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[REVIEW]

Diverse Systems for Pheromone Perception: Multiple Receptor Families in Two Olfactory Systems

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Traditionally, the olfactory epithelium is considered to recognize conventional odors, while the vomeronasal organ detects pheromones. However, recent advances suggest that vertebrate pheromones can also be detected by the olfactory epithelium. In the vomeronasal organ and the olfactory epithelium, structurally distinct multiple receptor families are expressed. In rodents, two of these receptor families, V1R and V2R, are expressed specifically in the vomeronasal organ and detect pheromones and pheromone candidates. A newly isolated trace amine-associated receptor detects some of the putative pheromones in the mouse olfactory epithelium. In addition, distinct second-messenger pathways and neural circuits are used for pheromone perception mediated by each receptor family. Furthermore, the function of these receptor families in these olfactory organs appears to differ among various vertebrate species. The systems for pheromone perception in vertebrates are far more complex than previously predicted.

Kew words: vomeronasal receptor, vomeronasal organ, vomeronasal system, olfactory epithelium, main olfactory system

INTRODUCTION

Pheromones have profound effects on vertebrate reproductive and social behavior, as well as on neuroendocrine responses. The term “pheromone” was originally defined as “substances secreted to the outside of 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” (Karlson and Luscher, 1959). This definition was mainly based on research on insect pheromone. The definition for vertebrate pheromone is somewhat different from that in insects because it is difficult to define a “specific reaction raised by pheromones”. Vertebrates have a more complex brain system, and vision and audition also influence vertebrates’ behavior when compared with insects. Therefore, it is difficult to quantitatively analyze vertebrates’ “specific reactions”. A lack of sensitive and simple methods to assess pheromone responses in vitro also makes it difficult to analyze them.

However, the identification of vomeronasal receptor families that are specifically expressed in the rodent vomeronasal organ (VNO) (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997) began a new era for pheromone research. The combination of molecular biological methodology and behavioral analyses led to profound insight concerning the molecular mechanisms underlying pheromone perception. In this review, I briefly summarize the recent advances concerning pheromone perception in the two olfactory systems, and describe the multiple receptors expressed in these two systems, based mainly on data obtained from rodents. Furthermore, I describe that the expression and functions of these receptor families in the two olfactory systems differ among various vertebrate species, thus suggesting that vertebrates have developed diverse systems for pheromone perception for the survival of both individuals and the species.

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Pheromone perception in the two olfactory systems

Vertebrate pheromones

Vertebrate pheromones (Table 1) are structurally classified into two categories: volatile molecules with low molecular weight, and nonvolatile molecules such as peptides or proteins. Volatile pheromones, such as (R, R)-3,4-dehydro-exo-brevicomin and 2-sec-butyl-4, 5-dihydrothiazole synchronize estrous, accelerate puberty, and promote male-male aggressive behavior in mice (Novotny et al., 1985; Jemiolo et al., 1986; Novotny et al., 1999); 2-heptanone extends estrous in female mice (Novotny, 2003); methylthiomethanethiol (MTMT) enhances urine attractiveness to female mice (Lin et al., 2005); and 5-α-androst-16-en-3-one facilitates a receptive mating stance in estrous pig females (Dorries et al., 1997). Nonvolatile pheromones, such as F prostaglandins, attract male goldfish (Sorensen et al., 1988);

Table 1. Vertebrate pheromones and pheromone candidates. NA, data not available.

		Effects	Mediator
Volatile			
(R, R)-3,4-dehydro-exo-brevicomin	mouse	synchronizes estrous, accelerates puberty induces male-male aggressive behavior	vomeronasal/main olfactory
2-sec-butyl-4, 5-dihydrothiazole	mouse	synchronizes estrous, accelerates puberty induces intermale aggression	vomeronasal/main olfactory
2-heptanone	mouse	extends estrus	vomeronasal/main olfactory
Methylthiomethanethiol (MTMT)	mouse	enhances attractiveness to females	main olfactory
Dimethyl disulfide	hamster	induces copulation in males	vomeronasal
5-α-androst-16-en-3-one	pig	induces standing response in females	main olfactory
2-methylbut-2-enal	rabbit	induces nipple search response in pups	main olfactory
Nonvolatile			
Prostaglandin $F_{2\alpha}$	goldfish	attracts males	main olfactory
Sodefrin	newt	attracts females	vomeronasal
Splendipherin	frog	attracts females	NA
Major urinary protein (MUP)	mouse	induces male-male aggressive behavior accelerates puberty	vomeronasal
MHC class I peptide	mouse	forms a pheromonal recognition memory in females, alters social preference of males	vomeronasal/main olfactory
Exocrine gland-secreting peptide 1 (ESP-1)	mouse	NA	vomeronasal

and newt sodefrin and frog splendipherin, which comprise 10 and 25 amino acid residues, respectively, attract females (Kikuyama et al., 1995; Wabnitz et al., 1999). Without its ligands, nonvolatile major urinary protein (MUP), which is known to carry small organic ligands, promotes aggressive male-male behavior (Bacchini et al., 1992; Brennan et al., 1999; Chamero et al., 2007) and accelerates puberty (Mucignat-Caretta et al., 1995). Major histocompatibility complex (MHC) class-I peptide is also regarded as a candidate for a nonvolatile pheromone that is involved in the formation of pheromonal recognition memory in female mice (Leinders-Zufall et al., 2004).

The main olfactory and vomeronasal systems

The majority of vertebrates possess two olfactory organs (Fig. 1): the olfactory epithelium (OE) and the VNO.

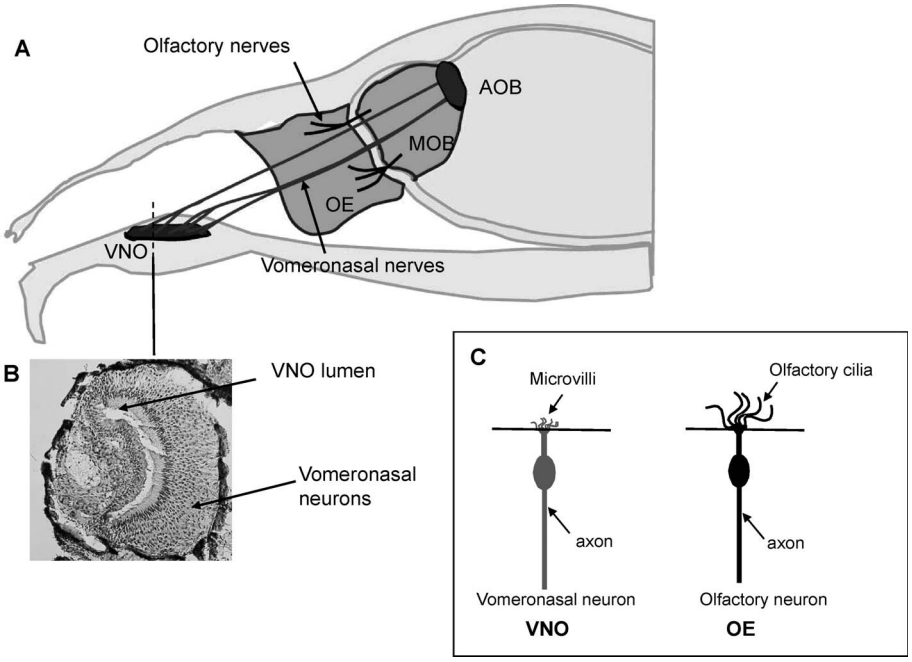


Fig. 1. Sagittal section of the mouse head showing the vomeronasal and main olfactory systems. **(A)** The vomeronasal organ (VNO) and its nerve projection to the accessory olfactory bulb (AOB), and the olfactory epithelium (OE) and its nerve projection to the main olfactory bulb (MOB). Dotted line indicates the position of the coronal section of the VNO. **(B)** Coronal section of the rodent VNO. **(C)** Schematic illustrations of the vomeronasal neuron (left) and olfactory neuron (right).

The OE is generally considered to recognize conventional odors, whereas the VNO primarily detects pheromones (Estes, 1972; Halpern and Martinez-Marcos, 2003). In rodents, bipolar olfactory neurons exist in the OE that lines the surface of the turbinate bones in the nasal cavity, and extend their dendrites to the epithelial surface (Fig. 1A and C, right). Long cilia emerge from the knobs of the apical dendrites of the olfactory neurons (Fig. 1C, right). Odor information detected by the surface of the cilia membrane is transmitted through the axons of the olfactory neurons to the main olfactory bulb (MOB) (Fig. 1A), and is relayed via the piriform cortex to reach the orbitofrontal cortex (Shipley et al., 2004). The vomeronasal neurons line the crescent-shaped lumen of the VNO (Fig. 1B), which has a paired, tubular structure divided by the nasal septum and is located at the base of the nasal cavity (Fig. 1A). Numerous microvilli emerge from the knobs of the apical dendrites of the bipolar vomeronasal neurons (Fig. 1C, left). Pheromone information detected by the surface of the microvillar membrane is transmitted through the axons of the vomeronasal neurons to the accessory olfactory bulb (AOB) (Fig. 1A), and is relayed via the amygdala to reach the hypothalamus, resulting in changes in endocrine status or affecting the autonomic nervous system (Johnston, 2000).

Although the main olfactory and vomeronasal systems are anatomically distinct (Halpern and Martinez-Marcos, 2003), both systems have been reported to be connected with the neurons that secrete gonadotropin-releasing hormone (GnRH). GnRH controls the peripheral and central aspects of reproduction, and affects a variety of mating behavior and fertility (Silverman et al., 1994; Dobson et al., 2003). The vomeronasal pathway is one of the major inputs to anatomical areas containing GnRH-expressing neurons (Meredith, 1998; Evans, 2003). By using genetically introduced tracing markers, two groups reported that there is a synaptic connection between the main olfactory pathway and GnRH-expressing neurons in mice (Boehm et al., 2005; Yoon et al., 2005). These results suggest that not only the vomeronasal but also the main olfactory pathway plays a role in reproduction and fertility.

Signal transduction in olfactory and vomeronasal neurons

Proposed models for signal transduction in rodent olfactory and vomeronasal neurons are as follows. In olfactory neurons, odor molecules bind to odorant receptors (OR) and trigger the activation of Golf, and this activation stimulates adenylyl cyclase type III (ACIII), resulting in increased intracellular cyclic adenosine 3', 5'- monophosphate (cAMP); increased cAMP then opens cyclic nucleotide-gated (CNG) cation channels, and the elicited influx of Ca^{2+} and Na^{+} depolarizes the membrane and opens Cl^{-} channels (Firestein, 2001) (Fig. 2A). Thus, odor information is converted to an electrical signal. In contrast, the signal transduction pathway in vomeronasal neurons is distinct from that in olfactory neurons in the main olfactory system. In vomeronasal neurons, pheromones bind to receptors and activate G protein; this activates phospholipase C (PLC), and PLC catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2), leading to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) formation (Kashiwayanagi, 2002; Halpern and Martinez-Marcos, 2003). Spehr et al. (2002) reported that arachidonic acid is synthesized by DAG lipase, and finally TRP2, a Ca^{2+}-permeable cation channel which is specifically expressed in the vomeronasal neurons, opens (Liman et al., 1999; Lucas et al., 2003). The pheromone signal is thereby converted to an electrical signal (Fig. 2B).

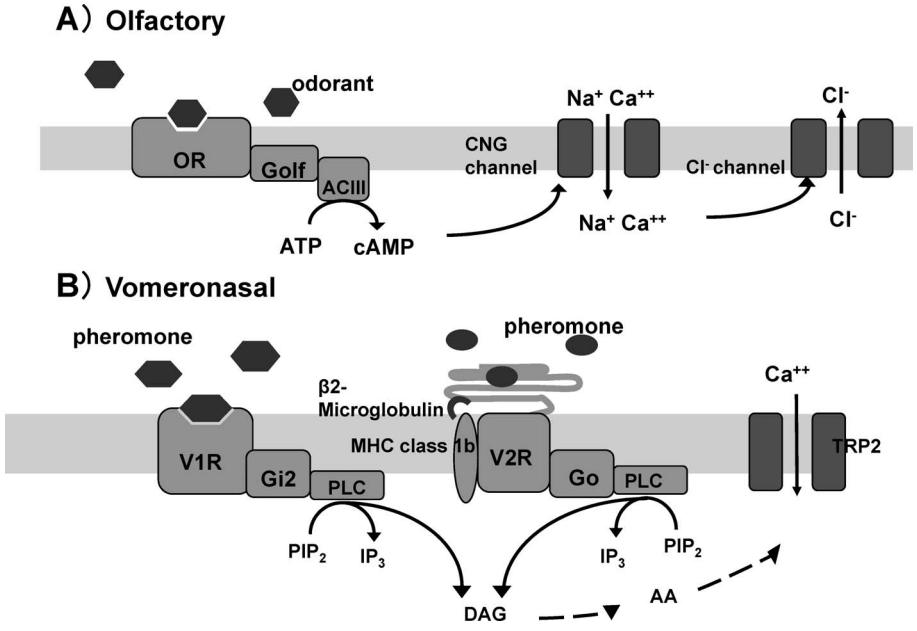


Fig. 2. Model of signal transduction pathways of the rodent olfactory and vomeronasal neurons. Signal transduction pathways of **(A)** the main olfactory system and **(B)** the vomeronasal system. OR, odorant receptor; ACIII, adenylyl cyclase III; CNG channel, cyclic nucleotide-gated cation channel; PLC, phospholipase C; DAG, diacylglycerol; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; AA, arachidonic acid.

Pheromone detection in the VNO

Surgical ablation of the rodent VNO severely impairs pheromone-induced behaviors such as mating and territorial defense, and also perturbs neuroendocrine responses such as the male testosterone surge and the female estrus cycle (Wysocki, 1979; Halpern, 1987). TRP2-deficient mice fail to display male-male aggression, and initiate sexual and courtship behaviors toward both males and females (Stowers et al., 2002). Recent analyses using electrophysiological and optical imaging techniques have shown that various pheromones activate mouse vomeronasal neurons: volatile pheromones, including (R, R)-3,4-dehydro-exo-brevicomin, 2-sec-butyl-4, 5-dihydrothiazole, and 2-heptanone (Leinders-Zufall et al., 2000), and nonvolatile pheromones, including MUP (Bacchini et al., 1992; Brennan et al., 1999; Chamero et al., 2007) and MHC class I peptide (Leinders-Zufall et al., 2004), were reported to activate vomeronasal neurons. These data strongly suggest that pheromones are detected in the VNO (Powers and Winans, 1975; Fleming et al., 1979; Wysocki, 1979; Clancy et al., 1984; Halpern and Martinez-Marcos, 2003; Kimchi et al., 2007).

Pheromone detection in the OE

In contrast, recognition of the volatile pheromones 5-α-androst-16-en-3-one (Melrose et al., 1971; Dorries et al., 1997) and 2-methylbut-2-enal (see Table 1) is mediated by the OE in pig and rabbit, respectively (Hudson and Distel, 1986; Schaal et al., 2003). Recently, Lin et al. (2005) showed that MTMT, which is only present in male mouse urine, evokes robust responses in a small subset of mitral cells in the MOB, indicating that MTMT is actually recognized by the main olfactory system. Furthermore, genetic ablation of ACIII (Wang et al., 2006) or the CNG channels (Mandiyan et al., 2005; Spehr et al., 2006), which are vital for transduction in most mouse olfactory neurons, causes deficient behavior such as impaired mating and fighting, as well as the lack of chemosensory investigation of 2-heptanone and MHC class I peptide. These data strongly suggest that both volatile (2-heptanone) and nonvolatile (MHC class I peptide) stimuli can be detected by the OE, and that the main olfactory system is also involved in pheromone perception. These data also show that 2-heptanone and MHC class I peptide are detected by both the VNO and OE, thus suggesting that the main and vomeronasal systems detect, at least in part, overlapping sets of chemical stimuli.

Pheromone detection using different second-messenger pathways

The response of vomeronasal neurons to 2-heptanone is strongly diminished in TRP2-deficient mice (Leypold et al., 2002), which suggests that 2-heptanone is perceived by a TRP2-mediated signal transduction pathway in the VNO. On the other hand, the response of olfactory neurons to 2-heptanone was also severely inhibited in ACIII- and CNG-deficient mice (Wang et al., 2006; Lin et al., 2004; Spehr et al., 2006), suggesting that recognition of 2-heptanone depends on the ACIII- and CNG-mediated signal transduction pathways in the OE. Similarly, it is suggested that the MHC class I peptide is recognized by the IP_3-mediated signal transduction pathway in the VNO, whereas it is recognized by the CNG-mediated signal transduction pathway in the OE (Leinders-Zufall et al., 2004; Spehr et al., 2006). These results suggest that the VNO and OE detect, in part, overlapping sets of chemical stimuli by using distinct signal transduction pathways.

Multiple receptors and their function

Experimental results to date indicate that both olfactory systems play a role in pheromone perception. But what types of receptor actually recognize pheromones in these olfactory organs? Recent investigations have revealed that structurally distinct multiple receptor families are expressed in these olfactory organs. Although not all of these receptor families have been completely confirmed as pheromone receptors, many experimental data imply or suggest that they play a role in pheromone reception.

V1R genes and their function

The rodent V1R family (Fig. 3) was first identified by Dulac and Axel (Dulac and Axel, 1995). They originally attempted to identify genes encoding vomeronasal receptors based on sequence similarity with the olfactory receptor, but this was unsuccessful. Based on the assumptions that receptor genes are specifically expressed in the VNO and that each individual neuron should express a different receptor, they compared the cDNA libraries from individual vomeronasal neurons and identified a novel family of 30–100 genes (V1R). The V1R genes encoded seven-transmembrane-domain protein receptors with short extracellular domains at their N-terminus, but they were distantly related to the genes of the olfactory receptors. They were expressed in a subpopulation of vomeronasal neurons localized in an apical zone in the vomeronasal epithelial layer. As

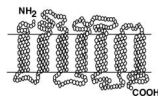
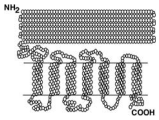
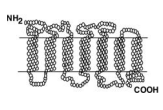
Receptors	V1R	V2R	TAAR	OR
Schematic structure of the receptor				
Number of intact genes	191*	121	15	1037
Chemical recognized	volatile	nonvolatile	volatile	volatile
G protein co-expressed	Gi2	Go	Golf	Golf
Expression site	VNO	VNO	OE	OE

Fig. 3. Chemoreceptors expressed in the mouse VNO and OE. The numbers of intact genes are derived from Grus et al. (2007) and *Zhang et al. (2007). The other descriptions are based on Mombaerts (2004), Liberles and Buck (2006), and Kimoto et al. (2005).

Gi2 and Go are expressed in subsets of vomeronasal neurons located in the apical and basal zones, respectively, of the vomeronasal epithelial layers (Berghard and Buck, 1996), the V1R-expressing neurons are likely to express Gi2.

At present, over 190 V1R genes have been identified in mice (Zhang et al., 2007). The intact V1R repertoire appears to be more species-specific than the olfactory receptor repertoire, even between mice and rats, and thus the V1R family, presumably due to its role in reproductive behavior, has evolved more rapidly in individual species (Zhang et al., 2007).

Although V1Rs appears to be good candidates as pheromone receptors, it was very difficult to probe due to the lack of functional assays for V1Rs. However, by using calcium imaging techniques, Leinders-Zufall et al. (2000) found that vomeronasal neurons located in the apical zone of the mouse vomeronasal epithelial layer (in other words, V1R-expressing neurons) were activated by some volatile pheromones. In addition, mice lacking a cluster of 16 V1R genes displayed deficits in a subset of VNO-dependent behaviors: expression of male sexual behavior and maternal aggression were substantially altered (Del Punta et al., 2002). The epithelium of the VNO in such mice does not detectably respond to specific pheromonal ligands such as 2-heptanone and 2,5-dimethyl-pyrazine (Del Punta et al., 2002). Furthermore, mouse vomeronasal neurons specifically expressing a member of the V1R family (V1Rb2) were found to respond to 2-heptanone, confirming that (volatile) pheromone detection is mediated by a V1R family member (Boschat et al., 2002). These results clearly confirm that a V1R actually functions as a pheromone receptor.

V2R genes and their function

As V1R genes are expressed in cells located in the apical zone of the vomeronasal epithelial layer, a new multigene family expressed in the basal zone of the rodent vomeronasal epithelial layer was investigated. Three groups subsequently identified the novel gene family now designated as V2R (Fig. 3) (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). The V2R genes encode G-protein-coupled seven-transmembrane domain protein receptors with a long extracellular N-terminal domain, and this domain of the V2Rs likely participates in ligand binding. Unlike olfactory receptors and V1R genes, the coding sequences for V2R genes contain introns. In addition, V2Rs do not share sequence similarity with either olfactory receptors or V1Rs, but are related to calcium-sensing receptors and metabotropic glutamate receptors. The V2R genes are widely distributed among the genomes of various vertebrates (Shi and Zhang, 2007).

Like V1Rs and the olfactory receptors, each V2R is expressed in a small subset of vomeronasal neurons (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). However, in situ hybridization experiments showed that V2R2 genes, a very specific subfamily of V2Rs, were broadly expressed in rodent Go-expressing vomeronasal neurons, and thus were co-expressed in the same cells as other members of V2R (Martini et al., 2001). The significance of expressing multiple receptors in a single neuron is unclear. Possible explanations are that V2R2s form heterodimers with other V2R members, or that yet another mode of chemosensory information processing may occur.

Two groups reported that V2Rs are also co-expressed with a family of MHC class1b molecules in the basal zone of the vomeronasal epithelial layer and that V2Rs form multimolecular complexes with MHC class-1b molecules and β2-microglobulin (β2m) in mice (Ishii et al., 2003; Loconto et al., 2003). In cultured cells, MHC class-1b molecules appear to function as escort molecules in the transport of V2Rs to the cell surface (Loconto et al., 2003). Mice deficient in β2m exhibit mislocalization of V2Rs in the VNO, and a specific defect in male-male aggressive behavior (Loconto et al., 2003). Interestingly, MHC class-1 peptide functions as a sensory stimulus for a subset of vomeronasal neurons located in the Go- and V2R-expressing zone of the vomeronasal epithelial layer (Leinders-Zufall et al., 2004), and the same peptides function as individuality signals underlying mate recognition in the context of the pregnancy block in mice (Leinders-Zufall et al., 2004). These results suggest the involvement of MHC class-1b molecules in pheromone perception. However, at present, no studies have confirmed this hypothesis.

Kimoto et al. (2005) reported that peptides with a molecular weight of about 7 kD specifically secreted from male exocrine glands (exocrine gland-secreting peptide 1; ESP1), stimulated mouse vomeronasal neurons, and that they were recognized by a specific V2R. These results indicate that V2R recognizes peptides. The recent finding that one of the protein pheromones, MUP, is recognized by Go-expressing (thus V2R-expressing) vomeronasal neurons (Chamero et al., 2007) also supports this notion.

Two vomeronasal subsystems in rodents

Immunocytochemical studies suggest that in rodents,

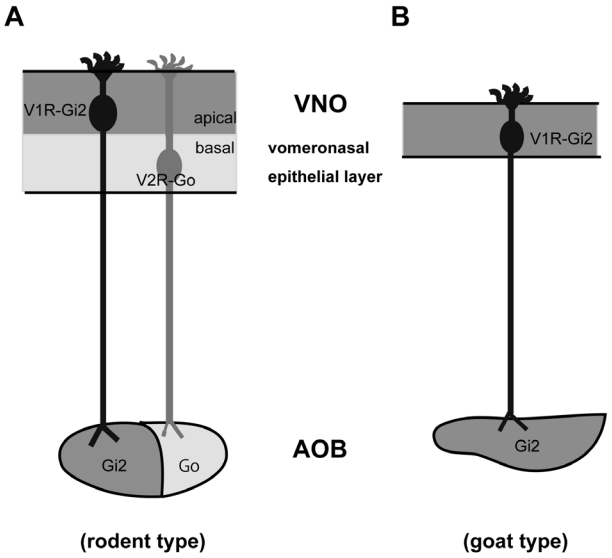


Fig. 4. Projection of vomeronasal neurons to the AOB. **(A)** Rodent-type projection. V1R-Gi2- and V2R-Go-expressing neurons are located in the apical and basal zones, respectively, of the vomeronasal epithelial layer and project to the anterior and posterior portions of the AOB, respectively. **(B)** Goat-type projection. Only V1R-Gi2-expressing neurons are detected in the VNO. Anterior-posterior segregation in the projection patterns of the vomeronasal neuron has not been identified in goats.

fibers reaching the AOB are likely to stay segregated based on whether they originate from the apical or basal zones of the VNO, with Gi2-positive fibers projecting to the anterior portion of the AOB and Go-positive fibers reaching the posterior half of the AOB (Fig. 4A) (Inamura et al., 1985; Ichikawa et al., 1994). Anterograde and retrograde tract-tracing methods have also shown differential projections from the anterior and posterior divisions of the AOB to the vomeronasal amygdala, thus suggesting that two vomeronasal subsystems are present in the rodent telencephalon (Mohedano-Moriano et al., 2007). However, the functional significance of these vomeronasal subsystems is yet to be analyzed.

TAARs and their function

Liberles and Buck (2006) reported the discovery of a family of receptors in the mouse OE. Genes encoding these receptors, called 'trace amine-associated receptors' (TAARs) (Fig. 2), are present in human, mouse, and fish, as well as in other vertebrates (Table 2) (Lindemann et al., 2005; Grus et al., 2007). Like odorant receptors, individual mouse TAARs are expressed in unique subsets of olfactory neurons dispersed in the OE (Liberles and Buck, 2006). Notably, at least three mouse TAARs recognize volatile amines found in urine: one detects a compound linked to stress, whereas the other two detect compounds enriched in male versus female urine, one of which is reportedly a pheromone (Liberles and Buck, 2006). The evolutionary conservation of the TAAR family suggests a chemosensory function distinct from odorant receptors (Liberles and Buck, 2006).

Three distinct families of pheromone receptors function in the olfactory organs

All of these receptor families, V1R, V2R, and TAAR, are G-protein-coupled seven-transmembrane domain receptors. However, they do not share sequence similarity, and are therefore likely to have evolved independently. As shown in Table 2, the numbers of genes for each receptor family differ markedly among vertebrate species, and there are considerable numbers of pseudogenes present in the genome of each species (Shi and Zhang, 2007). Thus, these receptors, particularly V1R and V2R, vary significantly in size and quality between vertebrate species, which suggests dynamic gene birth and death processes occurring during evolution. These processes may have contributed to the species-specific adaptation of these receptors.

Expression of vomeronasal receptors in various vertebrates

Although recent progress has revolutionized our knowledge of rodent pheromone perception, systems in other vertebrates differ from that in rodents. For example, some vertebrates, e.g., birds, old-world monkeys, and humans, do not possess the VNO, while in some reptiles, the VNO is used for trailing prey and feeding (Halpern and Martinez-Marcos, 2003). Recent studies have shown that the function of the two olfactory organs and the expression of multiple receptor families appear to be different among various vertebrate species.

Fish

The VNO is not present in fish. Fish pheromones are recognized in the OE (Eisthen, 1992). Fish possess approximately 100 of the olfactory receptor genes, six V1R genes and dozens of V2R genes (Alioto and Ngai, 2005; Saraiva and Korsching, 2007). Although the ligands for V1Rs have not yet been determined, those for V2Rs were found to be amino acids (Speca et al., 1999; Hansen et al., 2003). As amino acids are regarded as olfactory cues for fish, V2Rs apparently function as olfactory receptors in fish. In contrast, bile salts, which are thought to be involved in fish social interaction, is considered to be recognized by olfactory receptors (Hansen et al., 2003). Therefore, the functions of V2Rs and olfactory receptors are likely to be reversed between fish and rodents.

Amphibians

Evolutionarily, the VNO first appeared in amphibians (Bertmar, 1981; Bruner, 1984). Amphibians are adapted to both water and land, and can detect both water-soluble and volatile odorants (Altner, 1962). The vomeronasal and olfactory neurons extend their axons to the AOB and the MOB, respectively. Amphibians possess dual olfactory systems similar to those of rodents (Scalia et al., 1991; Saito and Taniguchi, 2000). A special feature of the olfactory systems of some amphibians, such as *Xenopus*, is that the OE is divided into two chambers: the primary chamber (PC) and the middle chamber (MC), in which volatile and water-soluble odorants are detected, respectively.

Recently, 21 V1R genes and approximately 250 V2R genes were identified in the genomes of *Xenopus tropicalis* and *Xenopus laevis* (Shi and Zhang, 2007; Date-Ito et al., 2008). The number of V1R and V2R genes increased from fish to amphibians, implying that a functional change may have occurred after the divergence of amphibians from fish. Analysis of the expression of these vomeronasal receptors revealed that V2Rs are expressed throughout the *Xenopus* vomeronasal epithelium (Fig. 5B), whereas expression of V1Rs was not observed (Fig. 5A) (Hagino-Yamagishi et al., 2004; Date-Ito et al., 2008). These results suggest that V2Rs function in the *Xenopus* VNO, whereas V1Rs do not. Considering that the newt pheromone sodefrin is recognized by vomeronasal neurons (Toyoda and Kikuyama, 2000),

Table 2. Sizes of the V1R, V2R, and TAAR repertoires in various vertebrates. Numbers indicate the number of intact genes. The data are derived from Grus et al. (2007) and *Zhang et al. (2007).

	Zebrafish	Xenopus	Chicken	Platypus	Opossum	Mouse	Dog	Cow	Human
V1R	2	21	0	270	98	*191	8	40	5
V2R	44	249	0	15	86	121	0	0	0
TAAR	57	2	3	4	22	15	2	17	6

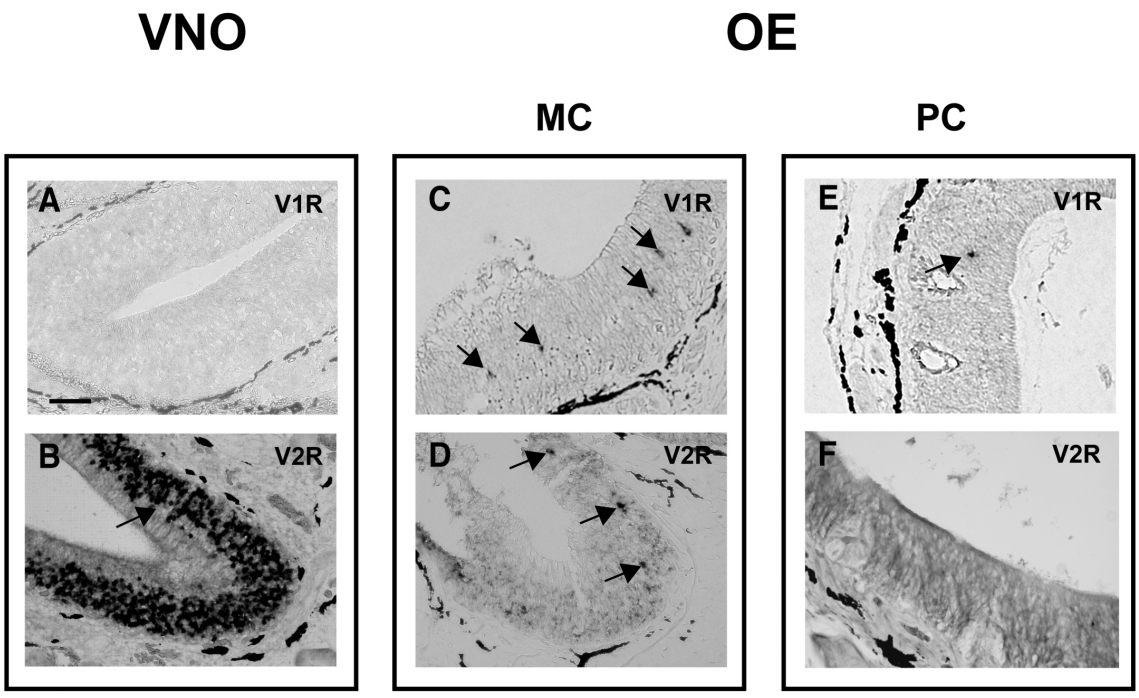


Fig. 5. Expression of vomeronasal receptor genes in the *Xenopus* VNO and OE. Coronal sections of the VNO **(A, B)**, MC **(C, D)**, and PC **(E, F)** of adult *Xenopus laevis* were hybridized with DIG-labeled antisense cRNA probes as described in Date-Ito et al. (2008). Antisense probes for V1R **(A, C, E)** and V2R **(B, D, F)** were used (Date-Ito et al., 2008). Arrows indicate receptor-expressing cells. Bar, 100 μm. The images are based on data from Date-Ito et al. (2008).

V2Rs expressed in the VNO are good candidates for pheromone receptors in amphibians. V2Rs might have gained additional function with the appearance of the VNO in amphibians.

Interestingly, some *Xenopus* V2R genes are expressed in the OE of the MC (Fig. 5D) (Hagino-Yamagishi et al., 2004), suggesting that the same chemical information is detected by V2Rs expressed in both the VNO and OE. These findings give rise to the idea that there is parallel processing of the same chemical cues in the main olfactory and vomeronasal systems.

Expression of *Xenopus* V1R genes was specifically detected in the OE of the MC and PC (Fig. 5C, E), but not in the VNO (Fig. 5A). The function of *Xenopus* V1Rs is uncertain, although they might play a role in chemoreception in the OE. We cannot rule out the possibility that *Xenopus* V1Rs detect pheromones in the OE.

Reptiles and Birds

The turtle vomeronasal system reportedly recognizes water-soluble odors and pheromones (Hatanaka and Matsuzaki, 1993; Fadool et al., 2001). The vomeronasal system of the garter snake plays a major role in trailing prey and feeding, as well as in reproductive behavior (Kubie et al., 1978; Inouchi et al., 1993). The vomeronasal neurons of garter snakes project to the AOB, and then project mainly to the medial amygdala and nucleus sphericus (Halpern and Martinez-Marcos, 2003); the nucleus sphericus also projects to the medial amygdala and the lateral cortex (Halpern and Martinez-Marcos, 2003). As the medial amygdala and the lateral cortex are the vomeronasal recipient and main olfactory recipient structures, respectively, garter snakes possess two distinct pathways from the AOB: one for recognizing odors and another for recognizing pheromones (Lanuza and Halpern, 1998). Although nonvolatile female and male sex pheromones were identified in garter snakes (Mason et al., 1989), the reptile pheromone receptors have not yet been reported.

Birds do not possess the VNO, and their OE and MOB are generally poorly developed (Malakoff, 1999). At present, there are no reports on avian pheromone receptors.

Mammals: ungulates

The goat vomeronasal epithelial layer is thinner than that in the mouse VNO. Gi2-expressing neurons, but not Go-expressing neurons, are detected in the vomeronasal epithelial layer (Wakabayashi et al., 2002), and the anterior-posterior segregation in the projection patterns of vomeronasal neurons observed in the rodent AOB (Shinohara et al., 1992) could not be identified in the goat (Takigami et al., 2000) (Fig. 4B). None of the V2R genes so far identified was functional, and only V1Rs were expressed in the VNO (Wakabayashi et al., 2002). Taken together, these observations suggest that the V2R-Go-mediated signal transduction pathway was lost in the goat vomeronasal system, and that only the V1R-Gi2-mediated signal transduction pathway seems to function (Fig. 4B).

A lack of Go-mediated pathways in the VNO has also been observed in various other mammals, such as suncuses (Insectivora), dogs (Carnivora), horses (Perissodactyla), and marmosets (Primata) (Takigami et al., 2004). In these animals, only Gi2-expression was observed in the AOBs.

Furthermore, no functional V2R genes exist in the genomes of dogs, cows, or humans (Shi and Zhang, 2007; Young and Trask, 2007), thus suggesting that the V2R-Go-mediated signal transduction pathway was lost and only the V1R-Gi2 mediated signal transduction pathway exists in these mammalian VNOs.

In goats and sheep, the pheromone produced by males induces out-of-seasonal ovulation in anestrous females, known as the “male effect”. Preventing the VNO from functioning does not affect the female responses to the male pheromone, thus suggesting that the main olfactory system plays a role in the perception of the pheromone, although the vomeronasal system also seems to function (Gelez and Fabre-Nys, 2004). Interestingly, in contrast to rodents, goat V1R genes are expressed in both the VNO and the OE (Wakabayashi et al., 2002; Wakabayashi et al., 2007). These results may support the notion that V1Rs are good candidates for the goat pheromone receptors.

Expression of vomeronasal receptors in the OE was observed in goats, humans (Rodriguez et al., 2000) and *Xenopus* (Date-Ito et al., 2008). Thus, expression of V1R and V2R genes in the OE may not be an exceptional feature in many vertebrates, and therefore it is possible that these vomeronasal receptors expressed in the OE are involved in pheromone perception in various animals.

Mammals: humans

The existence of human pheromones was first suggested by the demonstration that women living together in close proximity, such as roommates in dormitories, can develop synchronized menstrual cycles (McClintock, 1971). Stern and McClintock (1998) found that odorless compounds (putative pheromones) from the armpits of women regulate a specific neuroendocrine mechanism in other people. However, a functional VNO is not present in adult humans, although a VNO-like structure is present during early human embryogenesis (Boehm and Gasser, 1993).

At present, five V1R-like sequences have been identified in the human genome (Rodriguez and Mombaerts, 2002), and one human V1R, designated V1RL1, is expressed in the olfactory mucosa (Rodriguez et al., 2000). These results raise the possibility that human pheromones are detected by V1Rs expressed in human olfactory mucosa. However, it is difficult to confirm the possible involvement of V1Rs in pheromone perception due to the pseudogenization of human TRP2, which is thought to be a prerequisite for signal transduction mediated by V1Rs in rodents (Zhang and Webb, 2003). The complete pseudogenization of TRP2 occurred during the separation of new-world monkeys from catarrhines (Rouquier and Giorgi, 2007), and it coincides with the development of trichromatic color vision, which suggests that vision became more important than

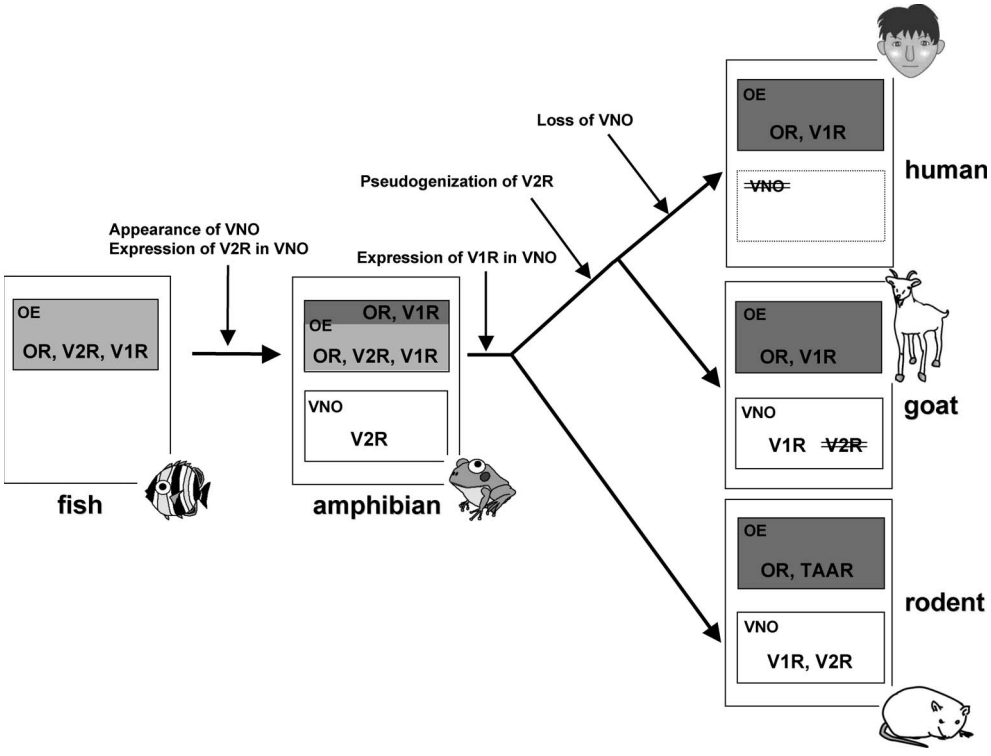


Fig. 6. Model of the diverse systems for pheromone reception in vertebrates. Fish lack a VNO. In the fish OE, pheromones are generally detected by olfactory receptors, whereas amino acids are detected by V2Rs. After the appearance of the VNO in amphibians, V2Rs are expressed in the VNO and possibly detect water-soluble pheromones. After the divergence of amphibians and mammals, V1Rs were expressed in the VNO and likely functioned as a pheromone receptor. However in many mammalian species, the VNO and/or V2R-Go-mediated signal transduction pathway have been lost. Alternatively, rodents may have evolved specialized and more complex functions for the VNO. Gray boxes indicate OE that detects mainly water-soluble pheromones or odorants. Dark boxes indicate OE that detects primary volatile pheromones or odorants. White boxes indicate the VNO, and the box with a dotted outline indicates the loss of a functional VNO in humans.

chemical communication (Rouquier and Giorgi, 2007).

Functional expression of human V1R genes in cultured cells showed that human V1Rs recognize C9-C10 aliphatic alcohols or aldehydes and activate cAMP signaling via Golf (Shirokova et al., 2008). It should be noted that some goat V1R-expressing cells in the OE co-express Golf (Wakabayashi et al., 2007), thus implying that a similar signal transduction pathway mediated by V1R-Golf exists in both humans and goats.

Evolutional shift in function in pheromone receptor families

Taken together with the results described above, the involvement of each receptor family in pheromone perception can be hypothesized from an evolutionary point of view (Fig. 6). Fish detect pheromones in the OE by using olfactory receptors, and V2Rs detect amino acids. After the appearance of the VNO in amphibians, V2R expression shifted to the VNO, and presumably V2R began to function in the detection of water-soluble pheromones. V1R expression probably shifted from the OE to the VNO after the divergence between amphibians and mammals. This shift may have been associated with the terrestrial adaptation of amphibian ancestors, and V1R appeared to function as a pheromone receptor for volatile pheromones. The expansion of the V1R gene repertoire and variation in the sequences of these genes might have contributed to maintaining species specificity. However, a vision-based signaling-sensory mechanism may have partially replaced the VNO-mediated chemical-based system in the social and reproductive activities of many non-rodent mammalian species, and the V2R-Go mediated signal transduction pathway was lost. Alternatively, rodents may have evolved specialized and complex functions for the VNO, probably because they possess poor sight.

Closing remarks

A growing body of evidence indicates that pheromones are detected by the two olfactory systems, multiple receptor families are expressed in the two olfactory organs and the functions of these receptor families seem to differ among various species. These findings raise many new questions. For example, what receptors recognize 2-heptanone and MHC class I peptides, which are detected in the mouse OE? Do olfactory receptors recognize such chemicals in the mouse OE? Do the V1Rs expressed in the *Xenopus*, goat, and human OEs recognize pheromones? How are pheromonal signals detected in the OE transmitted to the hypothalamus? Are GnRH neurons involved in the transmission of pheromonal signals to the hypothalamus? To solve these problems, various kinds of experimental approaches will need to be taken, using various vertebrate species.

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REFERENCES

Alioto TS, Ngai J (2005) The odorant receptor repertoire of teleost fish. BMC Genomics 6: 173–186

Altner H (1962) Untersuchungen über Leistungen und Bau der Nase des südafrikanischen Krallenfrosches Xenopus laevis (Daudin, 1803). Z vergl Physiol 45: 272–306

Bacchini A, Gaetani E, Cavaggioni A (1992) Pheromone binding proteins of the mouse, *Mus musculus*. Experientia 48: 419–421

Berghard A, Buck LB (1996) Sensory transduction in vomeronasal neurons: evidence for G alpha o, G alpha i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. J Neurosci 16: 909–918

Bertmar G (1981) Evolution of vomeronasal organs in vertebrates. Evolution 35: 359–366

Boehm N, Gasser B (1993) Sensory receptor-like cells in the human foetal vomeronasal organ. Neuroreport 4: 867–870

Boehm U, Zou Z, Buck LB (2005) Feedback loops link odor and pheromone signaling with reproduction. Cell 123: 683–695

Boschat C, Pelofi C, Randin O, Roppolo D, Luscher C, Broillet MC, Rodriguez I (2002) Pheromone detection mediated by a V1r vomeronasal receptor. Nat Neurosci 5: 1261–1262

Brennan PA, Schellinck HM, Keverne EB (1999) Patterns of expression of the immediate-early gene egr-1 in the accessory olfactory bulb of female mice exposed to pheromonal constituents of male urine. Neuroscience 90: 1463–1470

Bruner HL (1984) Jacobson's organ and the respiratory mechanism of amphibians. Morphol Jahrb 48: 157–165

Chamero P, Marton TF, Logan DW, Flanagan K, Cruz JR, Saghatelian A, Crevatt BF, Stowers L (2007) Identification of protein pheromones that promote aggressive behaviour. Nature 450: 899–902

Clancy AN, Coquelin A, Macrides F, Gorski RA, Noble EP (1984) Sexual behavior and aggression in male mice: involvement of the vomeronasal system. J Neurosci 4: 2222–2229

Date-Ito A, Ohara H, Ichikawa M, Mori Y, Hagino-Yamagishi K (2008) Xenopus V1R vomeronasal receptor family is expressed in the main olfactory system. Chem Senses 33: 339–346

Del Punta K, Leinders-Zufall T, Rodriguez I, Jukam D, Wysocki CJ, Ogawa S, Zufall F, Mombaerts P (2002) Deficient pheromone responses in mice lacking a cluster of vomeronasal receptor genes. Nature 419: 70–74

Dobson H, Ghuman S, Prabhakar S, Smith R (2003) A conceptual model of the influence of stress on female reproduction. Reproduction 125: 151–163

Dorries KM, Adkins-Regan E, Halpern BP (1997) Sensitivity and behavioral responses to the pheromone androstenone are not mediated by the vomeronasal organ in domestic pigs. Brain Behav Evol 49: 53–62

Dulac C, Axel R (1995) A novel family of genes encoding putative pheromone receptors in mammals. Cell 83: 195–206

Eisthen HL (1992) Phylogeny of the vomeronasal system and of receptor cell types in the olfactory and vomeronasal epithelia of vertebrates. Microsc Res Tech 23: 1–21

Estes RD (1972) The role of the vomeronasal organ in mammalian reproduction. Mammalia 36: 315–341

Evans C (2003) Vomeronasal Chemoreception in Vertebrates. A Study of the Second Nose. Imperial College Press, London

Fadool DA, Wachowiak M, Brann JH (2001) Patch-clamp analysis of voltage-activated and chemically activated currents in the vomeronasal organ of Sternotherus odoratus (stinkpot/musk turtle). J Exp Biol 204: 4199–4212

Firestein S (2001) How the olfactory system makes sense of scents. Nature 413: 211–218

Fleming A, Vaccarino F, Tambossee L, Chee P (1979) Vomeronasal and olfactory modulation of maternal behavior in the rat. Science 203: 372–374

Gelez H, Fabre-Nys C (2004) The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. Horm Behav 46: 257–271

Grus WE, Shi P, Zhang J (2007) Largest vertebrate vomeronasal

type 1 receptor gene repertoire in the semiaquatic platypus. Mol Biol Evol 24: 2153–2157
Hagino-Yamagishi K, Moriya K, Kubo H, Wakabayashi Y, Isobe N, Saito S, Ichikawa M, Yazaki K (2004) Expression of vomeronasal receptor genes in Xenopus laevis. J Comp Neurol 472: 246–256
Halpern M (1987) The organization and function of the vomeronasal system. Annu Rev Neurosci 10: 325–362
Halpern M, Martinez-Marcos A (2003) Structure and function of the vomeronasal system: an update. Progr Neurobiol 70: 245–318
Hansen A, Rolen SH, Anderson K, Morita Y, Caprio J, Finger TE (2003) Correlation between olfactory receptor cell type and function in the channel catfish. J Neurosci 23: 9328–9339
Hatanaka T, Matsuzaki O (1993) Odor responses of the vomeronasal system in Reeve's turtle, *Geoclemys reevesii*. Brain Behav Evol 41: 183–186
Herrada G, Dulac C (1997) A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 90: 763–773
Hudson R, Distel H (1986) Pheromonal release of suckling in rabbits does not depend on the vomeronasal organ. Physiol Behav 37: 123–128
Ichikawa M, Takami S, Osada T, Graziadei P (1994) Differential development of binding sites of two lectins in the vomeronasal axons of the rat accessory olfactory bulb. Dev Brain Res 78: 1–9
Inamura K, Mori K, Fujita S, Obata K (1985) Immunocytochemical identification of subgroups of vomeronasal nerve fivers and their segregated terminations in the accessory olfactory bulb. Brain Res 326: 362–366
Inouchi J, Wang D, Jiang XC, Kubie J, Halpern M (1993) Electrophpysiological analysis of the nasal chemical senses in garter snakes. Brain Behav Evol 41: 171–182
Ishii T, Hirota J, Mombaerts P (2003) Combinatorial coexpression of neural and immune multigene families in mouse vomeronasal sensory neurons. Curr Biol 13: 394–400
Jemiolo B, Harvey S, Novotny MV (1986) Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. Proc Natl Acad Sci USA 83: 4576–4579
Johnston RE (2000) Chemical communication and pheromones: the types of chemical signals and the role of the vomeronasal system. In "The Neurobiology of Taste and Smell" 2nd ed Ed by TE Finger, WL Silver, D Restrepo, Wiley, New York, pp 101–127
Karlson P, Luscher M (1959) Pheromones: a new term for a class of biologically active substances. Nature 183: 55–56
Kashiwayanagi M (2002) Molecular recognition and intracellular transduction mechanisms in olfactory and vomeronasal systems. In "Hormone, Brain and Behavior" Ed by D Pfaff, Academic Press, San Diego, pp 1–16
Kikuyama S, Toyoda F, Ohmiya Y, Matsuda K, Tanaka S, Hayashi H (1995) Sodefrin: a female-attracting peptide pheromone in newt cloacal glands. Science 267: 1643–1645
Kimchi T, Xu J, Dulac C (2007) A functional circuit underlying male sexual behaviour in the female mouse brain. Nature 448: 1009–1014
Kimoto H, Haga S, Sato K, Touhara K (2005) Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. Nature 437: 898–901
Kubie JL, Vagvolgyi A, Halpern M (1978) Role of the vomeronasal and olfactory systems in courtship behavior of male garter snakes. J Comp Physiol Psychol 92: 627–641
Lanuza E, Halpern M (1998) Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the snake *Thamnophis sirtalis*. Brain Behav Evol 51: 1–22
Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, Zufall F (2000) Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature 405: 792–796
Leinders-Zufall T, Brennan P, Widmayer P, et al. (2004) MHC class I peptides as chemosensory signals in the vomeronasal organ. Science 306: 1033–1037
Leypold BG, Yu CR, Leinders-Zufall T, Kim MM, Zufall F, Axel R (2002) Altered sexual and social behaviors in trp2 mutant mice. Proc Natl Acad Sci USA 99: 6376–6381
Liberles SD, Buck LB (2006) A second class of chemosensory receptors in the olfactory epithelium. Nature 442: 645–650
Liman ER, Corey DP, Dulac C (1999) TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc Natl Acad Sci USA 96: 5791–5796
Lin DY, Zhang SZ, Block E, Katz LC (2005) Encoding social signals in the mouse main olfactory bulb. Nature 434: 470–477
Lin W, Arellano J, Slotnick B, Restrepo D (2004) Odors detected by mice deficient in cyclic nucleotide-gated channel subunit A2 stimulate the main olfactory system. J Neurosci 24: 3703–3710
Lindemann L, Ebeling M, Kratochwil NA, Bunzow JR, Grandy DK, Hoener MC (2005) Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. Genomics 85: 372–385
Loconto J, Papes F, Chang E, Stowers L, Jones EP, Takada T, Kumanovics A, Fischer LK, Dulac C (2003) Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. Cell 112: 607–618
Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F (2003) A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction. Neuron 40: 551–561
Malakoff D (1999) Following the scent of avian olfaction. Science 286: 704–705
Mandiyan VS, Coats JK, Shah NM (2005) Deficits in sexual and aggressive behaviors in Cnga2 mutant mice. Nat Neurosci 8: 1660–1662
Martini S, Silvotti L, Shirazi A, Ryba NJ, Tirindelli R (2001) Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ. J Neurosci 21: 843–848
Mason RT, Fales HM, Jones TH, Pannell LK, Chinn JW, Crews D (1989) Sex pheromones in snakes. Science 245: 290–293
Matsunami H, Buck LB (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell 90: 775–784
McClintock MK (1971) Menstrual synchrony and suppression. Nature 229: 244–245
Melrose DR, Reed HC, Patterson RL (1971) Androgen steroids associated with boar odor as an aid to the detection of oestrus in pig artificial insemination. Br Vet J 127: 497–502
Meredith M (1998) Vomeronasal, olfactory, hormonal convergence in the brain. Cooperation or coincidence? Ann NY Acad Sci 855: 349–361
Mohedano-Moriano A, Pro-Sistiaga P, Ubeda-Banon I, Crespo C, Insausti R, Martinez-Marcos A (2007) Segregated pathways to the vomeronasal amygdala: differential projections from the anterior and posterior divisions of the accessory olfactory bulb. Eur J Neurosci 25: 2065–2080
Mombaerts P (2004) Genes and ligands for odorant, vomeronasal and taste receptors. Nat Rev Neurosci 5: 263–278
Mucignat-Caretta C, Caretta A, Cavaggioni A (1995) Acceleration of puberty onset in female mice by male urinary proteins. J Physiol 486: 517–522
Novotny MV (2003) Pheromones, binding proteins and receptor responses in rodents. Biochem Soc Trans 31: 117–122
Novotny MV, Harvey S, Jemiolo B, Alberts J (1985) Synthetic pheromones that promote inter-male aggression in mice. Proc Natl Acad Sci USA 82: 2059–2061
Novotny MV, Widong M, Wiesler D, Zidek L (1999) Positive identification of the puverty-accelerating pheromones of the house

mouse: the volatile ligands associating with the major urinary protein. Proc R Soc Lond B 266: 2017–2022
Powers JB, Winans SS (1975) Vomeronasal organ: critical role in mediating sexual behavior of the male hamster. Science 187: 961–963
Rodriguez I, Mombaerts P (2002) Novel human vomeronasal receptor-like genes reveal species-specific families. Curr Biol 12: R409–R411
Rodriguez I, Greer CA, Mok MY, Mombaerts P (2000) A putative pheromone receptor gene expressed in human olfactory mucosa. Nat Genet 26: 18–19
Rouquier S, Giorgi D (2007) Olfactory receptor gene repertoires in mammals. Mutat Res 616: 95–102
Ryba NJ, Tirindelli R (1997) A new multigene family of putative pheromone receptors. Neuron 19: 371–379
Saito S, Taniguchi K (2000) Expression patterns of glycoconjugates in the three distinctive olfactory pathways of the clawed frog, *Xenopus laevis*. J Vet Med Sci 62: 153–159
Saraiva LR, Korsching SI (2007) A novel olfactory receptor gene family in teleost fish. Genome Rese 7: 1448–1457
Scalia F, Gallousis G, Roca S (1991) Differential projections of the main and accessory olfactory bulb in the frog. J Comp Neurol 305: 443–461
Schaal B, Coureaud G, Langlois D, Ginies C, Semon E, Perrier G (2003) Chemical and behavioural characterization of the rabbit mammary pheromone. Nature 424: 68–72
Shi P, Zhang J (2007) Comparative genomic analysis identifies an evolutionary shift of vomeronasal receptor gene repertoires in the vertebrate transition from water to land. Genome Res 17: 166–174
Shinohara H, Asano T, Kato K (1992) Differential localization of G-proteins Gi and Go in the accessory olfactory bulb of the rat. J Neurosci 12: 1275–1279
Shipley MT, Ennis M, Puche AC (2004) Olfactory system. In "The Rat Nervous System" 3rd ed Ed by G Paxinos, Academic Press, San Diego, pp 923–964
Shirokova E, Raguse JD, Meyerhof W, Krautwurst D (2008) The human vomeronasal type-1 receptor family — detection of volatiles and cAMP signaling in HeLa/Olf cells. FASEB J 22: 1416–1426
Silverman AJ, Livne I, Witkin WJ (1994) The gonadotropin-releasing hormone (GnRH), neuronal systems: immunocytochemistry and in situ hybridization. In "The Physiology of Reproduction, Vol 1" Ed by E Knobil, JD Neill, Raven Press, New York
Sorensen PW, Hara TJ, Stacey NE, Goets FW (1988) F prostaglandins function as potent olfactory stimulants that comprise the postovulatory female sex pheromone in goldfish. Biol Reprod 39: 1039–1050
Speca DJ, Lin DM, Sorensen PW, Isacoff EY, Ngai J, Dittman AH (1999) Functional identification of a goldfish odorant receptor. Neuron 23: 487–498
Spehr M, Kelliher KR, Li X, Bohem T, Leiders-Zufall T, Zufall F (2006) Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. J. Neurosci 26: 1961–1970
Stern K, McClintock MK (1998) Regulation of ovulation by human pheromones. Nature 392: 177–179
Stowers L, Holy TE, Meister M, Dulac C, Koentges G (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science 295: 1493–1500
Takigami S, Mori Y, Ichikawa M (2000) Projection pattern of vomeronasal neurons to the accessory olfactory bulb in goats. Chem Senses 25: 387–393
Takigami S, Mori Y, Tanioka Y, Ichikawa M (2004) Morphological evidence for two types of mammalian vomeronasal system. Chem Senses 29: 301–310
Toyoda F, Kikuyama S (2000) Hormonal influence on the olfactory response to a female-attracting pheromone, sodefrin, in the newt, *Cynops pyrrhogaster*. Comp Biochem Physiol B 126: 239–245
Wabnitz PA, Bowie JH, Tyler MJ, Wallace JC, Smith BP (1999) Aquatic sex pheromone from a male tree frog. Nature 401: 444–445
Wakabayashi Y, Mori Y, Ichikawa M, Yazaki K, Hagino-Yamagishi K (2002) A putative pheromone receptor gene is expressed in two distinct olfactory organs in goats. Chem Senses 27: 207–213
Wakabayashi Y, Ohkura S, Okamura H, Mori Y, Ichikawa M (2007) Expression of a vomeronasal receptor gene (V1r) and G protein alpha subunits in goat, *Capra hircus*, olfactory receptor neurons. J Comp Neurol 503: 371–380
Wang Z, Balet SC, Li V, Nudelman A, Chan GC, Storm DR (2006) Pheromone detection in male mice depends on signaling through the type 3 adenylyl cyclase in the main olfactory epithelium. J Neurosci 26: 7375–7379
Wysocki CJ (1979) Neurobehavioral evidence for the involvement of the vomeronasal system in mammalian reproduction. Neurosci Biobehav Rev 3: 301–341
Yoon H, Enquist LW, Dulac C (2005) Olfactory inputs to hypothalamic neurons controlling reproduction and fertility. Cell 123: 669–682
Young JM, Trask BJ (2007) V2R gene families degenerated in primates, dog and cow, but expanded in opossum. Trends Genet 23: 212–215
Zhang J, Webb DM (2003) Evolutionary deterioration of the vomeronasal pheromone transduction pathway in catarrhine primates. Proc Natl Acad Sci USA 100: 8337–8341
Zhang X, Zuang X, Firestein S (2007) Comparative genomics of odorant and pheromone receptor genes in rodents. Genomics 89: 441–450

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