

# Genetic Analysis of Brain Circuits Underlying Pheromone Signaling

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## Key Words

vomeronal, olfactory, pheromones, accessory olfactory bulb, olfactory coding, sensory coding, vomeronasal receptors

## Abstract

Molecular approaches and genetic manipulations have provided novel insights into the processing of pheromone-mediated information by the olfactory and vomeronasal systems of mammals. We will review and discuss the specific contribution of each of the two chemosensory systems that ensure specific behavioral responses to conspecific animals.

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## INTRODUCTION

To ensure survival and transmission of their gene pool, animals must recognize their conspecifics and engage in gender- and species-specific social and reproductive interactions. In many species, communication within the animal group relies on the emission and detection of specific chemical cues, the pheromones. The term pheromone was coined in 1959 by Karlsson & Luscher from

the Greek *pherin*, meaning to bring or to transfer, and *hormon*, meaning to excite (10). Pheromones were defined as “substances that are excreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, for example a definite behavior or developmental process” (31). Pheromones have been shown in insect, fish, and mammal to trigger genetically preprogrammed sets of behaviors and endocrine responses that underlie the establishment of social hierarchy, mating rituals, and parental care. The high reproducibility and significant genetic component of the response to pheromones offers a unique experimental system to uncover how internal neural templates process sensory information.

The amenability of chemosensory systems to molecular approaches and genetic manipulations has led recently to a wealth of new information on the neural basis of olfactory detection and sensory processing. The nasal cavity of rodents contains two sets of chemosensory neurons located in the vomeronasal organ (VNO) and in the main olfactory epithelium (MOE), respectively. Although initial analysis had attributed specific and distinct functions to each of the two neuronal populations—the detection of odorants by the MOE and of pheromones by the VNO—recent evidence suggests that both olfactory structures and their associated central pathways are essential for pheromone-mediated responses. What then defines the functional difference between the two chemosensory systems, and what is the driving evolutionary force that has maintained two distinct sensory systems in order to process pheromonal information? Profound differences have recently been uncovered in the logic of how sensory signals processed by the olfactory and vomeronasal systems are relayed to the brain, suggesting that each system may employ a different strategy in the processing of chemical information. These differences may represent the most significant driving

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**VNO:** vomeronasal organ

**MOE:** main olfactory epithelium

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force underlying the functional split between vomeronasal and olfactory pathways.<sup>1</sup>

We review here the essential characteristics of MOE- and VNO-mediated detection and of the neural processing of pheromone information. We further discuss the functional significance of the integration of vomeronasal and olfactory information to ensure specific behavioral responses to conspecific animals.

## THE NEURAL CIRCUITS OF PHEROMONE DETECTION

The nasal cavity of mammals contains two anatomically distinct sets of chemosensory neurons (**Figure 1**). The MOE lines the posterior recess of the nasal cavity and is accessible to small volatile chemicals inhaled during breathing. MOE sensory neurons are bipolar neurons with an apical ciliated dendrite reaching the surface of the epithelium, and a basal axon projecting to the main olfactory bulb (MOB), where they synapse with mitral cells, the bulb output neurons. The second set of nasal chemosensory neurons lies in the neuroepithelium of the VNO, a bilateral tubular-shaped structure encased within bony capsules in each side of the ventral nasal septum, and first described in 1813

by the anatomist Ludvig Jacobson (29). The microvilli-based dendritic terminals of VNO neurons reach the liquid-filled lumen of the VNO, where aqueous-soluble molecules are actively pumped in from the nasal cavity. VNO axons bundled in the vomeronasal nerve project dorsally along the septum and the medial side of the olfactory bulb and terminate in the glomerular layer of the accessory olfactory bulb (AOB), where they synapse with AOB mitral cells.

Decades of investigation have pointed to sharp contrasts in the functional characteristics of the two olfactory structures and associated neural pathways (23, 24). Classical retrograde and anterograde tracing studies have evidenced the distinct central networks forming the vomeronasal and olfactory systems (**Figure 1**). Mitral cells of the MOB send fibers to different nuclei of the paleocortex (46), collectively forming the primary olfactory cortex and including the piriform cortex (Pir), entorhinal cortex (EC), anterior olfactory nucleus (AON), olfactory tubercle (OT), and lateral amygdala (LA). These structures are in turn connected to higher cortical brain centers, enlightening the role of the main olfactory system in the cognitive processing and perception of chemical cues, commonly referred to as the sense of smell. In contrast, AOB mitral cells bypass cortical structures and project instead to four nuclei of the limbic system: the bed nucleus of the stria terminalis (BNST), the nucleus of the accessory olfactory tract (NAOT), the medial amygdaloid nucleus (MeAN), and the posteromedial amygdaloid cortical nucleus (PMCN), the later two nuclei often named the vomeronasal amygdala (51, 83). Neurons from these areas project to hypothalamic nuclei such as the medial preoptic area (MPOA), the ventromedial hypothalamus (VMH), and the premammillary and supraoptic nuclei, which are associated with reproduction, aggression, and parental behavior (33, 34, 58). A summary of these pathways is shown in **Figure 1**. The hypothalamus is an essential integrator of

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**MOB:** main olfactory bulb

**AOB:** accessory olfactory bulb

**AON:** anterior olfactory nucleus

**BNST:** bed nucleus of the stria terminalis

**NAOT:** nucleus of the accessory olfactory tract

**MeAN:** medial amygdaloid nucleus

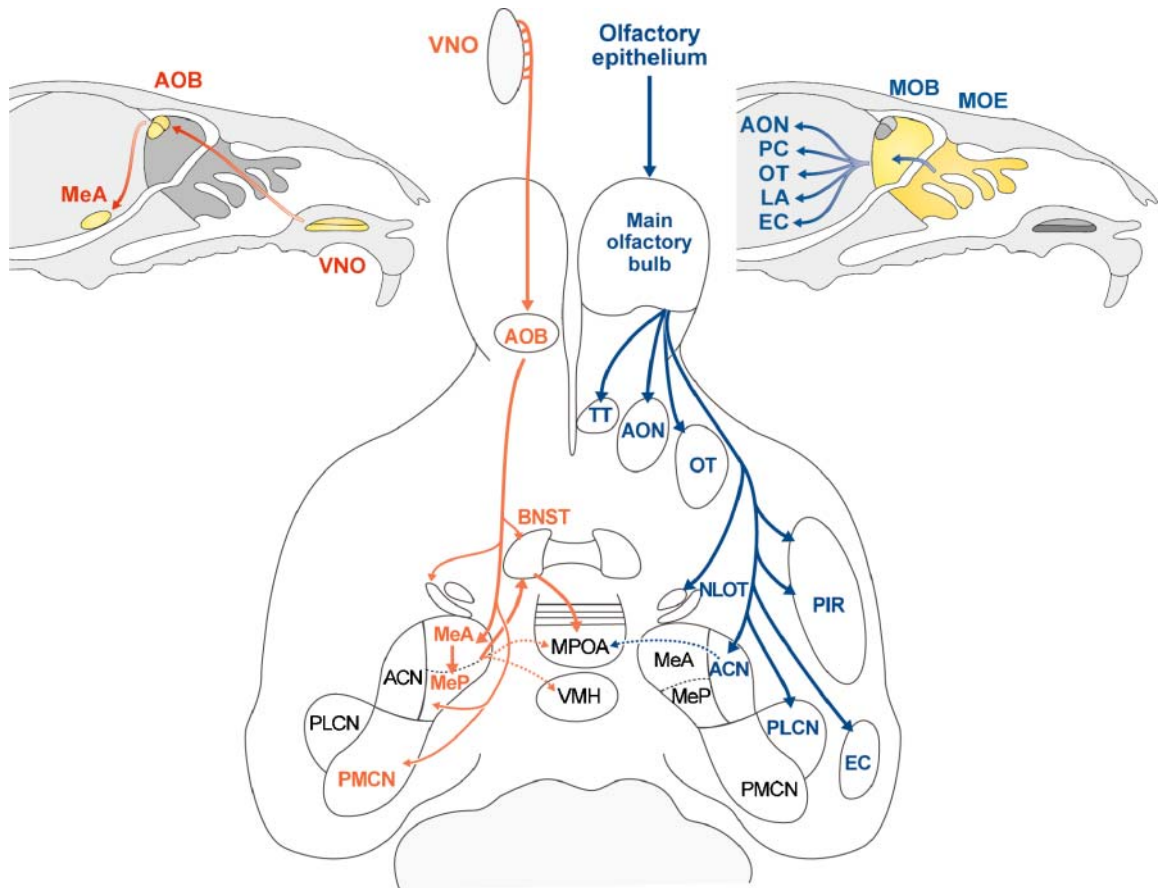
**PMCN:** posteromedial cortical amygdaloid nucleus

**MPOA:** medial preoptic area

**VMH:** ventromedial hypothalamus

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<sup>1</sup>Abbreviations used: 2MB2: 2-methylbut-2-enal; ACN: anterior cortical nucleus; AOB: accessory olfactory bulb; AON: anterior olfactory nucleus; AOT: accessory olfactory tract; BL: Barley lectin; BNST: bed nucleus of the stria terminalis; BNSTp: posterior bed nucleus of the stria terminalis; DAG: diacylglycerol; EC: entorhinal cortex; EPL: external plexiform layer; GCL: granule cell layer; GFP: green fluorescence protein; GL: glomerular layer; IP3: phosphatidylinositol-3-phosphate; LA: lateral amygdala; LHRH: luteinizing hormone releasing hormone; LOT: lateral olfactory tract; MeAN: medial amygdaloid nucleus; MHC: major histocompatibility complex; MOB: main olfactory bulb; MOE: main olfactory epithelium; MPOA: medial preoptic area; MTMT: (methylthio) methanethiol; NAOT: nucleus of the accessory olfactory tract; NL: nerve layer; NLOT: nucleus of the lateral olfactory tract; OCNC1: olfactory specific cyclic nucleotide gated channel; OR: olfactory receptors; OT: olfactory tubercle; Pir: the piriform cortex; PLCN: posterolateral cortical amygdaloid nucleus; PMCN: posteromedial cortical amygdaloid nucleus; TT: tenia tecta; VMH: ventromedial hypothalamus; VNO: vomeronasal organ.



**Figure 1**

Sensory epithelia and brain circuits mediating the detection of chemosensory cues in mice. The nasal cavity of mammals contains two anatomically and molecularly distinct collections of chemosensory neurons, each associated with a different central neuronal network. The vomeronasal system (*left*) comprises the accessory olfactory bulb (AOB), which receives sensory inputs from the vomeronasal organ (VNO) and projects to the medial (MeA/MeP) and posteromedial cortical amygdaloid nucleus (PMCN) with additional connections to the posterior bed nucleus of the stria terminalis (BNSTp). The main olfactory system (*right*) includes the main olfactory bulb (MOB), which get inputs from the main olfactory epithelium (MOE) and projects to the anterior cortical nucleus (ACN) and posterolateral cortical amygdaloid nucleus (PLCN), the anterior olfactory nucleus (AON), the olfactory tubercle (OT), the tenia tecta (TT), the piriform cortex (Pir), and the entorhinal cortex (EC). Both pathways connect to the hypothalamus.

central and environmental cues, ensuring the homeostasis of the organism, the coordination of visceral functions, and the initiation of genetically preprogrammed behaviors such as feeding, defense, and reproduction. Thus, specific changes in hypothalamic activity can in turn orchestrate both long-lasting endocrine changes and short-term behavioral

effects elicited by chemical cues. Besides its massive input to higher cortical areas, the MOB is also connected to areas of the limbic system distinct from vomeronasal projections such as the nucleus of the lateral olfactory tract (NLOT), the anterior cortical nucleus (ACN), and the posterolateral cortical amygdaloid nucleus (PLCN). These

**NLOT:** nucleus of the lateral olfactory tract

**ACN:** anterior cortical nucleus

nuclei project to hypothalamic areas, some of them shared with the vomeronasal pathway.

The gross connectivity of the vomeronasal system, bypassing cortical areas to connect directly to the limbic system, together with the small number of processing stages prior to reaching effector nuclei in the hypothalamus, raises specific predictions: The vomeronasal system is likely to mediate genetically pre-programmed responses rather than adaptive, experienced-based responses, and the processing of vomeronasal information is likely to be integrative and intensive from its early stages. Recent insights into the architecture of vomeronasal circuits largely support these predictions.

### **THE CONTRIBUTION OF THE OLFACTORY AND VOMERONASAL SYSTEMS TO PHEROMONE-EVOKED RESPONSES**

The behavioral phenotypes of animals with surgical or chemical destruction of olfactory or vomeronasal sensory structures have generally supported the predictions raised by the differential connectivity of the two systems, mainly the cognitive recognition of smell and the instinctive response to pheromonal cues by the olfactory and vomeronasal systems, respectively. Numerous studies have shown that animals in which the MOE was surgically ablated showed defects in odorant recognition, while VNO removal instead affected female estrus cycle, male and female mating behavior, and male-male and maternal female aggression (23, 85).

These initial observations were highly suggestive of divergent roles for the olfactory and the vomeronasal systems. However, the functional dichotomy between both systems was clearly not absolute and showed variations according to the species considered: male-female attraction in pigs and hamsters, for example, requires MOE function, whereas VNO function in snakes is required for prey

capture as well as for conspecific recognition (2). Furthermore, several studies have directly implicated the main olfactory system in pheromone-evoked responses, redefining its traditional role in sensing only odorant compounds. Experiments by Lin et al. using extracellular single unit recordings of MOB mitral cells identified responses to (methylthio) methanethiol (MTMT), a pheromone component of male urine that mediates attraction of female mice toward males (45). Similarly, detection of the male sex pheromone androstenone by female pigs (14, 15) and of the maternal mammary pheromone 2-methylbut-2-enal (2MB2) by newborn rabbits (66) is mediated by the olfactory epithelium.

Genetic approaches have substantiated the involvement of central olfactory pathways in pheromone responses. Two recent studies have used genetic tools to identify afferent pathways to neurons synthesizing luteinizing hormone releasing hormone (LHRH), a key neuro-hormone of reproduction produced in the hypothalamus and medial septum. In the first study (88), the conditional pseudorabies virus Ba2001 was injected into the medial preoptic area and the lateral septum of a transgenic mouse line expressing the Cre recombinase under the control of LHRH promoter. The exclusive retrograde propagation of Ba2001 along chains of synaptically connected neurons from its initial replication into Cre-expressing neurons, together with the ability to detect viral infection with a GFP (green fluorescence protein) reporter, led to the identification of the entire afferent networks of LHRH neurons (88). In contrast to the established view on the nature of LHRH neuronal inputs derived from classical tracer injections (68), the genetically controlled viral tracing identified a major projection pathway from the main olfactory system. This pathway includes all known central olfactory nuclei, and originates from a discrete population of olfactory sensory neurons in a restricted zone of the olfactory epithelium. Results fail to document any synaptic connectivity with the vomeronasal system (88). An independent

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**PLCN:**  
posterolateral  
cortical amygdaloid  
nucleus

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**OCNC1:** olfactory specific cyclic nucleotide gated channel

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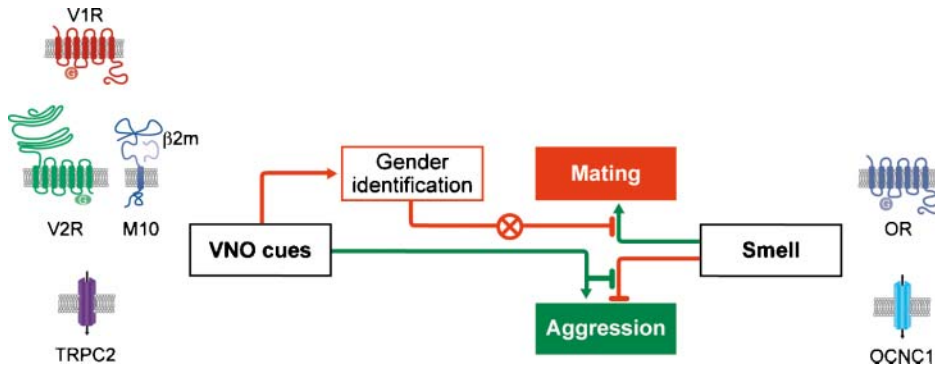
approach took advantage of the transneuronal transfer of Barley lectin (BL), and expressed BL under the control of LHRH promoter (6). Sites of BL expression, while also visualizing minor labeling along the vomeronasal pathway, also evidenced major and yet undescribed input from the main olfactory system. These results provide strong support to behavioral genetic experiments that uncovered the role of nonvomeronasal cues in the control of reproduction (19, 43, 70).

The studies summarized above clearly demonstrate the involvement of central olfactory nuclei in the relay of chemosensory information relevant to reproduction. Genetic silencing of VNO or MOE signaling resulting from the knockout of genes encoding either the TRPC2 or the OCNC1 (olfactory-specific cyclic nucleotide gated channel) ion-channel, respectively, provided additional evidence that the detection of pheromones leading to behavioral and endocrine changes relies on the activity of both the main olfactory and the vomeronasal systems (6, 18, 43, 45, 50, 70, 88). The TRPC2 channel is expressed by all VNO sensory neurons (44) and is required for their responses to pheromone stimuli. TRPC2<sup>-/-</sup> male mice appeared perfectly able to reproduce, showing no reduction in courtship and mating behavior with females. However, the TRPC2<sup>-/-</sup> male mutants showed profound defect in their ability to distinguish between males and females, and displayed mating behavior toward males and females with equal frequency (43, 70). In addition, TRPC2<sup>-/-</sup> male and female mice showed strong impairment in aggressive behavior. These results have suggested a model (**Figure 2**) in which sensory cues that trigger mating behavior do not require the VNO, whereas VNO function ensures the sex specificity of reproduction (70). Genetic or chemical manipulations impairing the detection of odorants by the main olfactory epithelium (50, 88) generate a profound defect in mating and aggressive behaviors, providing further support for the essential role of the main olfactory system in pheromone recognition.

## MOLECULAR BIOLOGY OF OLFACTORY AND VOMERONASAL CHEMOSENSORY DETECTION

Olfactory detection relies on the expression of a large family of about 1000 olfactory receptor (OR) genes (9). Each olfactory neuron expresses only one member of the OR family, and OR activation triggers a G protein-coupled signaling cascade resulting in the opening of an OCNC1, and subsequently of a chloride channel, leading to changes in membrane potential and electrical signaling. How widespread is the involvement of MOE neurons in pheromone responses? What fraction of the OR repertoire contributes to the detection of semiochemicals leading to changes in reproductive status? Two recent reports (45, 88) suggest that only a circumscribed population of olfactory neurons and mitral cells may be involved in MOE-mediated pheromone responses. The corresponding sensory neurons are likely to express genuine ORs and associated cyclic nucleotide-based cascade, although the involvement of other signaling pathways cannot be fully excluded at this point (88).

In the VNO, sensory neurons located in the apical layer of the neuroepithelium each express a single member of the *V1R* family of VNO receptors, whereas neurons of the basal half of the neuroepithelium express receptors of the *V2R* gene family (**Figure 2**). The mouse *V1R* and *V2R* gene families include about 150 functional genes each, and both belong to the G protein-coupled receptor (*GPCR*) gene superfamily. Both gene families are phylogenetically unrelated to each other and to the *ORs*. *V1Rs* share distant sequence similarity with T2R bitter taste receptors, whereas *V2R* genes are closely related to the metabotropic glutamate and GABA<sub>B</sub> receptors, as well as to the T1R sweet/umami taste receptors, characterized by a very large N-terminal extracellular domain. Recent studies indicate that *V1R*-expressing VNO neurons respond to low-molecular-weight organic molecules (11, 41)



**Figure 2**

The role of vomeronasal and olfactory signaling in gender discrimination and aggression. Neurons in the vomeronasal organ and in the main olfactory epithelium rely on distinct signaling components. VNO neurons express one member of one of the two families of vomeronasal receptors, V1Rs and V2Rs. V2Rs form a functional complex with the MHC class Ib molecules M10s. The activity of V1R- and V2R-expressing neurons requires the expression of the TRPC2 channel. MOE neurons express one member of the large family of olfactory receptors (OR), and require the expression of the OCNC1 channel. Behavioral analysis of TRPC2<sup>-/-</sup> males reveals the essential role of the VNO in controlling the sex specificity of reproductive behavior, and in male-male aggression. In contrast, analysis of OCNC1<sup>-/-</sup> males reveals that olfactory cues are essential to trigger mating behavior in mice.

while V2R-expressing cells respond to peptides (35, 40). Unlike the much larger family of main olfactory receptors (OR), both the V1R and V2R gene families show unusual diversity with multiple subfamilies or clades, which are extremely divergent phylogenetically (64, 86). The high degree of divergence between VR genes belonging to different clades is in sharp contrast to the smooth continuum in OR sequence variability, in which the whole space of sequence diversity is spanned. This distinct molecular organization of ORs and VRs likely reflects the different functional and evolutionary constraints exerted on olfactory and vomeronasal function, respectively.

A novel and unexpected layer of complexity has recently been added to our understanding of the chemical detection process in the VNO with evidence for a functional association between the V2R pheromone receptors, the M10 and M1 families of non-classical major histocompatibility complex (MHC) molecules, and beta2-microglobulin ( $\beta 2m$ ). M10s are exclusively expressed in the VNO, where they act as coreceptors of the

pheromone receptors V2Rs (47). The crystal structure of the ectodomain of M10.5, an M10 family member, is similar to that of classical MHC molecules (57). However, the M10.5 counterpart of the MHC peptide-binding groove is open and unoccupied, revealing the first structure of an empty class I MHC molecule. Similar to empty MHC molecules, but unlike peptide-filled MHC proteins and nonpeptide-binding MHC homologs, M10.5 is thermally unstable, suggesting that its groove is normally occupied. Future studies will determine the role of the M10 groove in the interaction with V2Rs or with other types of ligands with possible roles in the pheromone response.

V1R and V2R signaling relies on the activation of phospholipase C and results in generation of phosphatidylinositol-3-phosphate (IP3) and diacylglycerol (DAG) followed by the production of arachidonic acid (5, 26), which in turn activate the TRPC2 cation channel localized in the microvilli of VNO sensory neurons. Its role in pheromone detection was confirmed with the analysis of TRPC2 knockout mouse lines that exhibit

**EPL:** external plexiform layer

**LOT:** lateral olfactory tract

significant defects in VNO-evoked responses (43, 44, 70).

## NEURONS AND NEURONAL CIRCUITS OF THE MAIN AND ACCESSORY OLFACTORY BULBS

The cellular organization of the MOB and AOB is similar in large part and consists of (from dorsal to ventral) the superficial nerve layer (NL), the glomerular layer (GL), the external plexiform layer (EPL), the lateral or accessory olfactory tract (LOT, AOT), and the granule cell layer (GCL) (51).

The axon terminals of MOE and VNO sensory neurons, arriving through the NL, create globular neuropil structures called glomeruli. Glomeruli are a structural feature shared by all olfactory-related sensory systems across most multicellular phyla (1, 25). Mitral cells send elaborated dendritic tufts into glomeruli, on which sensory terminals synapse. MOB glomeruli are uniform in size (~50  $\mu\text{m}$  in diameter in mice) and lie side by side along the 1–2-glomeruli-deep glomerular layer. MOB glomeruli are encapsulated by periglomerular neurons and glial cells that provide clear anatomical and functional separation between individual glomerular units (36). In contrast, AOB glomeruli are highly variable in size (10–30  $\mu\text{m}$  in diameter) and are tightly clustered within the glomerular layer with only scarce periglomerular neurons distributed among them. While a few thousand sensory neurons converge on each of the ~2000 MOB glomeruli, there are only up to few hundred sensory terminals in each AOB glomerulus. The numerical relation between sensory terminals and mitral cells within glomeruli is around 1000:1 in the MOB and 100:1 in the AOB (24, 51).

Juxtglomerular interneurons lie below the glomerular layer, separating it from the EPL (74). The biology of these neurons is almost unexplored in the AOB, and any functional analogy with MOB juxtglomerular neurons, which include periglomerular, short-axon, and external-tufted cells, is mostly

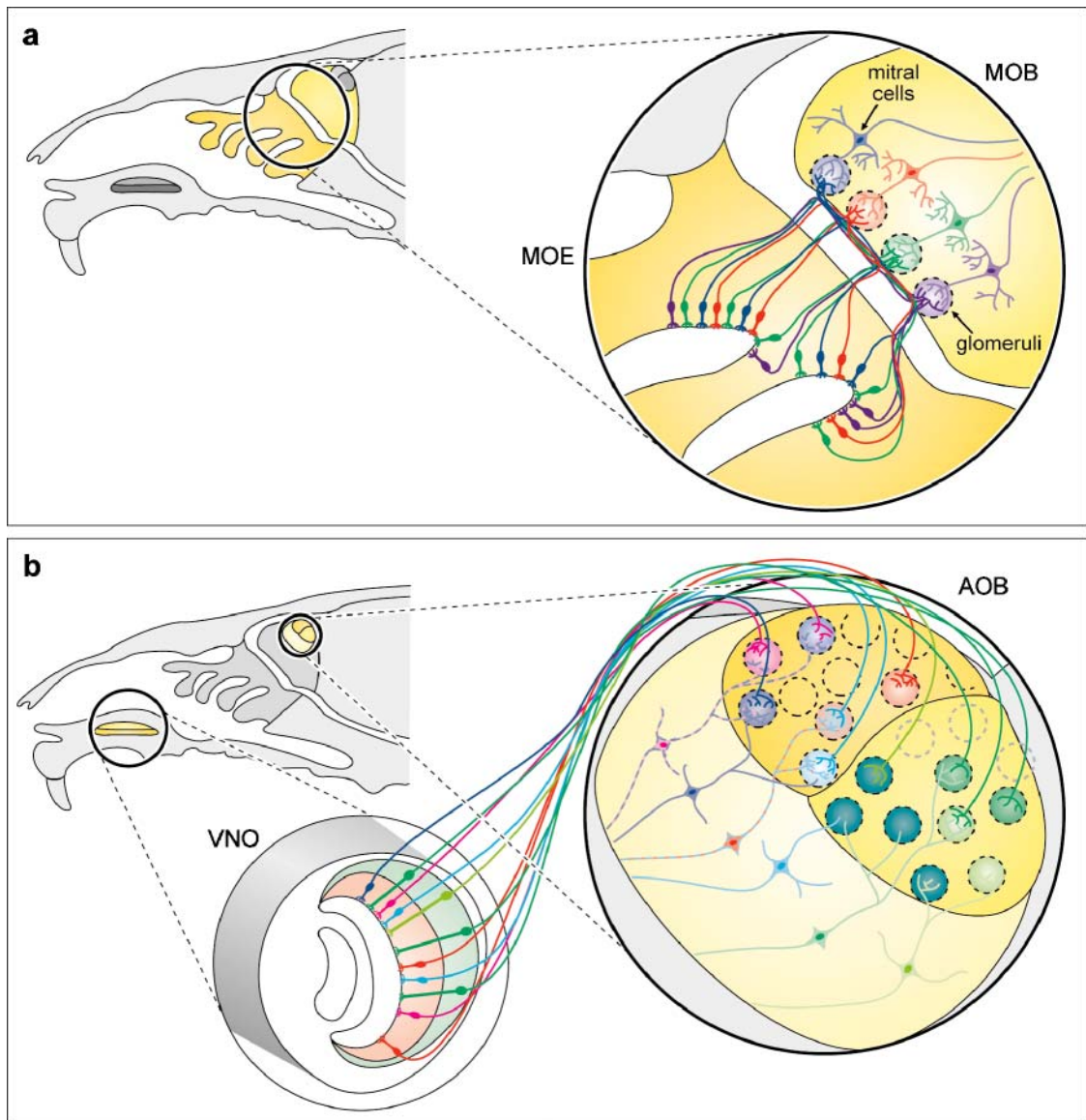
speculative (59, 60). AOB juxtglomerular neurons include both GABAergic and glutamatergic neurons (62, 74), but, in contrast to the MOB, do not seem to include dopaminergic interneurons (51).

The cell bodies of mitral cells are located mainly in the ventral part of the EPL, whereas their dendrites extend toward the glomeruli (76). Each MOB mitral cell sends one apical dendrite connecting to a single glomerulus (**Figure 3**) and multiple basal dendrites laterally on which granule cell dendrites create reciprocal excitatory/inhibitory synapses (67, 78). In sharp contrast, most AOB mitral cells (**Figure 3**) send multiple apical dendrites that contact 3 to 9 glomeruli in various locations of the AOB glomerular layer (75, 76). Moreover, AOB mitral cells send only 1–2 basal dendrites, and AOB granule cells synapse mainly on mitral cell apical dendrites (62), in a situation reminiscent of the fish olfactory bulb (16, 27, 37, 38) and the moth antennal lobe (30, 81). At first glance, the multiglomerular morphology of AOB mitral cells might suggest a significant integration of information coming from various inputs carried out by these cells (32). In addition, the dendritic tufts of AOB mitral cells are highly variable in shape and size, ranging from elaborated “ball of yarn”-like tufts similar to those of MOB mitral cells, through basket-like and bush-like tufts, tufts containing only few fine branches, and to tufts with a “dead” end (76). The functional significance of this variability is unknown, but it might imply a hierarchy between different inputs or a dynamic process of glomerulus-tuft connectivity.

## CENTRAL REPRESENTATION OF OLFACTORY AND VOMERONASAL SIGNALS

Spatially organized representations of sensory inputs enable dedicated brain areas to generate a neural code for stimuli detected in the environment. In most systems, such as the visual or somatosensory systems, the brain spatial representation, or map, of external





**Figure 3**

Cellular architecture of the main olfactory and vomeronasal systems. Olfactory sensory neurons in the MOE that express a given odorant receptor project to spatially conserved glomeruli in the MOB, where they synapse with mitral cells. Each mitral cell projects a unique dendrite to a glomerulus that is, in turn, innervated by only one neuron type. This organization provides little integration of signals at the level of the MOB. In contrast, sensory neurons in the epithelium of the VNO are segregated into apical and basal zones. Neurons with cell bodies located in the apical zone (shown in red) express members of the V1R family of receptors and project to multiple glomeruli in the anterior half of the AOB. Neurons in the basal zone (shown in green) express V2R receptors as well as M10 family members and project to multiple glomeruli in the posterior portion of AOB. AOB mitral cells send dendrites to multiple glomeruli as well.

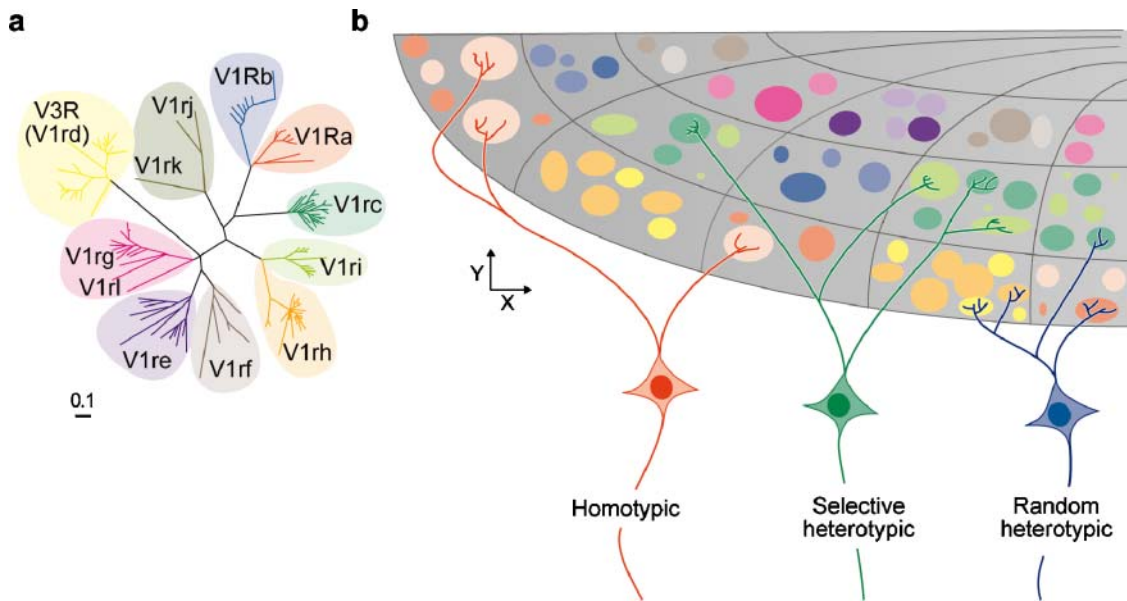
sensory stimuli, mirrors the topographical organization of the sensory cells themselves, for example, the position of ganglion and photoreceptor cells in the retina or the location of nerve terminals in the skin. This organization merely involves the conservation of the spatial organization of neurons in periphery into their central projections. However, the topographical representation of the sensory sheet in the brain would not provide any meaningful coding for olfactory and vomeronasal stimuli, because sensory neurons expressing distinct ORs and VRs are randomly dispersed within the VNO and MOE neuroepithelia. The nature of the central representations of olfactory and vomeronasal stimuli must therefore be different.

In situ hybridization of *OR* probes on MOB slices (63, 80) and genetic experiments expressing a reporter gene under the control of olfactory receptor loci (52) have shown that olfactory neurons expressing the same OR, although randomly distributed in the MOE, converge onto one or a few topographically invariant glomeruli in each side of the MOB, thus generating a chemotopic map (**Figure 3**). A variety of additional receptor loci modifications and imaging techniques (4, 7, 20, 28, 54, 56, 61, 71, 79, 90) have confirmed and complemented these results, and have further visualized two symmetrical chemotopic maps on each side of the bulb. Glomeruli corresponding to projections of neurons expressing a specific receptor are located in conserved positions across individuals, and neurons expressing closely related receptors project to neighboring glomeruli. Neural stimulation resulting from the activation of each OR is thus represented by the activation of one or few glomeruli at fixed positions in each side of the bulb. Hence, the organization in the olfactory bulb provides a transformation of the molecular identity of each OR into a spatial representation of glomerular position. The molecular logic underlying this transformation is not yet known, but electrophysiological and imaging studies have further uncovered the general features of the MOB sen-

sory map (73, 77). As recently summarized by Mori et al. (55), olfactory receptors sharing a common molecular-feature receptive field tend to occupy neighboring glomeruli, creating areas dedicated to the processing of specific primary molecular features, for example, functional groups such as aldehydes or alcohols. Within each area, secondary molecular features such as length or branching of carbon chain are represented by a local arrangement, for example, by a gradual shift in the position of the activated glomeruli when the carbon-chain length of the odorant increases.

The mode of organization of the AOB is very different. Several experiments (3, 12, 65), employing genetic strategies developed in the main olfactory system (52), have shown that cells expressing the same V1R or V2R converge onto subsets of AOB glomerular targets. However, in contrast to the projection of olfactory neurons in the MOB, VNO sensory neurons expressing the same *VR* send axons to 10 to 30 distinct AOB glomeruli. Glomeruli associated with a given *VR* are clustered in several large domains located in specific areas of the glomerular layer (anterior AOB for V1Rs, posterior AOB for V2Rs), thus creating a complex spatial representation of receptor activation in the AOB (**Figure 3**). In sharp contrast to the invariant positions of the MOB glomeruli, the locations of AOB glomeruli associated with specific V1R and V2R receptor populations appear only roughly conserved between individuals. Thus, the spatial representation of vomeronasal receptors in the AOB appears complex, prone to individual variation and without any obvious logical organization of sensory inputs. These characteristics are clearly counterintuitive in a sensory system assumed to mediate stereotypic and genetically preprogrammed responses to chemical stimuli.

A recent study reasoned that the AOB spatial representation of vomeronasal receptors might obey yet unseen topographical rules, which would emerge from comparison of the positions of multiple glomerulus types over the entire bulb. In order to pursue this



**Figure 4**

Ordered integration of VNO inputs in the AOB. Model for *V1R* subfamily-based organization of glomeruli in the anterior AOB. The *V1R*s are subdivided into 12 subfamilies, with a high degree of sequence divergence between *V1R* genes belonging to distinct clades. Glomeruli innervated by neurons expressing closely related receptors of the same subfamily are grouped together in several domains at conserved positions of the AOB GL. Three possible schemes for connectivity of mitral cells to these glomeruli are shown: homotypic, selective heterotypic, and random heterotypic. Direct injection of dyes into mitral cells suggests that the mitral cells tend to establish selective heterotypic connections with glomeruli associated with closely related receptors.

hypothesis, Wagner et al. (84) sought to monitor the representations of multiple receptors within the same animal. The approach took advantage of the clustering of *V1R*s in the mouse genome (11, 13), and used BAC (bacterial artificial chromosome) transgenesis (87) to genetically associate the expression of several *V1R*s with distinct fluorescent reporter proteins. To take into account the molecular organization of the *V1R* family into divergent clades (Figure 4), the loci of two receptors from the *V1Ra* subfamily and one receptor from the *V1Rb* subfamily were modified, generating the SW3M mouse line. In addition, three independent mouse lines were generated in which the loci of a single receptor of the *V1Re* subfamily, and of two receptors from the *V1Rb* subfamily were individually modified (84). The relative locations of the *V1Re*- and *V1Rh*-associated glomeruli were assessed

by crossing the corresponding mouse lines to a line in which the *V1Ra1* locus had been modified by gene targeting (3).

Analysis of these transgenic mouse lines revealed new features of the organization of the vomeronasal sensory map. In the SW3M transgenic mice, genetically labeled glomeruli appear clustered within specific domains that are common for receptors belonging to the same subfamily. For example, glomeruli representing the two *V1Ra* members are intermingled in a deeply located ventral strip while glomeruli representing the *V1Rb* member are positioned in a superficial dorsal strip (Figure 4). Similar organization was found by examining mouse lines in which two members of the *V1Rb* subfamily and one member of the *V1Eb* subfamily were modified (84). From this study a general model of AOB glomerular organization was proposed, according to

which the anterior AOB glomerular layer is divided in a grid-like manner with distinct columns and strips that obey the segregation lines of *VIR* subfamily sequences (**Figure 4**). Accordingly, members of each subfamily are represented in several domains, which are located in a single strip along the dorsal-ventral axis but within few columns. Within each large glomerular domain, multiple glomeruli representing a given receptor are seemingly randomly intermingled with glomeruli associated with the other members of the subfamily.

### **CODING PRINCIPLES IN THE OLFACTORY AND VOMERONASAL SYSTEMS**

The features described above demonstrate an ordered pattern of vomeronasal projections, a topographic map of receptor activation that follows specific molecular rules. The significance of this representation relies on how it is processed, or encoded, by the AOB mitral cells. In mammals, each MOB mitral cell sends one apical dendritic tuft into a single glomerulus from which it receives excitatory inputs, whereas modulatory signals are provided through secondary dendrites and interneurons. Thus, the olfactory information follows a labeled line of sensory processing that originates from a defined population of sensory neurons expressing a given OR, and is transmitted to a specific glomerulus, and in turn to a dedicated population of mitral cells (48). The multiglomerular mitral cells of fish (16, 21, 27, 37, 38) and the projection neurons in the antennal lobe of some insects (30, 82) are likely to process the olfactory information differently. Similarly, the extensive divergence of inputs arising from neurons expressing a given vomeronasal receptor and sent into many AOB glomeruli, combined with the multiglomerular nature of AOB mitral cell connectivity, have raised the prediction that extensive integration of excitatory activity may take place in the AOB (32). The early integration of multiple recep-

tors signals at the level of the AOB appears quite advantageous in the vomeronasal pathway because it contains fewer relay stations than the olfactory system. However, an initial report showed examples of AOB mitral cells projecting dendrites to glomeruli receiving identical receptor inputs (12). The authors concluded that, despite their multiglomerular nature, AOB mitral cells receive information from multiple glomeruli associated with the same VR, and suggested a homotypic model of mitral cell connectivity (**Figure 4**). According to this model, the vomeronasal information follows a labeled line of sensory processing similar to the one demonstrated in the main olfactory system (48). The nature of AOB mitral cell connectivity was further examined using the SW3M mice in which the projection of multiple receptor populations can be visualized. Injections of intracellular dyes into AOB mitral cells identified by patch clamp showed that mitral cells connected to glomeruli associated with the *VIRa1* or the *VIRa3* receptors also project to genetically unlabeled glomeruli, presumably associated with distinct receptors (84). Furthermore, an example of a mitral cell connected to two glomeruli associated with distinct receptors, as well as to additional unlabeled glomeruli, was documented. These results show that a significant fraction of AOB mitral cells receive inputs from glomeruli associated with more than one receptor, and thus exhibit heterotypic connectivity (**Figure 4**). Further studies monitoring the distances between dye-filled dendritic tufts and genetically labeled glomeruli suggest that mitral cells have a preference toward certain domains within the AOB glomerular layer (84) and that this preference may be geared toward inputs from members of the same receptor subfamily (**Figure 4**).

It is now possible to describe the sensory representations and coding principles of the olfactory and vomeronasal information in a more general way. A central representation of stimuli, or map, according to which the relative location of sensory inputs is predictive of the exact nature of the stimulus, provides

a spatial code. In contrast, an identity code is provided by a representation of sensory inputs, or map, in which the relative location of inputs does not bear information about the exact nature of the represented stimuli (39). Accordingly, the labeled line of information flow in the MOB organized according to the nature of individual ORs is predictive of the molecular features of OR ligands (55) and thus provides a spatial code of the olfactory information. The organization of the AOB appears dramatically different. AOB innervation is organized in clusters or domains that mirror the sequence clustering of *VIR* genes into phylogenetically divergent clades. This contrasts with the spatially ordered distribution of glomerular positions in the MOB that reflects the continuous *OR* sequence variation and correlated change in the molecular features of OR ligands. Moreover, the *VIR* map appears highly repetitive: Each subfamily-related cluster of glomeruli is represented in multiple locations of the AOB glomerular layer, and each subfamily-based domain contains multiple glomeruli corresponding to a given receptor. Additionally, glomeruli representing receptors belonging to the same subfamily are intermingled within each domain with no intradomain spatial order. Thus local computation of *VIR*-related information is likely to take place within each subfamily-related domain, a design that facilitates integration of, and discrimination between, chemicals within a blend. One can therefore conclude that, since the location of a signal in the AOB glomerular layer is predictive of the identity of a group of cues associated with a particular receptor subfamily, the AOB sensory map provides an identity rather than a spatial code.

## THE DUAL ROLES OF THE MAMMALIAN CHEMOSENSORY SYSTEMS

The characteristics of animal chemosensory systems are likely to result from complex and sometimes opposite functional constraints.

At the level of sensory detection, both generalist as well as selective recognition of chemical cues is required. On the one hand, the thorough appraisal of changes in the environment, including the detection of rare scents, implies the ability to detect the vast arrays of chemical cues that largely exceeds the number of genes encoding chemosensory receptors. Therefore, receptors with relatively low selectivity are more suitable than narrowly tuned detectors. Accordingly, olfactory receptors show typically poor selectivity over a range of concentrations and display detection thresholds in the micromolar range (17, 49). On the other hand, highly selective and sensitive receptors are required to identify unique molecules that bear important information for survival of the species, for example, specialized food sources, suitable mates, or known predators. Dedicated sensory channels must therefore ensure the exclusive and sensitive recognition of selected compounds. The narrow tuning and high sensitivity of vomeronasal receptors to pheromones, with no change in specificity over a large range of concentrations and sensitivity in the picomolar range (40, 41), indicate that the vomeronasal system may be perfectly adapted to play this role.

At the level of information processing, both synthetic and analytic representations of chemical cues are necessary. Olfaction is unique in its synthetic ability to give meaning and memorize any combination of odorants. The synthetic representation requires integrative processing of information that enables the animal to remember a new location by its unique blend of odorants. On the other hand, the system should also be able to recognize meaningful cues in the background of others, for example, to identify and locate food in a complex environment. Thus, an analytical processing is needed to isolate each component of the blend. The mammalian olfactory bulb seems especially designed for analytical processing of odorants. The convergence of thousands of MOE sensory terminals of a given type into specific glomeruli,

together with the single apical tuft of mitral cells and the lateral inhibition of granule cells on basal dendrites, provides a powerful analytical process. Indeed, experiments monitoring the responses of rodents' MOB mitral cells have shown that one component can dominate the response whether applied alone or within a mixture (22). The synthetic integration of these lines is likely to occur in higher cortical areas in which extensive convergence of information has been described (91). The wiring of homologous structures in nonmammalian species such as the olfactory bulb of fish or the antennal lobe of some insects is different. Fish mitral cells send several apical dendrites to multiple glomeruli but do not have basal dendrites, such that granule cells synapse on the apical dendrites shafts, a wiring pattern similar to that of the mammalian AOB. This wiring scheme seems less analytical and more integrative, suitable for detecting blends rather than single compounds. Indeed, it has been shown in zebrafish that the response of mitral cells to food extracts is not dominated by any single component (72). Similar results were found in projection neurons from moth antennal lobes (82).

Thus, the characteristics of the mammalian olfactory and vomeronasal systems strongly contrast with each other. At the level of chemosensory detection, olfactory receptors show relatively low selectivity over a large range of concentrations and can therefore mediate responses of MOE sensory neurons to inordinate numbers of odorants (17, 49). In contrast, vomeronasal receptors are narrowly tuned and highly sensitive, and can therefore mediate highly selective genetically programmed sensory responses in VNO neurons (40, 41). At the level of central processing, MOB mitral cells are uniglomerular and receive inhibitory connections on their lateral dendrites (67), enabling analytical information processing. In contrast, AOB mitral cells are mutiglomerular with granule-cell mediated inhibition on their apical dendrites (76), which may reflect integrative information processing.

## CONCLUDING REMARKS: THE ROLE OF THE VOMERONASAL SYSTEM IN PHEROMONE-MEDIATED RESPONSES

Pheromonal stimuli provide complex information about an animal's social and sexual status: Specific sets of cues encode the strain, gender, health, age, hormonal state, and genetic and familial identity of the individual. What are the respective contributions of the olfactory and vomeronasal systems to the detection of these various cues and to orchestrating behavioral responses to pheromones? Chemicals carrying pheromonal information are typically small organic molecules that seem likely to reach equally well the olfactory and the vomeronasal epithelia: Small hydrophobic molecules are efficiently transported in nasal mucus by odorant-binding proteins of the lipocalin family while compounds activating VNO neurons display a distinctive "smell," suggesting activation of MOE neurons as well. Thus, the distinction between the volatile or nonvolatile natures of olfactory and vomeronasal cues is likely to be inaccurate and should be abandoned. The dramatic differences uncovered in the wiring diagrams of the MOB and the AOB suggest that the contribution of the olfactory and the vomeronasal system in pheromone-mediated responses lies instead in how the chemical information is processed. Pheromones have been shown to act as single molecules or as a blend of chemicals. We propose that the nature of the pheromone signal as a single molecule or a blend of components determines whether it will be efficiently processed through the main or the vomeronasal system.

Single molecules carry specific information regarding the social or sexual status of an individual. For example, MTMT, a single compound released in mouse male urine and absent in females (45), provides gender information and mediates attraction of females toward males. Similarly, androstenone in pigs mediates male attraction toward females (14).

This strategy, however, is “expensive” and likely to be limited: It is difficult to imagine every physiological or social state represented by the presence or absence of a distinct molecule. Furthermore, information about the specific state or identity of the animal is also efficiently provided by ratio differences between molecular components of a blend. Variations in ratios of chemicals in animal secretions result from differences in hormonal, physiological, or even social states, which in turn influence individual metabolisms (69). Thus, an animal can gather precise information about conspecifics by analyzing subtle variations in the ratio differences of metabolites present in bodily secretions.

Detection of single compounds or blends may be better achieved using different architectures of the sensing systems. Precise identification of single molecules requires labeled line analytical processing, whereas identification of chemical ratios within blends involves simultaneous analysis and comparison of multiple compounds. Hence, the MOB ar-

chitecture appears more appropriate for single molecule detection, whereas the AOB architecture seems preferable for identification of blends. Assuming that receptors with closely related sequences bind related ligands, the tendency of AOB mitral cells to receive inputs from members of the same receptor subfamily (84) may be directed at analyzing blends of structurally related molecules. In support of the proposed functional distinction between the olfactory and vomeronasal systems, all single molecules shown to trigger pheromone responses in mammals, such as androstenone in pigs (14, 15), 2MB2 in rabbits (66), and MTMT in mice (45), act via the MOS. Similarly, the few mammalian compounds known to activate the VNO and to trigger behavioral responses act mostly as groups of structurally related molecules (8, 19). We therefore propose that the vomeronasal pathway has evolved as a unique neural template to efficiently identify chemical blends relevant to behavioral responses and recognition of conspecifics.

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