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Is the vomeronasal system really specialized for detecting pheromones?

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Many academics, clinicians and lay readers of science incorrectly assume that vomeronasal processing is equivalent to pheromone processing. We review the abundant data concerning the roles of both the olfactory and the vomeronasal systems in the processing of both pheromones and other odorants, demonstrating that this 'equivalency hypothesis' is untenable. This conclusion has important implications for the design and interpretation of experiments examining vomeronasal and olfactory system function. We describe some of the problems that arise from assuming that this equivalency holds. Two alternative hypotheses have been offered, but the available data do not enable us to accept or reject either one. Perhaps no single functional description can adequately characterize the role of the vomeronasal system.

Introduction

The vomeronasal organ is a peripheral chemosensory structure in vertebrates that is commonly assumed to be specialized for detecting pheromones. Pheromones were first defined by Karlson and Luscher as 'substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction.' [1] Although the first modern experimental study of vomeronasal system function demonstrated that destruction of the olfactory bulbs abolishes all sexual behavior in male hamsters (*Mesocricetus auratus*), and that deafferentation of the vomeronasal organ also causes severe sexual deficits, at first no link was made between the vomeronasal system and the exclusive detection of pheromones [2]. However, such an association was later uncovered and the possible relationship was discussed in an influential review paper by Wysocki [3], who concluded that the vomeronasal system could mediate responses to pheromones.

Removal of the vomeronasal organ does interfere with pheromone responses in some species, apparently leading several researchers to accept the hypothesis that the system is specialized for pheromone detection [4–6]. Many neurobiologists now believe that vomeronasal processing is functionally equivalent to pheromone processing, assuming either explicitly or implicitly that the receptor neurons of the vomeronasal organ are specialized for detecting pheromones and that stimulation of vomeronasal receptor neurons indicates the presence of pheromones. Results not fitting with this view are often overlooked, downplayed or dismissed as irrelevant.

The hypothesis that the vomeronasal system is specialized for pheromone detection is not without solid theoretical underpinning. A hard-wired system would ensure that the receiver of a pheromone would react in a specific way: even in a highly unpredictable olfactory environment, the receiver of a pheromonal signal would respond consistently. Individuals not responding appropriately to pheromones could have reduced reproductive fitness, so it seems reasonable to postulate that selection would favor the evolution of a specialized olfactory subsystem dedicated to detecting pheromones. Insects are generally believed to possess such a hard-wired system for pheromone detection (but see [7]), prompting researchers to seek evidence of an analogous system in vertebrates.

But do the data support the view that vomeronasal processing and pheromone processing are functionally equivalent? To draw such a conclusion, one would need behavioral and physiological data demonstrating that (i) pheromone signals are processed by the vomeronasal system only, and (ii) the vomeronasal system mediates no other functions. In fact, the available behavioral and physiological data contradict both of these points. Here, we briefly describe the organization of the vomeronasal system and then focus on what is known about vomeronasal system function and vertebrate pheromone detection.

Organization of the vomeronasal system

A discrete vomeronasal organ is not present in fishes; evolutionarily, early tetrapods were probably the first animals to have separate olfactory and vomeronasal organs [8]. However, not all tetrapods have a vomeronasal organ, and when the organ is present its anatomical relationship with the olfactory cavity varies considerably. For example, in most salamanders the vomeronasal organ extends anteriorly from the lateral edge of the nasal cavity, whereas in frogs it tends to be medial, beneath the olfactory vestibule [9]. In snakes, the vomeronasal organ is ventral to the olfactory organ, and in some species it contains more sensory neurons than does the olfactory epithelium [10]. In mammals, the vomeronasal organ is generally enclosed in the vomer bone, with the sensory epithelium located on the medial wall of the organ [11]. The presence of a functional vomeronasal system in humans is dubious [12,13]. The basic anatomy of

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the peripheral olfactory and vomeronasal systems in frogs, snakes and rodents is illustrated in Figure 1.

The olfactory and vomeronasal organs maintain separate central projections through several synapses [11]. The axons of the vomeronasal and olfactory receptor neurons project to the accessory and main olfactory bulbs, respectively. The telencephalic projections of the main olfactory bulb are far more extensive than those of the accessory olfactory bulb. Interestingly, in amphibians, reptiles and mammals, the main and accessory olfactory bulbs generally project to different parts of the amygdala, which could be a convergence point for olfactory and vomeronasal input [14]. The consistent presence of separate projections suggests that the two systems have different roles in physiology and behavior.

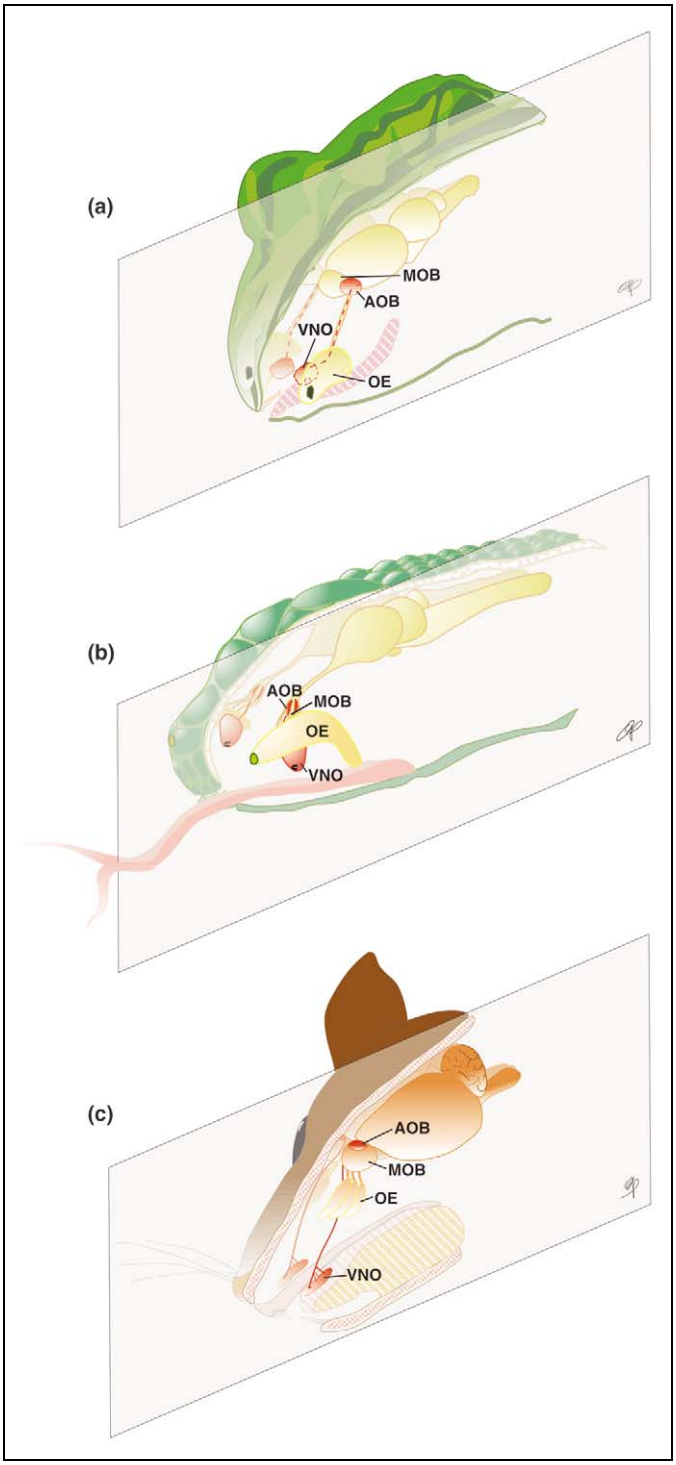

Figure 1. Anatomy of the olfactory and vomeronasal organs and their central projections in a generalized frog **(a)**, snake **(b)** and rodent **(c)**. Abbreviations: AOB, accessory olfactory bulb; MOB, main olfactory bulb; OE, olfactory epithelium; VNO, vomeronasal organ. Illustration by Gianluca Polese.

The transduction mechanisms used within the olfactory and vomeronasal organs also differ. In mammals, the vomeronasal receptor neurons express genes encoding seven-transmembrane-domain receptors of either the V1R or the V2R family, which are coupled to different G proteins [11]. A low degree of sequence similarity suggests that genes encoding the olfactory, V1R and V2R receptors arose independently from within the larger family of G-protein-coupled receptors, and some have hypothesized that the vomeronasal receptor genes evolved to detect different odorants from those detected by the main olfactory system [15,16]. Nevertheless, the olfactory and vomeronasal systems have overlapping functions and, as will be discussed in this review, both are involved in responses to both pheromones and general odorants [11,17,18].

Vomeronasal responses to pheromones

The vomeronasal organ clearly mediates some responses to pheromones. The vomeronasal system mediates both the acceleration of reproductive maturation in female mice exposed to male pheromones and the increase in circulating luteinizing hormone in male mice exposed to a protein from urine of female mice [19–21]. In female salamanders (*Cynops pyrrhogaster* and *Plethodon jordani*), attraction pheromones produced by males elicit physiological responses from the vomeronasal organ but not from the olfactory epithelium [22,23]. In some snake species, the male vomeronasal organ detects female pheromones in scent trails, and these cues are used to assess reproductive status [24].

The sensitivity of vomeronasal receptor neurons to pheromones has rarely been examined, but vomeronasal receptor neurons in mice have been shown to respond to identified pheromones at picomolar concentrations [25]. Although these data could be interpreted as indicating that the vomeronasal system is hard-wired to pinpoint tiny quantities of pheromones against a complex background of odorants, the sensitivity of olfactory receptor neurons to the same mouse pheromones or to other odorants was not examined, rendering a comparison of the sensitivity of the two systems impossible. The results of a separate study suggest that individual olfactory receptor neurons respond to quantities as small as a single odorant molecule [26].

Vomeronasal responses to non-pheromonal stimuli

The view that vomeronasal processing and pheromone processing are equivalent is contradicted by data demonstrating that the vomeronasal system responds to non-pheromonal stimuli. For example, mice lacking the olfactory-specific adenylyl cyclase show behavioral responses to some general, non-pheromonal odorants that elicit electrophysiological responses from the vomeronasal, but not olfactory, epithelium [27]. Similarly,

imaging data demonstrate that general odorants can elicit responses from individual vomeronasal receptor neurons in mice [28].

Heterospecific stimuli cannot be considered pheromones [1,18]; thus, chemical cues involved in foraging are not pheromones. Snakes have long been model organisms for understanding vomeronasal function, and studies conducted at the behavioral, electrophysiological and molecular levels demonstrate that the vomeronasal system is crucial in mediating responses to foraging cues [11]. The characteristic tongue-flicking behavior of snakes brings molecules into the vomeronasal organ [29], and the prevalence of tongue-flicking during hunting suggests that responses to prey odorants are mediated by the vomeronasal system [30]. Earthworms, a common prey item of garter snakes (*Thamnophis sirtalis*), produce a 20-kDa protein that is sensed by the garter snake vomeronasal organ [31,32]. When the vomeronasal nerve is sectioned, the snakes stop following prey odor trails and eventually stop eating prey [33]. Similarly, without a functional vomeronasal organ, rattlesnakes (*Crotalus viridis*) show a decreased rate of striking at prey and fail to eat prey after striking [34]. Electrophysiological studies have further confirmed the role of the vomeronasal system in prey discrimination in snakes [11]. The vomeronasal organ is also crucial for foraging behaviors of other reptiles, such as the lizard *Calcides ocellatus*, which will attack prey but not eat it if chemical cues are prevented from reaching the vomeronasal organ [35].

Although few such studies have been conducted using other tetrapods, the importance of the vomeronasal system in foraging and feeding is not restricted to reptiles. For example, plethodontid salamanders require a functional vomeronasal organ to capture stationary prey [36], and opossums fail to demonstrate food preferences after access to the vomeronasal organ has been blocked [37].

Responses to pheromones by other systems

Even if the vomeronasal system functioned exclusively to detect pheromones, other sensory systems could also respond to pheromones. Nevertheless, many researchers appear to subscribe to a strict equivalence between vomeronasal function and pheromone detection, assuming that all pheromone responses are mediated by the vomeronasal system (e.g. [5]). This view is not supported by the available data.

The olfactory system also mediates responses to pheromones. For example, induction of nipple search and attachment in rabbit pups is mediated by a maternal pheromone, 2-methylbut-2-enal (2MB2), that is probably detected by the olfactory system [38,39]. Androstenone, a pheromone found in boar saliva, facilitates adoption of a mating posture in estrous female pigs (*Sus scrofa*). Blockage of the vomeronasal duct has no effect on behavioral detection of androstenone or elicitation of pheromone-induced mating postures [40], indicating that this well-characterized mammalian pheromonal effect is mediated by the olfactory system. Female hamster vaginal secretions contain pheromones that induce mating behaviors in males and are correlated with an increase of *fos* expression in the medial nucleus of the amygdala and preoptic area, areas involved in the initiation of copulation. Destruction of the olfactory epithelium but not the vomeronasal organ eliminates the increase in *fos* expression, again indicating that the olfactory system mediates the response [41]. In mice, both the main and accessory olfactory bulbs are stimulated by both pheromones and general odorants [42].

Further, pheromones can exert effects without mediation by the nasal chemosensory systems. For example, some pheromones can be detected by the taste system, a possibility that has not been explored in vertebrates [18]. Moreover, in plethodontid salamanders, males increase the receptivity of female conspecifics by injecting pheromones directly into the bloodstream [43].

Alternatives: the learning and volatility hypotheses

The substantial differences in the anatomy and physiology of the vomeronasal and main olfactory systems strongly suggest that the two serve different functions. What could differentiate these functions? One hypothesis is that the vomeronasal system mediates unlearned responses to odorants which, through experience, can become associated with the olfactory system [44]. *fos* immunoreactivity indicates that central chemosensory pathways and the medial preoptic area are activated during initiation of copulation in male hamsters. After lesions of the vomeronasal organ, olfactory input stimulates the medial preoptic area in sexually experienced males but not in naïve males [45]. In mice and prairie voles (*Microtus ochrogaster*), the detrimental effects of vomeronasal organ removal on odor-induced ultrasonic calling and hormone surges can be overcome in sexually experienced males but not in naïve animals [46]. Because this hypothesis has received relatively little experimental attention, its validity is currently difficult to assess.

Another intriguing hypothesis is that the vomeronasal system mediates responses to molecules of low volatility, whereas the olfactory system mediates responses to more volatile molecules. When guinea pigs (*Cavia porcellus*) are given free access to conspecific urine containing a nonvolatile fluorescent dye, the dye is transferred to the vomeronasal and septal organs but not the olfactory epithelium [47]. Vaginal discharge of estrous female hamsters contains both volatile compounds that signal the presence of a female and nonvolatile compounds that elicit mating behaviors in males. In male hamsters, lesions of the olfactory nerve reduce investigation of both females and vaginal discharge, suggesting that the olfactory system detects the volatile components; lesions of the vomeronasal nerve impair initiation of mating behavior, suggesting that the vomeronasal system detects components of lower volatility [48].

Some of the mammalian and amphibian pheromones that have been isolated to date are large, high-molecular-weight molecules, which might explain why the effects of pheromones are frequently mediated by the vomeronasal organ. For example, the newt pheromone sodefrin is a large decapeptide that stimulates the vomeronasal organ [22], a result consistent with the volatility hypothesis. Low-molecular-weight pheromones such as 2MB2 and

Table 1. Sensory systems mediating responses to selected vertebrate pheromones, categorized by molecular weight[a]

Compound	MW	Species	Effect	Carrier?[b]	Mediator	Refs
3-Amino-s-triazole	84.1	Mouse (*Mus musculus*)	Attracts females	NA	NA	[73]
2-Methylbut-2-enal	84.1	Rabbit (*Oryctolagus cuniculus*)	Attracts pups to nipples	No (?)	Olfactory	[39,49]
Dimethyl disulfide	94.2	Hamster (*Mesocricetus auratus*)	Induces copulation in males	Aphrodisin	Vomeronasal	[54,62,74]
2,5-Dimethylpyrazine	108.1	Mouse (*M. musculus*)	Delays puberty in females	NA	Vomeronasal	[75]
2-Heptanone	114.2	Mouse (*M. musculus*)	Extends estrus	NA	Vomeronasal	[76]
4-Ethylphenol	122.2	Mouse (*M. musculus*)	Attracts females and repels males	NA	NA	[73]
2-*sec*-Butyl-4,5-dihydrothiazole	143.3	Mouse (*M. musculus*)	Attracts females and repels males; induces Whitten effect[c]	MUP-IV?	Vomeronasal	[57,58,77]
2,3-Dehydro-*exo*-brevicomin	154.2	Mouse (*M. musculus*)	Attracts females and repels males; induces Whitten effect	NA	Vomeronasal	[58,77]
(*Z*)-7-Dodecenyl acetate	226.4	Asian elephant (*Elephas maximus*)	Attracts males	Yes, unidentified	Vomeronasal?	[53]
5-α-Androst-16-en-3-one	272.4	Pig (*Sus scrofa*)	Facilitates mating in females	No (?)	Olfactory	[40]
Sodefrin	1071.2	Newt (*Cynops pyrrhogaster*)	Attracts females	No (?)	Vomeronasal	[22]

[a]Abbreviations: MW, molecular weight; NA, data not available.
[b]A carrier is a larger molecule bound to the pheromone when it is transported to the sensory organ. '(?)' indicates cases in which the pheromone alone is sufficient to produce the response in intact, behaving animals, suggesting that a larger carrier molecule is not required for transport into the sensory organ.
[c]The Whitten effect is the induction of estrous in anestrous females.

androstenone are detected by the olfactory system [38–40,49], again consistent with the volatility hypothesis. Table 1 summarizes data concerning vertebrate pheromones, their molecular weights and the sensory organs that mediate the responses to them.

Two important hurdles must be overcome before we can determine the feasibility of the volatility hypothesis. The first involves our lack of understanding of the mechanics of stimulus dispersal. For example, the volatility hypothesis appears to be contradicted by the presence of a vomeronasal organ in aquatic amphibians and reptiles, because volatility is irrelevant in water [50]. This problem might be solved if instead of volatility we focus on molecular weight, which is relevant in both air and water; thus, perhaps the vomeronasal system mediates responses to high-molecular-weight molecules, and the olfactory organ detects low-molecular-weight compounds [8]. Even so, molecules of varying molecular weights would presumably have access to both the olfactory and vomeronasal epithelia in aquatic animals, and recent data suggest that large particles, such as viruses, can reach the olfactory epithelium in terrestrial animals [51]. Few studies have compared the movements of large and small molecules in external environments and inside chemosensory organs in either air or water, yet this information is potentially crucial for our understanding of vomeronasal system function.

The second major gap in our knowledge concerns the functional relationship between small signaling molecules and larger proteinaceous compounds found in urine, glandular secretions and mucus. Large binding proteins appear to serve several functions: in some cases larger molecules hold the smaller ones in place and release them over time, as occurs in mouse scent marks [52,53]; larger molecules can also transport smaller molecules into urine or into the vomeronasal organ [53]; and large and small molecules together can form complexes with receptors [54,55].

The importance of understanding the relative roles of volatiles and carrier proteins is exemplified by two pheromones from male mice, 2,3-dehydro-*exo*-brevicomin (DHB) and 2-*sec*-butyl-4,5-dihydrothiazole (SBT), that are excreted in urine and bound by members of the major urinary protein (MUP) family [56]. Another MUP with high binding affinity for SBT is present in nasal mucus, suggesting that one or both of these pheromones could be transported to the vomeronasal epithelium in a bound state, rendering them effectively nonvolatile [57]. A study by Guo and colleagues found that DHB and SBT stimulate the accessory olfactory bulb only in the presence of MUPs [58], but another study found that different portions of the accessory olfactory bulb are stimulated separately by the volatile pheromones and MUPs [59]. Both DHB and SBT can elicit responses from vomeronasal neurons *in vitro* in the absence of MUPs [25,60], and the MUPs themselves might function as cues for individual recognition [61]. These two pheromones and the associated MUPs have been intensively studied for a decade or more, and represent one of the better-understood examples of pheromones and binding proteins in vertebrate physiology and behavior. Nevertheless, from the data available, it is still not clear whether DHB and SBT are part of a nonvolatile complex with a MUP when they enter the vomeronasal organ, or whether the presence of a MUP is necessary for stimulation of the vomeronasal system by DHB and SBT.

If experiments were to demonstrate unambiguously that responses to some small molecules are mediated by the vomeronasal organ and that these molecules arrive at the organ unbound to larger molecules, such a result would plainly contradict the volatility hypothesis. The available data provide tantalizing and contradictory information

concerning the likelihood of this scenario. One small molecule, the hamster pheromone dimethyl disulfide, exerts its effects through the vomeronasal system [62] but might arrive at the vomeronasal epithelium bound to a larger carrier molecule, aphrodisin [63]. Similar to MUPs, aphrodisin could have a more complicated role than simply being a carrier protein, because it elicits mating behavior in addition to responses from vomeronasal neurons and the accessory olfactory bulb [54,64,65]. In another example, the mouse pheromones 2-heptanone, 2,5-dimethylpyrazine, *E,E*-α-farnesene and *E*-β-farnesene can elicit responses from vomeronasal neurons *in vitro* [25], but elicit no response from mitral cells in the accessory olfactory bulbs of awake, behaving mice [66]. This suggests that these small molecules must be bound to larger carrier molecules to gain access to or stimulate the vomeronasal organ under natural conditions, but this conclusion is not supported directly. As illustrated in Table 1, several volatile pheromones exert their effects through the vomeronasal system, and we might yet find that each arrives bound to a carrier protein. If this is the case, it is not clear why other volatile pheromones, such as 2MB2 and androstenone, exert their effects through the olfactory system.

Concluding remarks

Those who adhere to the hypothesis that vomeronasal processing is equivalent to pheromone processing often focus on supporting data, overlooking contradictory data. This view has become so prevalent that some researchers apparently do not expect to be presented with conflicting results; for example, Sam *et al.* wrote in 2001 'Surprisingly, mouse vomeronasal neurons also detect odorants' [28]. Nevertheless, a similar observation was published in the early 1970s [67].

Uncritical acceptance of the equivalency hypothesis can lead to a situation in which studies of vomeronasal system function involve circular logic. By assuming that the vomeronasal system exclusively detects pheromones, some studies of vomeronasal function use only stimuli associated with conspecific responses and reproductive behaviors, and do not test other potential stimuli. For example, both Leypold *et al.* [68] and Stowers *et al.* [69] examined social and mating behaviors and electrophysiological responses to urine and pheromones in mice in which the TRP2 channel, which is involved in transduction in vomeronasal receptor cells, had been knocked out. Neither study included an examination of potential changes in feeding preferences or responses to predator-related odorants. Another example was presented by Luo *et al.* [66], who found that neurons in the accessory olfactory bulb are active while mice sniff the mouth region of anesthetized conspecifics. Although the authors suggested that the mice might be exploring cues associated with sex or individual identity, an alternative explanation is that the mice perceive the anesthetized conspecific as ill and examine its breath to learn what it recently ate so that they can avoid eating the same food [70]. Perhaps this possibility would have been considered by Luo *et al.* if the idea that the vomeronasal system functions as a pheromone detector were not such a firmly ingrained assumption.

Contemporary studies sometimes involve experiments that do not have appropriate controls, rendering the results difficult to interpret. Researchers need to use at least a two-by-two design, testing more than one type of biologically-relevant odorant on both the olfactory and vomeronasal organs. For example, Leinders-Zufall *et al.* [25] tested six putative pheromones on vomeronasal neurons but did not test general odorants on vomeronasal neurons or the putative pheromones on olfactory neurons. Similarly, Holy *et al.* recorded responses to urine from neurons only in the vomeronasal organ, and not in the olfactory epithelium, of mice [71]. Without appropriate data available for comparison, the specificity of vomeronasal responses cannot be assessed.

Concomitantly, the role of the vomeronasal system in mediating responses to non-pheromonal cues deserves

Table 2. The equivalency hypothesis is not well supported by the relevant behavioral, physiological and endocrinological data

Observation	Consistent with equivalency hypothesis?	Consistent with volatility hypothesis?	Refs
Increase in circulating luteinizing hormone in male mice exposed to a protein in female urine is mediated by the vomeronasal system	Yes	Yes	[20]
Attraction to sodefrin is mediated by the vomeronasal system in newts	Yes	Yes	[22]
Small-molecule pheromones elicit responses from vomeronasal neurons *in vitro* but not in awake, behaving mice	No	Yes	[25,67]
Small-molecule general odorants elicit responses from the vomeronasal organ in mice	No	No	[27,28,42]
A 20-kDa earthworm protein is detected by the vomeronasal organ in snakes	No	Yes	[31,32]
2MB2-stimulated nipple attachment is mediated by the olfactory system in rabbit pups	No	Yes	[38,39]
Adoption of mating posture in response to androstenone is mediated by the olfactory system in pigs	No	Yes	[40]
Lesioning the olfactory nerve in male hamsters reduces investigation of estrous females and their vaginal discharge elicited by volatile compounds	No	Yes	[48]
Lesioning the vomeronasal nerve in male hamsters impairs mating behaviors elicited by nonvolatile compounds	Yes	Yes	[48]
Mounting stimulated by dimethyl disulfide and aphrodisin is mediated by the vomeronasal system in male hamsters	Yes	Yes	[53,74]
DHB- and SBT-stimulated estrous cycling in female mice is mediated by the vomeronasal system	Yes	No	[77]

more attention. This is not a unique perspective: Rodriguez *et al.* [72] have suggested that the size and diversity of the V1R gene family indicate that these receptors might be used for more than just pheromone detection. The role of the vomeronasal system in foraging in mammals should be investigated, for example by testing for involvement of the vomeronasal system in predation. In addition, changes in behavioral and physiological responses to chemical cues resulting from sexual experience should be explored more deeply, and the effects of foraging experience on responses to olfactory and vomeronasal input should be determined. At a minimum, researchers should not assume that a newly-discovered feature of the vomeronasal system underlies pheromonal processing without obtaining direct behavioral or physiological evidence of such a role.

To date, the volatility hypothesis does not appear to be on a much firmer footing than the equivalency hypothesis, perhaps because significant gaps remain in the data. In particular, adequate understanding of the functional relationship between large carrier molecules and smaller molecules is lacking, complicating attempts to generate specific predictions using the volatility hypothesis. Table 2 provides a comparison of the fit of experimental observations to the pheromone equivalency and volatility hypotheses. Neither hypothesis is fully supported by the available data, but the equivalency hypothesis is clearly contradicted.

The view that experience has a role in vomeronasal system function does not generate easily testable predictions, but we can make some predictions concerning differences in the roles of olfactory and vomeronasal input before and after sexual or foraging experience. Of course, we should keep in mind that the volatility and experience hypotheses are not mutually exclusive.

Here, we have focused on progress towards a more coherent and accurate classification of the functions of the vomeronasal and olfactory systems. Trying to distinguish specific and wholly separate functions of the two systems can be frustrating. Although it might be tempting to equate the vomeronasal system with pheromone processing, this hypothesis is contradicted by the available data, and cannot be accepted.

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