily. The four groups of rats were exposed to +/- limonene, butanol/pentanol, decanal/dodecanone or mineral oil only, respectively. After the enrichment phase, all rats were tested using the habituation/cross-habituation test again. Five rats from each group were randomly chosen to assess the expression of Zif268 in the granule cell layer. Zif268 is an immediate early gene whose expression is driven by sensory activity (Inaki et al. 2002; Mandairon et al. 2006b). C. Enrichment with +/-limonene and pentanol/butanol increase the density of Zif268-positive cell in the granule cell layer compared to decanal/dodecanone group and non-enriched control rats (Mandairon et al., 2008). Di. Each graph shows rats’ investigation time during four sequential presentations of +limonene, separated by 5 min, followed by a single presentation of -limonene. The two enantiomers of limonene are confused before the
enrichment period in all groups. Dii. Rats enriched with decanal/dodecanone do not discriminate between the two enantiomers of limonene after the 10-day enrichment phase. In contrast, rats enriched with +/- limonene or pentanol/butanol discriminated between the two enantiomers of limonene after the enrichment phase.

**Figure 4.** Correlated network processing and perceptual changes due to manipulation of bulbar cholinergic inputs. A. Enhanced cholinergic modulation in the OB effects glomerular computation by directly activating PG cells (Castillo et al. 1999; Ravel et al. 1990), depolarization of mitral cells (Castillo et al. 1999) and modulation of granule cell inhibitory effects onto mitral cells (Pressler et al. 2007 ). B. Schematic depiction of setup for local infusion of neostigmine into the OB during in vivo experiments. C. During behavioral experiments, control and neostigmine infused rats were habituated to a given odorant during four sequential trials and then presented with novel, chemically related, test odors. Rats with increased bulbar acetylcholine (neostigmine) discriminated between chemically highly similar odorants significantly better than saline infused control rats (Chaudhury et al. 2009). D. OB mitral cell responses to chemically highly similar odorants differed significantly more in neostigmine infused as compared to saline infused rats (Chaudhury et al. 2009).
**Input:**
- Convergence
- Signal-to-noise ratio
- Contrast
- Normalization

**Output:**
- Dynamics
- Synchronization
- Plasticity
- Contrast

**OSN**
- **Ach:** activation of PG cells
- **NA:** modulation of Mi-GC interaction
- **NA/ACh:** modulation of Mi cell activation
- **Cortical:** Projections to GC cell layer

*Mandairon and Linster, Figure 1*
Glomerular activation patterns are modulated by experience.

Mitral cell odor responses sparsen in response to experience.

Granule cell odor responses increase in response to experience.

Dynamics are modulated through learning.

Newborn cell survival is modulated.

Activating nicotinic receptors modulates perception.

Activation of NMDA receptors enhances perceptual discrimination.

Noradrenergic modulation of odor discrimination.

NA receptors support short-term habituation memory.

Cortical feedback modulates dynamics and supports odor-reward learning.

**B**

Pseudo conditioned group (+)L/(-)L

Conditioned group (+)L/(-)L

**C**

Investigation time (s)

NMDA

Mandairon and Linster, Figure 2
GC responsiveness to odor stimulation changes in correlation with perceptual changes
Activation of PG cells increases contrast. Depolarization of Mi cells counterbalances increased inhibition. Modulation of GC-Mi interactions modulates signal-to-noise ratio.

Mandairon and Linster, Figure 4