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Peripheral Modulation of Smell: Fact or Fiction?

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Abstract

Despite studies dating back 30 or more years showing modulation of odorant responses at the level of the olfactory epithelium, most descriptions of the olfactory system infer that odorant signals make their way from detection by cilia on olfactory sensory neurons to the olfactory bulb unaltered. Recent identification of multiple subtypes of microvillar cells and identification of neuropeptide and neurotransmitter expression in the olfactory mucosa add to the growing body of literature for peripheral modulation in the sense of smell. Complex mechanisms including perireceptor events, modulation of sniff rates, and changes in the properties of sensory neurons match the sensitivity of olfactory sensory neurons to the external odorant environment, internal nutritional status, reproductive status, and levels of arousal or stress. By furthering our understanding of the players mediating peripheral olfaction, we may open the door to novel approaches for modulating the sense of smell in both health and disease.

Keywords

olfactory epithelium; sustentacular cells; microvillar cells; purinergic; cholinergic; sniff rates; trigeminal

Introduction

The emotional, reproductive, circadian, nutritional, and external environmental states of an animal are constantly changing. Sensory input is matched to the internal needs and external environment through modulation at different levels of the processing pathway. It is widely accepted that at least some component of sensory modulation occurs at the level of the peripheral receptor cells in all sensory systems except for one—the olfactory system. The vast majority of publications on the olfactory system dismiss modulation of olfactory signals at the level of the olfactory epithelium. Instead, published reports often include such words as ‘the olfactory bulb is the first level of processing in the olfactory system’ (the olfactory bulb being the CNS target for axons from olfactory sensory neurons in the olfactory epithelium).

What is so different about olfaction that the scientific community clings to the notion that peripheral signals into the brain from olfactory sensory neurons is invariant while accepting peripheral modulation in other sensory systems? What is the evidence for peripheral modulation in the olfactory system? When is peripheral modulation of odor sensitivity

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brought into play? What mechanisms take place to modulate peripheral odor reception? Where are these mechanisms active in the olfactory epithelium? How does peripheral modulation affect behavioral responses to odors? Not all of these questions are easily addressed. However, recent progress in our understanding of peripheral modulation in olfaction suggests that it is time to rethink sensory processing in the olfactory system. The theme of this review is that new findings may lead to new strategies to advance our understanding of how internal and external signals impinge upon and modulate peripheral olfactory sensory processing.

Anatomy and physiology of the olfactory system

To understand the origin of the dogma that olfactory information processing occurs first in central nervous system circuits, and to put the studies that demonstrate peripheral sensory modulation into context, it is important to understand the anatomy and physiology of the olfactory system. There are a number of chemosensory structures in the nose that are related to olfaction, including the vomeronasal organ, septal organ, Grunenberg ganglia, and solitary chemoreceptors (reviewed in Ma, 2010). However, the following review will focus on the main olfactory epithelium of vertebrates. The olfactory mucosa is comprised of a pseudostratified neuroepithelium containing ciliated bipolar sensory neurons, microvillar cells, glial-like sustentacular cells, duct cells, neuronal progenitor cells, and basal stem cells (Figure 1A). A basement membrane separates the epithelium from the glands, olfactory nerve bundles, and extrinsic innervation of the submucosa or lamina propria. Like most epithelia, the olfactory neuroepithelium is avascular. However, a rich vascular bed lies just below the basement membrane (Fig. 1B).

How are odors transduced?

Odorants bind to G protein-coupled odorant receptors (ORs) localized to the long slender cilia that extend from the apical dendritic knobs of olfactory sensory neurons into the mucus covering the nasal cavity (Buck and Axel, 1991). According to the canonical model for olfactory transduction, odorants binding to specific odorant receptors activate a transduction cascade that includes activation of the olfactory specific G_{olf} (Jones and Reed, 1989), the α subunit of which increases activity of an olfactory specific adenylyl cyclase III (Bakalyar and Reed, 1990; Pace et al., 1985) in the cilia. Activation of adenylyl cyclase III increases cAMP production. Cyclic nucleotide gated ion channels (Dhallan et al., 1990), also present in the olfactory cilia, bind intracellular cAMP, open, and allow a rapid influx of Na^+ and Ca^{2+} . Influx of these cations generates a depolarizing receptor potential. Moreover, the influx of Ca^{2+} in particular opens Ca^{2+} -dependent Cl^- channels, further depolarizing the cilia by allowing the efflux of anions (Cl^-). Ca^{2+} -activated Cl^- efflux may be less important in mammals than in fresh water aquatic animals (Billig et al., 2011). If the odorant-generated receptor potential in the cilia is sufficiently large, it will trigger action potentials in the soma. Impulses are propagated along the unmyelinated axons of olfactory sensory neurons to their distant synaptic terminals in glomeruli in the olfactory bulb.

In addition to the canonical cAMP-based transduction pathway, there are subpopulations of sensory neurons that express other transduction proteins. These proteins include guanylyl cyclase D, an enzyme present in certain olfactory sensory neurons and that responds to CO_2 (Leinders-Zufall et al., 2007); or TRPM5-PL β 2 in olfactory sensory neurons that respond to social odorants (Lin et al., 2007). Thus, within the population of vertebrate ciliated olfactory sensory neurons, there are subgroups of neurons that utilize different transduction pathways. These subgroups project to distinct regions of the olfactory bulb.

The termination of odorant responses in peripheral olfactory sensory neurons involves multiple steps including (a) removal of odorants from the air or mucus leading to unbinding

from receptors; (b) degradation of cAMP (Firestein et al., 1991); (c) desensitization of CNG channels via Ca^{2+} -calmodulin (Song et al., 2008); and (d) rapid extrusion of Ca^{2+} via a $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Reisert and Matthews, 1998). During prolonged odor stimulation, slow adaptation occurs in olfactory sensory neurons. Adaptation involves mechanisms including odorant receptor desensitization and activation of soluble guanylate cyclase by nitric oxide (Brunert et al., 2009; Pietrobon et al., 2011). Activating soluble guanylate cyclase generates cGMP throughout the cell and this leads to a prolonged elevation of $[\text{Ca}^{2+}]_i$ (Pietrobon et al., 2011) which, in turn, activates downstream enzymes that reduce odorant sensitivity. Pathways activated by odor stimulation and desensitization alike provide multiple substrates for modulation that could increase or decrease the output of olfactory sensory neurons.

How are different odorants perceived and discriminated?

The identification of odorant receptor genes (Buck and Axel, 1991) allowed molecular and genetic approaches to begin unraveling olfactory coding. Each olfactory sensory neuron expresses only one of the ~1000 odorant receptor genes found in rodents (~350 in humans). Each olfactory sensory neuron throughout the olfactory epithelium that expresses a particular receptor projects on average to 2 glomeruli in each one of the paired olfactory bulbs (Mombaerts et al., 1996). Conversely, each glomerulus in the main olfactory bulbs receives thousands of converging inputs from olfactory sensory neurons that all express the same odorant receptor. Thus, there is an exquisite targeting of odorant receptor specific axons onto glomeruli in the olfactory bulbs. Moreover, there is a loose chemotopic organization in the olfactory epithelium such that different classes of odorant receptors can be mapped to domains of glomeruli in the olfactory bulbs (Bozza et al., 2009; Tsuboi et al., 2006). Because odorant receptors recognize specific features of odorant molecules, multiple odorants can bind to a single receptor with varying affinities. Conversely, multiple classes of receptors respond to the same odorant, again with varying affinities. In addition, because odors that we perceive as a single percept (e.g., the aroma of coffee) generally are a complex mixture of many different odorants, odors typically activate (or inhibit) multiple classes of odorant receptors (Brunert et al., 2010; Ukhonov et al., 2011; Kawai et al., 1997). The outcome is that odors are believed to generate a specific pattern of activity in thousands of olfactory sensory neurons that converge onto a particular collection of glomeruli in the olfactory bulbs and is encoded there by neurons that project to the olfactory (piriform) cortex and other brain centers including the amygdala and hypothalamus. Surprisingly, the odorant specificity of connections between the olfactory epithelium and the olfactory bulbs does not appear to be maintained at the higher cortical levels. Tracers injected into a single glomerulus in the olfactory bulbs are taken up by dendrites of projection neurons and transported to cortical neurons broadly distributed across the piriform cortex (Ghosh et al., 2011; Sosulski et al., 2011). Modeling studies, combined with viral tracing and electrophysiological recordings, suggest that cortical representations of odors appear as sparsely distributed activity patterns. Furthermore, these patterns reflect extensive inhibitory processing and the convergence of inputs from multiple glomeruli (Wilson and Sullivan, 2011; Miyamichi et al., 2011). In short, the bulk of olfactory coding utilizes distributed activity however labeled lines for certain amino acids and trace amines also exist. Understanding the central processing of olfactory information is an active area of investigation. New optogenetic tools that allow precise activation or inhibition of specific neuronal populations in behaving animals promise to greatly advance our comprehension of odor coding. Nonetheless, as new approaches are adopted to analyze odor coding at the systems level and increased attention is paid to central coding, it is important to understand that the physiological status of the animal contributes importantly to the measured parameters. Significant modulation of these parameters occurs at all levels of the olfactory system, including at the level of the peripheral sensory receptor neurons.

Peripheral Olfactory Modulation

What is the historical context that led to the general acceptance of the olfactory bulb as the first site where olfactory input is modulated? The mammalian olfactory neuroepithelium has been traditionally described as having 3 classes of cells: ciliated bipolar sensory neurons, sustentacular support cells, and basal stem/progenitor cells. The lack of vasculature for providing hormonal input and the relatively modest abundance of predominantly afferent nerve fibers contributed to the notion that there is little or no peripheral sensory information processing. So-called perireceptor factors that might influence peripheral signaling, such as mucus, enzymes, and binding proteins, are generally absent during investigations on isolated olfactory sensory neurons, cell lines, or even tissue slice preparations. Hence, the view that the peripheral olfactory system is relatively homogenous, simple, and without obvious efferent input has contributed to the text book interpretation that it lacks the capability for sensory information processing.

Peripheral modulation via extrinsic innervation of the olfactory mucosa

The olfactory submucosa and the neuroepithelium alike receive extrinsic innervation (Graziadei and Gagne, 1973). There are four sources of extrinsic innervation of the olfactory mucosa: sympathetic, parasympathetic, trigeminal, and terminal nerves (Bojsen-Moller, 1975). Perhaps the dearth of studies on the physiology of the neuroepithelial innervation has contributed to the concept that there is no peripheral processing in olfaction. As detailed below, the majority of studies on innervation of the olfactory mucosa have focused on glands, blood vessels, and sustentacular cells, and show that autonomic stimulation evokes release of mucus. The fluidity and depth of mucus covering the OSNs will modulate odor sensitivity via perireceptor events described later.

Autonomic innervation of the olfactory epithelium

Blood vessels and glands of the vertebrate olfactory submucosa receive sympathetic (Kawano and Margolis, 1985; Zielinski et al., 1989) and parasympathetic innervation (Getchell and Getchell, 1992; Zielinski et al., 1989). Sympathetic innervation originates from the superior cervical ganglion (Kawano and Margolis, 1985). Sympathetic input alters in response to changing environmental conditions. For instance, levels of the sympathetic neurotransmitters dopamine and norepinephrine in the mucus covering the olfactory epithelium transiently increase following CO₂ stimulation (Lucero and Squires, 1998). Fibers containing tyrosine hydroxylase and presumed to be adrenergic sympathetic axons extend to the basement membrane and are present within glands (Fig. 3E). Dopamine or norepinephrine released from these fibers may reach olfactory sensory neurons by diffusing across the basement membrane or via mucus released from innervated glands. The concentration of these catecholamines will increase or decrease depending on arousal state of the animal. Parasympathetic innervation of nasal glands and blood vessels arises from the ciliary and pterygopalatine ganglia (Weite and Ashwell, 2004). As with catecholamines secreted from sympathetic axons, acetylcholine released from parasympathetic fibers may reach cholinergic receptors on olfactory sensory neurons via diffusion from the submucosa or from secretion into mucus.

Researchers have proposed that sympathetic adrenergic innervation of the olfactory mucosa enhances odor contrast during ‘flight or fright’ responses. Parasympathetic input is believed to increase odorant sensitivity during food finding or feeding (Hall, 2011). More specifically, adrenergic stimulation of olfactory sensory neurons reduced T-type Ca²⁺ currents and enhanced voltage-gated Na⁺ currents in the neurons. The net effect was that the threshold for impulse generation was increased, but once reached, neuronal firing was increased. This dual action was mediated through cAMP-dependent phosphorylation and resulted in a contrast enhancement such that responses to low odor concentrations would be

silenced, but responses to odorants presented at high concentrations would be enhanced (Kawai et al., 1999). In contrast, dopamine released into mucus during exposure to noxious air environments appears to reduce odor responsiveness of olfactory sensory neurons (Hegg and Lucero 2004; Wetzel et al., 2001). This reduction appears to be mediated through D2 dopamine receptors and is caused by decreasing the depolarizing current I_h . Reducing I_h hyperpolarizes the resting membrane potential and increases the threshold for firing of action potentials (Vargas and Lucero, 1999; Vargas and Lucero, 2002), explaining the reduction in odor responsiveness. Dopamine also reduces voltage-gated Na^+ currents through a D1 receptor pathway (Wetzel et al., 2001). The dual and opposing actions of D1 and D2 dopamine receptors on cAMP levels in the same cell is reconciled by the observation that cAMP can be specifically localized to subdomains of a cell. These subdomains depend on the localization of dopamine receptors and phosphodiesterases (Karpen and Rich, 2004). To date, dopamine receptors have not been localized to olfactory cilia. It is likely that dopaminergic modulation of olfactory sensory neurons occurs in the soma where voltage-gated channels are localized, rather than in cilia where odorant transduction takes place.

Trigeminal innervation of the olfactory epithelium

In addition to the autonomic innervation of the submucosa, the main olfactory epithelium is innervated by the ophthalmic and maxillary branches of the trigeminal nerve (Getchell and Mellert, 1991; Finger et al., 1990). The more medial branch of the ophthalmic nerve, called the ethmoid nerve, innervates olfactory epithelium and olfactory bulb, alike (Schaefer et al., 2002; Fig. 2). Although trigeminal innervation of the olfactory epithelium is considered to be sensory (Damann et al., 2006; Rothermel et al., 2011; Silver and Moulton, 1982; Gerhold and Bautista, 2009), these fibers contain and may release Substance P (Fig. 3E,F), CGRP (Finger et al., 1990), and ATP through axonal reflex. Surprisingly, little work has been done to examine neuropeptide/neurotransmitter release by trigeminal fibers within the olfactory epithelium, although in vivo and in vitro studies have shown that trigeminal ganglia can release ATP and substance P (Matsuka et al., 2001). Viral tracing studies have identified trigeminal ganglion cells that innervate nasal epithelium and characterized their electrophysiological and pharmacological properties (Damann et al., 2006). These trigeminal neurons responded to ATP and responses were consistent with expression of P2 $\times$ 2 purinergic receptors. Ultrastructurally, trigeminal fibers in the olfactory epithelium have not been shown to directly innervate olfactory sensory neurons. However, trigeminal fibers pass in close proximity to sustentacular cells, ducts of Bowman's glands (Finger et al., 1990; Getchell and Getchell, 1992), and certain microvillar cells found in the olfactory epithelium (Hegg et al., 2010), raising the possibility that those tissues and cells are targets for ATP or peptide release.

Trigeminal innervation of the olfactory and respiratory epithelium is important for detecting irritants or bacteria and for initiating protective reflexes such as coughing and sneezing (Silver and Finger, 2009; Tizzano et al., 2010). In the olfactory epithelium, release of substance P, CGRP, or ATP by trigeminal fibers in response to noxious stimuli might provide a fast feedback system for modulating the sensitivity of olfactory sensory neurons during exposure to high concentrations of odorants, to toxic chemicals, or to xenobiotics. In addition to acutely regulating the activity of olfactory sensory neurons, these same trigeminal substances could signal damage to the olfactory epithelium and initiate mechanisms for regional protection (Carr et al., 2001; Hegg and Lucero, 2006) or regeneration (Jia and Hegg, 2011; Jia et al., 2011b; Jia et al., 2011a; Jia and Hegg, 2010; Jia et al., 2010; Jia et al., 2009; Gao et al., 2010).

Terminal nerve innervation of the olfactory mucosa

The terminal nerve is a cranial nerve that is present in most vertebrates but is very poorly understood. The somata of terminal nerve ganglion cells are found in multiple cranial ganglia. These neurons originate in the olfactory placode. Ganglion cells of the terminal nerve are identified by expression of GnRH/LHRH (Schwanzel-Fukuda, 1999). In some species, acetylcholine and peptides such as NPY are coexpressed with GnRH in the neurons (Zheng and Jourdan, 1988; Schwanzel-Fukuda, 1999; Mousley et al., 2006). Axons of the terminal nerve pass through the submucosa of the olfactory epithelium en route to the vomeronasal organ (VNO), with some branches approaching the basement membrane, glands and blood vessels (Wirsig-Wiechmann, 1993). Interestingly, olfactory sensory neurons in axolotls and mudpuppies become more responsive to GnRH during mating season (Park and Eisthen, 2003; Zhang and Delay, 2007). The effects of GnRH are relatively slow and long lasting, as might be expected for peptidergic modulation. Electroolfactogram (EOG) recordings from the olfactory epithelium of axolotls show reduced responses to food odors during mating season. In mudpuppies, a similar seasonal variation in GnRH sensitivity as well as ion channel modulation were observed. However, the effects of GnRH on odorant responses were biphasic with an initial increased excitatory transient followed by inhibition (Zhang and Delay, 2007; Kawai et al., 2009).

Peripheral modulation occurs during sniffing

Perireceptor events that alter odorant responses include processes that change the access, concentration, or chemical structure of the odorant molecules. These factors include mucus thickness and composition (Getchell et al., 1988; Getchell and Mellert, 1991), the presence of P450 enzymes in supporting cells that can alter the concentration and chemical structure of odorants (Chen et al., 1992; Dahl, 1988), and odorant binding proteins that may alter access (increase or decrease) of odorants to the sensory cells (Pevsner et al., 1988). There are many aspects of perireceptor modulation. An especially intriguing topic is the mechanical event determining how and when odorants gain access to the olfactory epithelium, namely sniffing (Schoenfeld and Cleland, 2005; Kepecs et al., 2006; Mainland and Sobel, 2006; Schaefer and Margrie, 2007; Wachowiak, 2011; Wachowiak et al., 2009; Scott, 2006). With each breath or sniff inhaled through the nose, a complex mix of air, dust, odorants, and xenobiotics enters the nasal passages. The air is humidified, warmed, and filtered before it reaches the olfactory epithelium on the posterior septal, ventral, and dorsal recesses of the nasal cavity. Recent studies show that modulating the rate and speed of airflow over the olfactory epithelium (sniffing) dramatically alters the odor-related signals that reach the olfactory bulb (Carey et al., 2009; Verhagen et al., 2007). Hence, an important form of peripheral olfactory modulation occurs as a result of central motor regulation of breathing and the kinetics of odorant delivery to the olfactory epithelium. Interestingly, ghrelin, a peptide hormone from the stomach that is released into the blood stream during the hunger state, has recently been shown to enhance exploratory sniffing behaviors in rodents and humans (Tong et al., 2011).

Adsorptive model for airflow modulation of odorant delivery

One model for how airflow rates affect OSN activity is based on the adsorptive properties of the odorant molecules themselves (Moulton, 1976). The concept is that the moist nasal passages act as a chromatograph, absorbing small hydrophilic molecules that readily dissolve in mucus in the anterior-septal regions, and allowing larger, non-polar molecules to reach olfactory epithelium in more posterior-ventral regions (Hornung and Mozell, 1977; Schoenfeld and Cleland, 2005). During high flow rates, mucus in more anterior nasal regions quickly saturates and allows hydrophilic polar molecules to reach the olfactory epithelium, thereby increasing their effectiveness as odor stimuli. In contrast, high flow rates have less effect on the ventral/lateral turbinates, and thus non-polar molecules are less

affected by airflow (Scott et al., 2006). The expression and distribution of odorant receptors in the olfactory epithelium seem to follow this pattern. Odorant receptors that bind hydrophilic organic acid molecules (class I odorant receptors) are expressed by olfactory sensory neurons that have an anterior dorsal location; olfactory sensory neurons that express receptors for more hydrophobic odorants such as acetophenone are distributed more broadly (Bozza et al., 2009; Tsuboi et al., 2006).

However, two recent studies dispute these models. Abaffy and Defazio (2011) tested 100 odorants and found no evidence for a match between dorsal versus ventral olfactory receptors and the solubility/volatility of odorants. In a separate study, Johnson et al. (2007) found that odorants that are more water soluble did not appear to activate olfactory sensory neurons that project to the anterior dorsal olfactory bulbs, as would be expected. Instead, these odorants preferentially activated olfactory sensory neurons that projected more posteriorly into the olfactory bulbs. Thus, although airflow does affect responses of olfactory sensory neurons, it is becoming less clear as to whether or how chemotopy (the spatial organization of odorant receptors in the olfactory epithelium), is related to airflow dynamics in the nasal cavity.

A second model for how sniffing and airflow might modulate the sensitivity of olfactory sensory neurons is based on the concept that at high sniff frequencies, an elevated background concentration of odorants in the nasal passages desensitizes various steps of the transduction pathway (Verhagen et al., 2007). Verhagen *et al* (2007) used genetically altered (SynaptopHlorin) mice to image synaptic transmission in the nerve terminals of olfactory sensory neurons. These researchers showed that during slow rates of sniffing (< 3 Hz), responses recorded in the nerve terminals reliably followed each sniff. Surprisingly, at higher sniff rates (>4 Hz), where the concentration of odorants in the nasal passages is elevated and one might expect larger responses from olfactory sensory neurons, signals were in fact reduced. Adding a second (new) odorant during low frequency sniffing of a first odor produced additive responses. In contrast, adding a second odorant during high frequency sniffing of the first odor reduced responses to the first (background) stimulus and thus enhanced the difference between background and the second (new) odorant (Verhagen et al., 2007). This sniff-dependent modulation is clearly mediated at the level of the peripheral olfactory epithelium because it still occurred when synaptic transmission between olfactory sensory neurons and their targets, neurons in the olfactory bulb, was inhibited (Pirez and Wachowiak, 2008; Lecoq et al., 2009).

Autocrine, paracrine, and endocrine modulation in the olfactory epithelium

Is there also endocrine or paracrine modulation of olfactory sensory neurons? Interestingly, a number of studies document the expression and/or release of neurotransmitters, neuropeptides, nucleotides, and hormones in the olfactory epithelium. Key molecules that have already been shown to modify odorant responses are listed in Table 1. Table 2 lists other important candidate bioactive substances yet unexplored that are found in or near olfactory sensory neurons and for which receptors are expressed in these neurons. Paracrine or autocrine release of certain of these modulators, including nitric oxide (Brunert et al., 2009), NPY (Kanekar et al., 2009), and ATP (Hegg and Lucero, 2006), coincides with external stimuli that cause irritation such as strong odors or noxious air environments. Other modulators such as insulin (Lacroix et al., 2008), orexin (Caillol et al., 2003), leptin (Savigner et al., 2009; Baly et al., 2007), and PACAP (Hansel et al., 2001b; Hegg et al., 2003a) may be linked to stress or to the internal nutritional and hormonal status of an animal. Receptors for circulating hormones which help maintain salt and water balance such as arginine vasopressin (Levasseur et al., 2004) and endothelin (Gouadon et al., 2010) are expressed by olfactory sensory neurons and provide a potential endocrine mechanism for modulating peripheral odor sensitivity.

Who are the major players and where are they located within the olfactory epithelium?

Our present view of the complexity of cell types and signaling within the olfactory epithelium has been greatly aided by the generation of mice expressing GFP or other fluorescent markers in cells expressing specific receptors, ion channels, and transduction molecules. One of the most intriguing players to emerge from this genetically guided analysis has been the enigmatic microvillar cell (Carr et al., 1991; Fig. 3).

Microvillar cells as paracrine modulators of OSNs

Microvillar cells in the olfactory epithelium of fish are well-characterized bipolar olfactory neurons that send axons to the olfactory bulb (Muller and Marc, 1984; Hansen and Zielinski, 2005). These chemosensory cells respond to amino acids (Lipschitz and Michel, 2002). Microvillar cells are also present in olfactory epithelium in rodents. However, until recently the chemosensory capabilities of microvillar cells in mammals were less clear. Conflicting studies claimed that microvillar cells were (Elsaesser et al., 2005) or were not chemosensory (Carr et al., 1991; Lin et al., 2008; Hansen and Finger, 2008), and did or did not possess axons. Now, though, these discrepant reports are becoming resolved as fluorescent markers, immunocytochemistry, and confocal calcium imaging in slices of olfactory epithelium are beginning to reveal that there are multiple subclasses of microvillar cells. The majority of microvillar cells respond to odorants (Hegg et al., 2010; Ogura et al., 2011) but do not send axons to the olfactory bulbs (Lin et al., 2008; Ogura et al., 2011; Hansen and Finger, 2008; Asan and Drenckhahn, 2005; Hegg et al., 2010). Markers injected into the olfactory epithelium in rats labeled a small population of bipolar microvillar cells that do, in fact, project to the olfactory bulbs (Rowley et al., 1989). Subsets of microvillar cells express IP3R3, are innervated by trigeminal axons that contain substance P, and respond to odorants as well as to exogenously applied substance P and ATP (Hegg et al., 2010; Fig. 3E). Microvillar cells innervated by trigeminal fibers may signal odorant information to the olfactory bulbs via a branch of the trigeminal nerve that appears to connect the olfactory epithelium and olfactory bulbs (Schaefer et al., 2002; Fig. 2). However, direct evidence that trigeminal fibers which innervate microvillar cells project to the olfactory bulbs remains to be shown. Microvillar cells associated or not with trigeminal axons contain NPY (Fig. 3J-L) and purinergic stimulation results in NPY production and release. These data suggest that microvillar cells play a role in providing trophic factors to the olfactory epithelium under the control of odorant or trigeminal nerve stimulation, or both (Hansel et al., 2001a; Jia and Hegg, 2011; Jia and Hegg, 2010; Kanekar et al., 2009; Montani et al., 2006).

The location of microvillar cells at regular intervals along the anterior olfactory epithelium positions them as putative nodes for paracrine release of modulatory factors under odorant-stimulated control (Fig. 3B). An example of such paracrine signaling comes from identification of a subset of microvillar cells that co-express TRPM5 and choline acetyltransferase (ChAT, the enzyme required for synthesizing acetylcholine, ACh) (Ogura et al., 2011; Fig. 3B,C). These microvillar cells do not receive trigeminal innervation (Hansen and Finger, 2008). They respond to odor and thermal (cold) stimulation by mobilizing intracellular Ca^{2+} and release ACh into the olfactory epithelium (Ogura et al., 2011).

What are the targets in olfactory epithelium for acetylcholine? Sustentacular cells are a likely target. They express M3 (muscarinic) ACh receptors and increase intracellular Ca^{2+} in a muscarinic dependent manner (Hegg et al., 2009; Ogura et al., 2011). Interestingly, olfactory sensory neurons express inhibitory muscarinic ACh receptors M4 and M5 and are inhibited by ACh (Ogura et al., 2011). Olfactory sensory neurons also express M3 receptors and enhance odorant receptor activity (Li and Matsunami, 2011). A model can be generated where strong odorants activate microvillar cells that express TRPM5/ChAT which, in turn,

release ACh to mobilize Ca^{2+} in sustentacular cells and reduce cAMP in olfactory sensory neurons (Ogura et al., 2011). Muscarinic activation appears to reduce the “gain” of olfactory sensory neurons, allowing them to respond to higher concentrations of odorants. Simultaneously, cholinergic stimulation may activate neuroprotective and neuroregenerative pathways in sustentacular cells (described below) to minimize damaging effects of the odorants. Given that there are multiple subtypes of microvillar cells, it is likely that release of additional peripheral modulators will be discovered in the future.

Sustentacular Cells as Paracrine Modulators

In addition to microvillar cells as candidates for paracrine release of modulators of olfactory sensory neurons, sustentacular cells are also likely players in peripheral sensory modulation. These glial-like support cells possess many of the signaling properties of CNS glial cells and are capable of conducting both Ca^{2+} and electrical signals from the apex to base of the epithelium (Hegg et al., 2009; Vogalis et al., 2005b). Sustentacular cells express metabotropic P2Y purinergic receptors (Dooley et al., 2011; Gao et al., 2010; Hegg et al., 2003) and muscarinic receptors as described above. Like glia in the CNS, sustentacular cells are a source of neurotrophic and neuromodulator factors and have been shown to produce insulin (Lacroix et al., 2008), PACAP (Hegg et al., 2003a), NPY (Hansel et al., 2001a), endocannabinoids (Breunig et al., 2010), ATP, and they secrete mucus in response to odorants (Getchell et al., 1987). Their cellular continuity from the basement membrane to the apical epithelial surface may allow bidirectional movement of nutrients, wastes and hormones between capillaries and throughout the avascular olfactory epithelium (Rafols and Getchell, 1983). For instance, olfactotoxins such as methimazole are taken up from the blood stream and drawn into the olfactory epithelium via sustentacular cells (Bergstrom et al., 2003). The distribution of aquaporin channels in the olfactory epithelium supports a pathway for water transport from the vasculature through the epithelium and into the nasal cavity via sustentacular cells (Solbu and Holen, 2012). It is presently unknown whether neuropeptides, neurotransmitters, or hormones are transported from capillaries into the olfactory epithelium via sustentacular cells. However, given that many of these molecules are effective at nanomolar concentrations, such a delivery mechanism seems plausible. Moreover, many of the signaling molecules present in and released by sustentacular cells themselves modulate the sensitivity of olfactory sensory neurons (Table 1).

In addition to the aforementioned signaling pathways, lateral coupling of sustentacular cells via gap junctions provides a syncytium for Ca^{2+} , IP3, and other molecular signals to spread radially across the epithelium (Rash et al., 2005; Vogalis et al., 2005a; Zhang, 2011; Zhang et al., 2000).

Widespread communication between sustentacular cells by all the above mechanisms is important for signaling damage to the olfactory epithelium (heat shock protein upregulation (Carr et al., 2001; Hegg and Lucero, 2006) and for activating proliferation (Jia et al., 2009) via ATP/NPY release in the OE.

Olfactory sensory neurons as autocrine modulators of odorant sensitivity

As listed in Tables 1 and 2, olfactory sensory neurons can be a source as well as a target for certain neuromodulators. This suggests that there is autocrine modulation of peripheral olfactory sensitivity. ATP, endocannabinoids, and PACAP are all produced in olfactory sensory neurons and act on these same cells in an autocrine manner. ATP and PACAP alike may be released in response to stress or olfactotoxins. Although the actions of PACAP on odorant-evoked responses has not yet been tested directly, PACAP dramatically mobilizes calcium in olfactory sensory neurons. Given the Ca^{2+} sensitivity of several steps in olfactory sensory transduction, PACAP would be expected to reduce odorant sensitivity as a

neuroprotective response, similar to the actions of dopamine (Hegg and Lucero, 2004) and ATP (Hegg et al., 2003).

How does peripheral modulation affect behavioral responses to odorants?

Availability of mice deficient in certain peptides or receptors is providing further evidence that odor-evoked responses are modulated at the level of the olfactory epithelium. Indeed, in mouse models where either the leptin receptor or leptin itself were knocked out, mice were able to locate hidden food pellets faster than were wild type control mice (Getchell et al., 2006). Injecting leptin, a peptide hormone important for regulating food intake, restored the normal search times in leptin-deficient mice (Getchell et al., 2006). Much of the effect of leptin could be central. However, the presence of leptin receptors on olfactory sensory neurons (Baly et al., 2007) and the reduced sensitivity to odorants in the fed state suggest that leptin may modulate peripheral receptor sensitivity. Furthermore, this modulation would be dependent on the animal's nutritional status. The mechanism for the reduced sensitivity of leptin-treated olfactory sensory neurons is thought to be caused by an increased basal activity that reduces the signal to noise of odorant responses (Savigner et al., 2009). Insulin is another peptide hormone related to food consumption and appetite control. It, too, has similar mechanisms for reducing odorant sensitivity in the fed state (Savigner et al., 2009).

In contrast to the reduced odorant sensitivity produced by leptin and insulin, endocannabinoid signaling increases appetite and heightens olfactory sensitivity. In fasted *Xenopus* larvae, sustentacular cells increase their production and release of endocannabinoid, resulting in a decreased odorant threshold and thus enhanced odorant sensitivity (Czesnik et al., 2007; Breunig et al., 2010). Knocking out receptors for the orexogenic peptide NPY results in mice that have reduced olfactory capabilities. However, this may be due to reduced replacement of olfactory sensory neurons without a concomitant, NPY-mediated proliferation (Jia and Hegg, 2011) and thus unrelated in any direct manner to peripheral modulation of sensory transduction.

The examples given above are related to feeding behaviors. Other behaviors mediated by odors and relating to stress and reproduction are modulated at the level of peripheral sensory processing (Table 1 & 2). Fig. 4 is a summary diagram showing sites of action of neuromodulators referenced in Tables 1 & 2. Although greatly simplified, this diagram illustrates that there are several mechanisms that shape odor-evoked signals in the peripheral olfactory epithelium. Future studies with knockout mice and genetically identified markers for these modulators will greatly expand our understanding of the multiple and complex modulation that occurs within the olfactory epithelium.

Summary and Conclusions

The evidence is mounting that important modulation of olfactory sensory neurons occurs in the peripheral olfactory system. Recent reviews of autonomic modulation of olfactory sensory neurons (Hall, 2011) and olfactory networks (Leinwand and Chalasani 2011) reflect the broadening awareness that there are abundant sites for peripheral modulation of smell. From modulation of sniff rates; perireceptor events; changes in odorant receptor expression/coupling/affinity; Ca^{2+} - or phosphorylation-dependent modification of receptors, enzymes, or ion channels, there are tightly regulated feedback mechanisms that exert control over the sensitivity and specificity of information that is relayed from olfactory sensory neurons to the brain. The neurotransmitters norepinephrine and acetylcholine from sympathetic and parasympathetic innervation (Getchell et al., 1988) or released locally from microvillar cells (Ogura et al., 2011); dopamine released into the mucus during exposure to irritants (Lucero and Squires, 1998); substance P secreted by trigeminal neurons (Finger et al., 1990); NPY from microvillar cells (Kanekar et al., 2009); leptin and insulin (Baly et al., 2007; Savigner

et al., 2009) which may gain access to the olfactory epithelium via glands, mucus or from the vasculature; and ATP released by OSNs, sustentacular cells (Hegg and Lucero, 2006), and possibly by trigeminal fibers -- are all examples of candidates for peripheral modulation of olfactory sensory neurons. The signal processing that occurs at the level of olfactory sensory neurons is responsive to both external and internal states and dynamically adjusts to changes in physiological state.

As our understanding of the molecules and mechanisms for peripheral sensory processing evolves, new strategies become available for either increasing odorant sensitivity in the case of olfactory loss or temporarily decreasing sensitivity as an appetite suppressant, an anti-nausea treatment or in a malodorous environment. Clearly, it is time to revise the text books as to the initial site of sensory processing. Peripheral modulation of olfactory neurons is a fact, not fiction.

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Our current view of peripheral olfactory processing is changing rapidly
Neurotransmitters released by extrinsic innervation modulate odor sensitivity
Many neuromodulators are synthesized and released within the olfactory mucosa
Peripheral smell modulation matches odor sensitivity to internal and external states
Understanding the complexities of peripheral modulation generates novel therapeutics

Fig. 1.

The olfactory mucosa is comprised of an avascular pseudostratified neuroepithelium containing olfactory sensory neurons (OSNs), sustentacular cells, ducts of Bowman's glands, microvillar cells, immature OSNs, and basal cells. **A**, in the *lamina propria* there is a rich network of capillaries, venules and arterioles within a stroma consisting of Bowman's glands, fibroblasts, and extrinsic nerve fibers. **B**, the olfactory turbinate of a postnatal day 3 mouse was stained with anti-aquaporin1 (AQP1, green) which labels blood vessels and fibroblasts of the *lamina propria* (Solbu, and Holen., 2012). Abbreviations: Sustentacular cells (SUS), olfactory sensory neurons (OSNs), Basal cells (BC), *Lamina propria* (LP), Bowman's Gland (BG), capillaries (cap).

Fig. 2.

Diagram illustrating the peripheral and central locations of tracer injections used to examine trigeminal innervation of the nasal epithelium and olfactory bulb. To address whether trigeminal fibers in the olfactory bulb are collaterals of trigeminal axons that innervate the nasal epithelium, Nuclear Yellow was injected into the dorsal nasal epithelium and True Blue was injected into the ipsilateral olfactory bulb (1). For triple retrograde labeling, an additional injection was performed by placing either Evans Blue or Rhodamine Dextran into the ventral spinal trigeminal tract (Sp5) to determine whether trigeminal fibers in the olfactory bulb terminate in the brainstem (2). Modified from Fig. 1, Schaefer et al., 2002.

Fig. 3.

Examples of cholinergic (choline acetyltransferase, ChAT), catecholergic (tyrosine hydroxylase, TH), and peptidergic (Substance P, NPY)-expressing microvillous cells. **A**, schematic drawing of a sagittal section of the mouse nose, showing the distribution of GFP-expressing microvillous cells in the main olfactory epithelium (MOE) and septal organ (SO) (blue regions) in ChAT(BAC)-enhanced GFP (eGFP) and TRPM5-GFP mice. OB, olfactory bulb; VNO, vomeronasal organ. **B**, GFP-expressing microvillous cells imaged in a strip of MOE from a ChAT(BAC)-eGFP mouse. **C**, confocal image from a MOE section of a ChAT(BAC)-eGFP mouse labeled with an antibody against the neuronal marker PGP 9.5 to visualize olfactory sensory neurons (OSNs) (red). Note that the ChAT (GFP)-expressing microvillous cells are not labeled by the PGP 9.5 antibody. The labeled cells are located in the superficial layer that consists primarily of cell bodies of supporting cells. **D**, image of TRPM5 (GFP)-expressing microvillous cells immunostained with anti-espin, viewed from the mucosal surface. **E**, nerve fibers in the submucosa immunostained with anti-TH (blue arrow) and anti-Substance P (red) in sections of the olfactory epithelium from an IP3R3-GFP mouse. **F**, higher magnification of IP3R3-GFP-labeled microvillous cells shows trigeminal axons containing Substance P (red arrow). **J, K, L**, Immunocytochemistry shows colocalization of anti-NPY (green) labeling with IP3R3 in microvillar cells (red). Scale bars: *B* = 50 μm, *C* = 20 μm, *D* = 5 μm. *A-D* Modified from Fig. 1, Ogura et al., 2011; *E-F* modified from Fig. 7, Hegg et al., 2010; *J-L* modified from Fig. 5, Jia & Hegg, 2010.

Fig. 4.

Summary diagram showing the complex modulation of odorant responses in OSNs. The numbers in black represent the source and numbers in blue are the target of each of the neuromodulators listed below. 1. Acetylcholine (ACh), 2. ATP, 3. Endocannabinoids, 4. dopamine or catecholamines, 5. GnRH or LHRH, 6. insulin, 7. leptin, 8. nitric oxide, 9. NPY, 10. odorants (triangles). The trigeminal and terminal nerves are combined for simplicity. Gland refers to both Bowman's glands and deeper nasal glands.

Table 1

Modulators with demonstrated effects on odorant responses in OSNs

Modulator	Sources	Target Cells	Modulatory Effect	Mechanism	Vertebrate model	References
1. ACh	Parasympathetic innervation Terminal Nerve in some species	Bowman's Glands, blood vessels,	Increase mucus secretion which changes access of odorants to receptors	Innervation of acinar glands. Activation causes secretory vesicle release	Salamander	Hedlund & Shepherd 1983 Firestein & Shepherd 1992
	Subset of non-innervated TRPM5 ⁺ /ChAT ⁺ Microvillar Cells	Olfactory sensory neurons	Enhanced sensitivity of OSNs to odors in frog Reduce sensitivity of OSNs to odorants in mouse Increase expression or activity of ORs	M4/5 mACh receptor inhibition of ACIII M3 mACh receptor mediated increase in [Ca ²⁺] _i	frog	Bouvet et al. 1987 Frings 1993
2. ATP	Sustentacular cells OSNs	Sustentacular cells	Promote Ca ²⁺ -dependent release of modulatory factors (cannabinoids, ATP)	M3 mACh receptor mediated increase in [Ca ²⁺] _i	mouse	Hegg et al. 2009 Ogura 2011 Li & Matsumani 2011
		Sustentacular cells OSNs Microvillar cells	Reduce odorant sensitivity in OSNs Promote release of NPY from microvillar cells Signal stress in OE	P2Y activation increases [Ca ²⁺] _i in OSNs & sustentacular cells P2X in OSNs and microvillar cells	Mouse Rat Frog	Hegg et al. 2003 Dooley et al. 2011 Hassenlover et al. 2008 Czesnik et al. 2006
3. Cannabinoids	Sustentacular cells OSNs	OSNs	Increase odorant sensitivity by reducing odorant threshold	Starved animals increase endocannabinoid release onto OSNs which show reduced odor thresholds	<i>Xenopus</i> larvae	Breunig et al. 2010 Czesnik et al. 2007
4. Dopamine or Catecholamines	Sympathetic innervation of glands and blood vessels/Mucus following CO ₂ stimulation	Adrenergic receptors on sustentacular cells, Bowman's glands, blood vessels	Reduce sensitivity to odorants With adrenalin, weak odorant responses suppressed more that strong. Reduce sensitivity to odorants	Increased mucus secretion changing access to odorants Adrenalin inhibits weak odor responses via increased basal activity of OSNs and reduced signal to noise	Newt Salamander Rat Mouse	Getchell & Getchell 1984 Kawai et al. 1999 Vargas & Lucero 1999 Hegg et al. 2002 Wetzel et al. 2001
		D ₂ and D ₁ receptors on OSNs		D2 dependent reduction in Ih D1 increase in basal activity reducing signal to noise		
5. GnRH	Terminal nerve	OSNs	Reduced EOG response to food odors during mating season More GnRH responsive cells during mating season	Variable: in axolotl OSNs GnRH increased Na currents and decreased outward currents Mudpuppy GnRH biphasic effects on INa and increased outward	Axolotl Mudpuppy	Eisthen, 2000 Park & Eisthen 2003 Zhang & Delay 2007

Modulator	Sources	Target Cells	Modulatory Effect	Mechanism	Vertebrate model	References
				currents		
6. Insulin	Sustentacular cells Nasal glands	OSNs sustentacular cells basal cells Blood vessels	Increased spontaneous activity, reduced EOG response to odorants during fed state	Reduced signal to noise due to increased basal activity	Rat	Lacroix et al. 2008 Savigner et al. 2009
7. Leptin	Olfactory mucosa sustentacular cells, Bowman's glands, nasal glands Circulation increases with fasting	OSN cilia Immature OSN Sustentacular cells Bowman's glands	Absence of leptin signaling speeds food finding Increased spontaneous activity, reduced EOG response to odorants during fed state	Reduced signal to noise due to increased basal activity	Mouse Rat	Getchell et al. 2006 Savigner et al. 2009 Baly et al. 2007
8. Nitric Oxide	eNOS in OSNs	OSNs	Increase odor adaptation	eNOS—/— reduced adapt.	Mouse	Brunert et al. 2009
9. NPY	Terminal nerve Microvillar cells	OSNs Glands or blood vessels Basal cells	Increase Na current, reduce EOG odorant responses NPYY1 receptors on globose basal cells increase proliferation via p44/42 ERK	in hungry animals, may link feeding to olfaction reduced olfaction in NPY Y1 KO ATP-mediated release of NPY following damage	Aquatic salamander (axolotl) Mouse	Mousley et al. 2006 Doyle et al. 2008 Montani et al. 2006 Jia et al. 2011
10. Odorants	air	OSNs Sustentacular cells	Inhibition of odorant responses in odor mixtures (mixture suppression) Increase mucus secretion	GPCR increase in PI3K increases PIP3 which directly inhibits CNG channels Competition at odorant receptors Ca activated K channels Suppression of GPCR basal activity by an inverse agonist	Rat rat mouse rat	Spehr et al. 2002 Zhainazarov et al. 2004 Brunert et al. 2010 Ukhanov et al. 2011 Takeuchi et al. 2009 Morales et al. 1994 Getchell et al. 1987

Table 2

Modulators with Inferred Effects on Odorant Responses in OSNs

Modulator	Sources	Target Cells	Modulatory Effect	Mechanism	Vertebrate model	References
11. arginine vasopressin	OE and primary culture of OE	Bowman's glands, ducts, Blood vessels OSNs, basal cells	Increase mucus secretion which changes access of odorants to receptors Increase Ca^{2+} in OSNs Neuromodulator? Neuroproliferation? maturation, protection?	V1a receptor in OSNs mediated increase in $[Ca^{2+}]_i$ via PLC/IP3 store release V1b receptor in Globose basal cells Bowman's glands based on immunostaining	rat	Levasseur et al. 2004
12. Orexin	OSNs, sustentacular cells and lamina propria	Cilia of OSNs Microvilli of sustentacular cells	Activation of both cAMP and IP3 pathways	Increased Ca^{2+} MAP kinase activation	Rat Odora cells	Caillol et al. 2003 Gorjankina et al. 2007
13. PACAP	Basal cells OECs, OSNs, Sustentacular cells	OSNs, immature OSNs, basal cells	Increase Intracellular Ca Reduced IA Increased Na current density Proliferation Protection of mature OSNs	PAC1 receptor activation resulting in increased cAMP and PLC activity	Rat mouse	Hansel et al. 2001 Au et al. 2003 Han & Lucero 2005 Han & Lucero 2006 Hegg et al. 2003 Illing et al. 2002 Kanekar et al. 2010
14. Substance P/CGRP	Trigeminal fibers terminating just below tight junctions of OE	Sustentacular cells, blood vessels, glands, microvillar cells	Increased Ca in microvillar cells	Promote release of modulatory substances from microvillar or sustentacular cells	Mouse Rat	Finger et al. 1990 Hegg et al. 2010