REVIEW

The Vomeronasal Organ

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The vomeronasal organ (VNO) is a chemoreceptor organ enclosed in a cartilaginous capsule and separated from the main olfactory epithelium. The vomeronasal neurons have two distinct types of receptor that differ from each other and from the large family of odorant receptors. The VNO receptors are seven-transmembrane receptors coupled to GTP-binding protein, but appear to activate inositol 1,4,5-trisphosphate signaling as opposed to cyclic adenosine monophosphate. The nature of stimulus access suggests that the VNO responds to nonvolatile cues, leading to activation of the hypothalamus by way of the accessory olfactory bulb and amygdala. The areas of hypothalamus innervated regulate reproductive, defensive, and ingestive behavior as well as neuroendocrine secretion.

The VNO, located at the base of the nasal cavity, has the appearance of a paired, tubular structure divided by the nasal septum, each side having a crescent-shaped lumen (Fig. 1) (*1*). It is a chemosensory organ distinctly separated from the nasal cavity in most amphibia, reptiles, and nonprimate mammals, but is absent in birds and adult catarrhine monkeys and apes (*2*). The VNO is enclosed in a bony or cartilaginous capsule and opens through a duct into the base of the nasal cavity. A connection with the oral cavity may be present through the nasopalatine duct, especially in carnivores and ungulates. The crescent-shaped lumen of the VNO is lined with receptor neurons on the medial concave side and is filled with fluid from the vomeronasal glands. Lateral to the lumen are large blood vessels and sinuses that are innervated by the autonomic nervous system which, together with nitric oxide, and vasoactive intestinal polypeptide nerve fibers (*3*), induce vasodilation and vasoconstriction, thereby producing a pumplike action for stimulus access to the lumen (*4*). The vomeronasal neurons are therefore isolated from the airstream that passes through the nasal cavity during normal respiration, and stimulus access requires some form of arousal to activate the vascular pump. In horses, sheep, cows, and goats, a pronounced curl of the lips and closure of the external nares (flehmen behavior) is thought to be associated with promoting stimulus access to the VNO (*5*).

Measurable quantities of noradrenaline (NA) have been found in the VNO of mice after exposure to male urine (*6*). This release of NA may alter the vascular tone, or change the glandular secretions containing pheromone transporters, and such paracrine secretion might also increase the sensitivity of the sensory epithelium in particular behavioral contexts. Such an effect of NA has recently been demonstrated for the main olfactory epithelium as a result of increasing the amplitude of Na^+ current and decreasing the amplitude of Ca^{2+} current (*7*). Functioning of the VNO is thus not simply a passive chemosensory event, but is actively regulated by centrifugal projections.

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VNO Sensory Epithelium

The medial, concave side of the vomeronasal lumen is lined by a pseudostratified epithelium that differentiates into receptor cells, supporting cells, and basal cells. The basal stem cells are located along the basement membrane and cluster near the boundary with nonsensory epithelium. Supporting cells are found in the more superficial layer of the sensory epithelium, while the receptor cells form two overlapping populations that can be distinguished by in situ hybridization for a number of different markers [receptor genes (*8*), GTP-binding proteins (G proteins), N-CAMS (*9*)]. The volume of the sensory epithelium increases from birth to puberty but remains fairly constant in size from 2 months after birth. The receptor neurons possess apical microvilli as opposed to the cilia seen on the main olfactory receptors, and their axons merge together, forming vomeronasal nerves that run between the paired olfactory bulbs and enter the accessory olfactory bulb (AOB) at the posterior dorsal aspect of the main olfactory bulb (MOB) (Fig. 1). Transneuronal tracers appear not to reach the AOB mitral cells until the postnatal period, suggesting that the system does not become active until after birth (*10*).

The vomeronasal sensory cells originate in the olfactory placode together with luteinizing hormone–releasing hormone (LH-RH) neurons and γ-aminobutyric acid (GABA)–containing neurons (*11*). The GABA-containing and olfactory sensory neurons have resting potentials around −50 mV and appear not to migrate with the LH-RH neurons that traverse the developing AOB and migrate to the medio-basal hypothalamus. The vomeronasal sensory neurons maintain a functional relationship with these areas of the medio-basal hypothalamus in the adult mammal, influencing neuroendocrine function and behavior (*12*). Because of the considerable experimental evidence linking the vomeronasal organ with pheromone detection, the vomeronasal receptors are frequently referred to collectively as pheromone receptors (*13*). However, this does not exclude the olfactory receptors from a functional role in detecting certain pheromones.

Two multigene families of G protein–linked receptors (V1 and V2), each expressed in a distinct region of the VNO, have been identified (*8*). These seven-transmembrane receptors are only distantly related to the main olfactory receptors, suggesting that they may respond to very different ligands. These two families of putative VNO receptors differ, not only in their linkage to distinct G proteins, but in the length of their extracellular NH_2-terminal domains. The V1 receptors (V1Rs) are linked to $G\alpha_{i2}$, have a relatively short NH_2-terminal, and have greatest sequence diversity in their transmembrane domains (*14*). The V2Rs are linked to $G\alpha_o$ and comprise a family of about 140 genes distinguished by their long extracellular NH_2-terminal that is thought to bind ligands (*15*). Of the V2R genes, a number are pseudogenes that lack one or more exons or have mutations that prevent transcription of functionally normal receptors. Calculations based on probes that both recognize genes and hybridize with $G\alpha_o$ suggest that only one receptor is expressed in each sensory neuron (*8*).

Subdivisions of the vomeronasal projections to the glomerular layer of the AOB have been reported through the use of a wide variety of techniques (*16*, *17*) and in a wide variety of species (mouse, rat, rabbit, guinea pig, and garter snake). These features underlie a zone-to-zone projection with the vomeronasal sensory neurons from the apical regions (expressing V1Rs) projecting to the rostral (anterior) part of the AOB. VNO neurons in the basal region (expressing V2Rs) project to glomeruli in the posterior AOB. The former also express the olfactory cell adhesion molecule (OCAM) that belongs to the immunoglobulin superfamily with structural homologies to N-CAM (*18*). N-CAM is expressed in both sets of VNO sensory neurons. These cell adhesion molecules are involved in stabilization, growth, and plasticity of synaptic connections. OCAM-positive

vomeronasal axons exclusively invade glomeruli occupied by dendrites of OCAM-negative mitral cells, whereas OCAM-negative axons terminated on OCAM-positive mitral cells (*19*). A splice variant of the axonal associated cell adhesion molecules (AxCAMs) lacking the glycosyl-phosphatidylinositol–anchoring domain has been identified in the VNO neurons (*20*). Maximum expression was observed in mature VNO sensory cells, and because this splice variant seems to be a soluble form, it may be involved in promoting neurite outgrowth.

VNO Transduction by Pheromones

Distinct signaling mechanisms appear to be operating in the VNO sensory neurons (*21*) in contrast to main olfactory sensory neurons where the G protein–coupled receptors activate adenylyl cyclase and ultimately the opening of cyclic nucleotide gated (CNG) ion channels (*22*). Work on a number of different species suggests that inositol 1,4,5-trisphosphate (IP_3) signaling is activated by pheromones. In the hamster, oestrous vaginal discharges modulate male attraction and mounting behavior. Purified aphrodisin, a protein isolated from hamsters vaginal secretions, increases IP_3 production in the VNO membranes without altering cyclic adenosine monophosphate (cAMP) production (*23*). This appears to be specific to the VNO and has no effect on second messengers in the main olfactory epithelium. The female pig shows a stereotyped rigid stance to boar seminal fluid and urine, and receptor membranes isolated from her VNO and incubated with boar seminal fluid or urine produce an increase in IP_3 (*24*). In the garter snake chemoattractive proteins from their prey, earthworms, act by way of a G protein–coupled receptor, increasing IP_3 levels but reducing levels of cAMP (*25*). Adenylate cyclase in the snake VNO is very sensitive to Ca^{2+}, which is mobilized from intracellular stores on increases in IP_3 (*26*). In the rat, stimulation of VNO membrane preparations with male and female urine induces activation of both vomeronasal G-protein subtypes (G_i and G_o) and accumulation of IP_3 (*27*). Upon stimulation with lipophilic volatile odorants only G_i proteins are activated, whereas G_o activation is elicited by α-2-globulin, a rat urinary lipocalin protein (*28*). This suggested that the two types of vomeronasal receptors may be activated by distinct ligands, with the V2Rs responding to nonvolatile proteins. Studies have failed to identify CNG channels in VNO neurons, but specific expression of rTRP2 (transient receptor potential) in VNO sensory microvilli is consistent with IP_3 activation leading to opening of TRP2 channels and alteration in membrane potential (*29*).

Physiology of VNO Sensory Neurons

Patch-clamp recordings from single receptor cells in vitro have provided information on the sensitive nature of vomeronasal neurons, which fire action potentials in response to current injection as small as 1 pA (*30*). The ease of eliciting action potentials may be enhanced by a neuronal resting potential (around −80 mV in frog, −60 mV in mouse and rat) that is close to the firing threshold (*30–32*). Spontaneous firing activity has rarely been seen in these dissociated VNO neurons. Mouse VNO neurons fire tonically to a maintained current injection of as little as 1 pA and show very slow adaptation (*33*). This is in contrast to olfactory sensory neurons, which fire a single or short burst of action potentials and require pulsatile current injection to sustain firing. The firing rate of VNO neurons increases linearly with increasing current injection up to 10 pA, which suggests that they have the potential to encode stimulus strength. However, persistent stimuli even of small amplitude are also effective (*33*), a finding that is congruent with the necessity for prolonged exposure to pheromones in order to induce the reproductive changes seen in mice.

Twenty six percent of dissociated VNO neurons respond to chemical stimulation (dehydro-exo-brevicomin, a putative pheromone) with an outward current at negative holding potentials. This chemical stimulation produces membrane hyperpolarization and a reduction in the firing of action potentials. The current reverses when the VNO neurons are clamped at +4 mV and chemically stimulated (*34*). These different findings may represent different populations of neurons because the application of this pheromonal component produces a response in fewer neurons (26%) than urine itself (50%) (*30*, *34*). It would therefore seem likely that the slow-adapting, tonically active VNO neurons are the $G\alpha_o$ neurons containing V2Rs. V2Rs have sequence homologies with the metabotropic glutamate receptors, which also have a large extracellular NH_2-terminal, are slow adapting, and do not readily down-regulate (*35*).

The subdivisions of the AOB with respect to the two types of receptor neuron (V1Rs and V2Rs) show very different responses to VNO nerve stimulation (*36*). Activation of neurons expressing V1Rs elicit field potentials with weak oscillatory responses exclusively in the anterior AOB, whereas distinct oscillations are provoked by electrical stimulation of the axons from VNO neurons that project to the posterior aspect of the AOB (V2Rs). Stimulation of the nerve layer innervating the anterior AOB produces neural activity only within the anterior

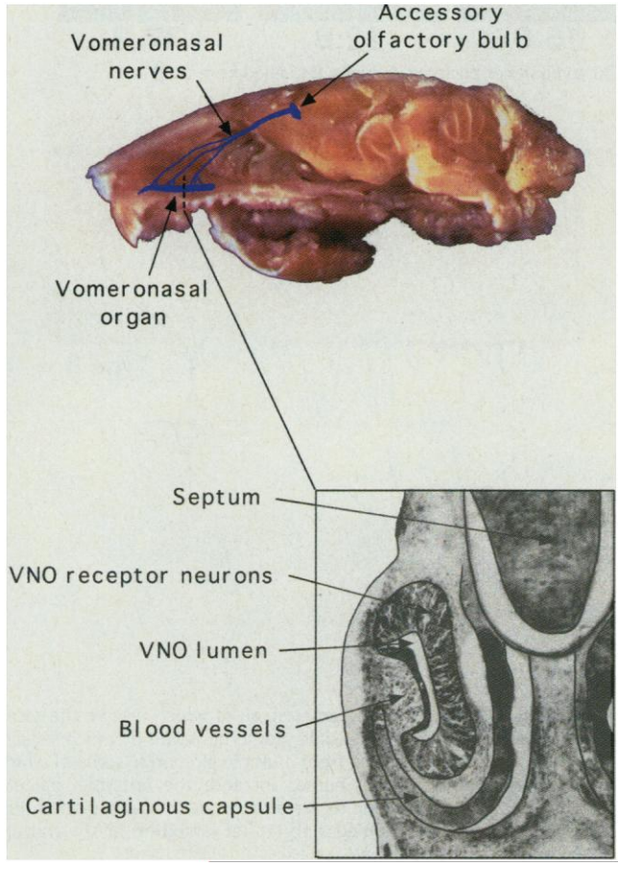

Fig. 1. Sagittal section of the mouse head showing position of the VNO and nerve projections to the AOB. Dotted line indicates position of coronal section of the VNO (shown below).

portion of the AOB (Fig. 2). This activity spreads in the anterior-posterior direction, producing differing levels of excitement throughout the anterior AOB. Strong excitation of the granule cell layer can be seen, which may be responsible for the dampening of the oscillatory field potential in the anterior AOB. V2R-linked posterior VNO stimulation evokes periodic neural activity, the timing of which coincides with oscillatory field potentials, but only in the posterior AOB. Both the field potentials and the real-time optical imaging have demonstrated a precise boundary in each subdivision of the AOB that corresponded with the boundary defined by the two types of VNO G protein–linked receptor.

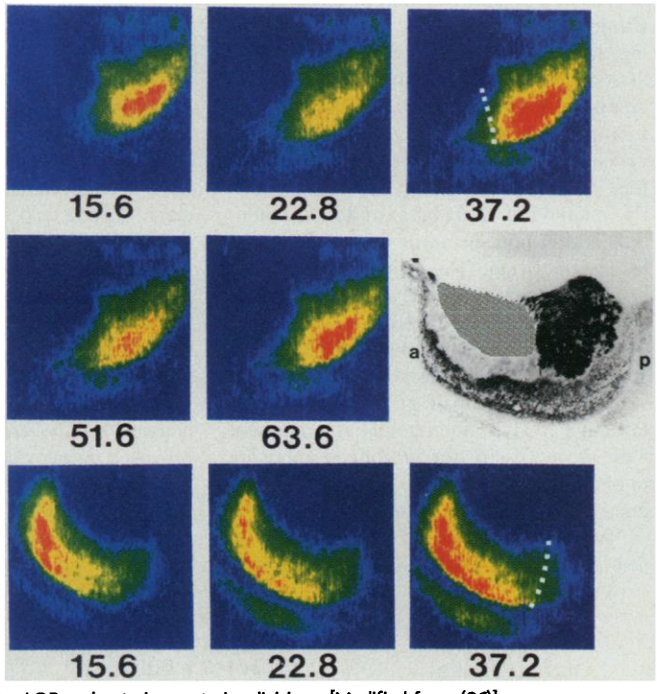

Fig. 2. Signal propagation in the AOB after anterior (lower three images) and posterior AOB (upper and middle five images) vomeronasal nerve stimulation. Excitation in the posterior AOB appears as an oscillatory wave (two cycles shown with peak activity at 15.6, 37.2, and 63.6 ms) that does not cross into the anterior division of AOB. Excitation in the anterior AOB spreads in the anterior-posterior direction throughout the anterior AOB but does not cross into the posterior division (indicated by white dotted line at 37.2 ms). Increasing excitation in the granule cell layer can also be seen, which may explain the dampening of oscillations in the anterior AOB. Photomicrograph shows sagittal section of the AOB and anterior-posterior divisions. [Modified from (*36*)]

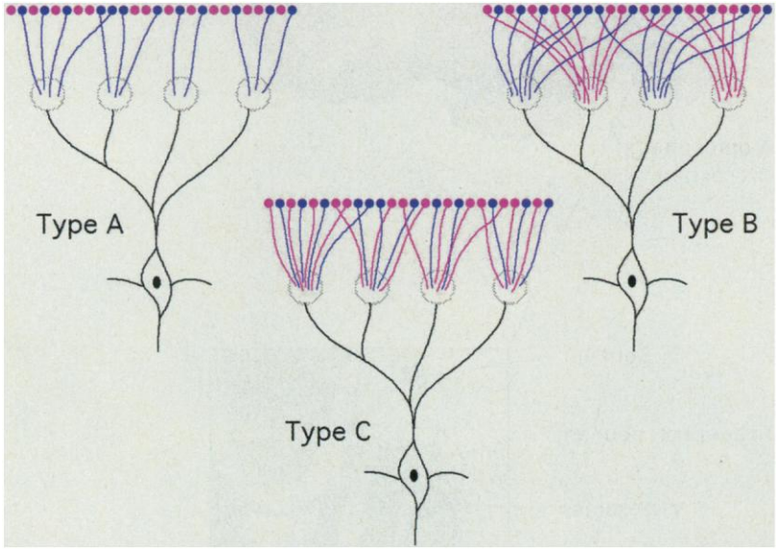

Fig. 3. (**A**) Mitral cell receives information from multiple glomeruli, all of which receive the same VNO receptor input. (**B**) Mitral cell receives input from multiple glomeruli, some of which receive different VNO receptor input. (**C**) Mitral cells receives input from multiple glomeruli, some of which receive mixed receptor input. Combinations of these schemes increase the potential pattern generation. If combinations of inhibitory and excitatory inputs were to invade the same glomerulus, small differences in signal components might be amplified, and lateral inhibition at the mitral–granule cell interaction would further enhance this signal.

VNO Projections and Behavior

In contrast to the main olfactory system, the VNO has relatively small families of receptor genes. Nevertheless, this limited repertoire of receptors is able to code for mouse individual recognition that occurs at the first VNO relay in the AOB (*37*). Mouse pheromones have more common than different components across individuals. Therefore, in order to achieve recognition, amplification of differences is required in both the spatial and temporal activity patterns in the AOB. With the relatively small repertoire of receptor types expressed in VNO neurons, this can be achieved by increasing the complexity of the spatial interconnections in the AOB. In the MOB, olfactory neurons with one receptor type converge on one glomerulus, and each output neuron makes synaptic contracts with only one glomerulus, thereby maximizing precision and sensitivity. The interconnections of the AOB are more variable, as has recently been shown by gene targeting to visualize the patterns of VNO projections to the AOB (*38*). Neurons expressing the same receptor gene project to many different glomeruli, while a single glomerulus may receive input from more than one receptor type (Fig. 3). This is further complicated by the AOB output neurons (mitral cells) contacting more than one glomerulus through their apical dendrites. Although certain glomeruli receive inputs from more than one receptor type, it is possible that spatial segregation within this glomerulus could match to the apical dendrites of distinct mitral cells, providing more organization than is now apparent. Nevertheless, it would appear that a relatively small population of receptor types is capable of generating distinct patterns of neural activity from which different pheromone compositions can be recognized. Development of a network capable of generating this kind of complexity at the first relay presents problems for precise spatial mapping, especially because the VNO neurons subsequently undergo turnover and regeneration. It is therefore not surprising that, although glomeruli that receive inputs from a given receptor type are found in spatially restricted areas, the spatial maps appear to have both common and variable components. The precision of this mapping is far removed from that of the main olfactory projections and there is not even precise symmetry between the two AOBs of the same animal. Although individual recognition may be thought to require extreme precision in the sensory map, the recognition of a mouse's pheromone mix is thus able to withstand flexibility at the first relay. Recognition by way of the MOB is required to attribute meaning and categorize odors—complex neural events that are achieved by integration at cortical levels. In contrast, the AOB communicates principally

with the hypothalamus and neuroendocrine neurons, and in the context of individual recognition only needs to serve as a difference detector. Output neurons are activated only if the signal is different; a repeated learned signal is gated at the level of the AOB (*39*).

Mating in both males (*40*) and females (*41*) produces activation of immediate-early gene markers in the AOB and in the central projections of the AOB (medial amygdala, bed nucleus of the stria, medial pre-optic area, ventro-medial hypothalamus, and arcuate nucleus) (*42*). The medial hypothalamic projections of the accessory olfactory amygdala are especially prominent and selectively innervate parts of the three systems that control the expression of reproductive, defensive, and ingestive behaviors (*43*). Moreover, there is a sexual dimorphism in the VNO and its central projections (*44*), but the extent to which these relate to anterior, posterior AOB, or different VNO receptor types is not known. These sexual dimorphisms in the morphology of the VNO projection circuit are not reflected in the functional responsiveness of neurons in this circuit to chemosensory cues from male versus female conspecifics (*45*). In mice, exposing females to male bedding (pheromones) increases the number of fos-positive neurons in the VNO and in the AOB, and this effect is enhanced in oestrous females especially after mating (*40*). Two independent studies have shown that in female mice the biologically relevant cues from males especially activate the anterior part of the AOB, suggesting preferential activity of V1Rs (*46*, *41*).

Male mice excrete a large quantity of major urinary proteins (MUPs) (*47*) that have been proposed to have an important pheromonal role either alone or by way of their bound ligands (*48*). These proteins induce early puberty (*49*) and are involved in the pheromone block to pregnancy in mice (*37*), as well as conveying the strain recognition signal of the male pheromone (*46*). Using the immediate-early gene marker *egr-1*, it has been shown that the MUPs and male mouse urine induce expression in the anterior and posterior region of the AOB, whereas MUP ligands (brevicomin, dihydrothiazole) without their carrier activate the medial and lateral margins of only the posterior AOB (Fig. 4) (*46*). This would suggest that the anterior and posterior halves of the AOB are processing different aspects of the male pheromone signal, and that the anterior region, which responds strongly to the MUPs, but not their ligand alone, is principally concerned with individual recognition, which is an essential component of the pheromone block to pregnancy.

Human Pheromones

The very nature of human behavior is exemplified by its emancipation from any simple or single determining factor. Reproductive behavior is independent of oestrous-promoting hormones, maternal behavior may occur without pregnancy and parturition, and there is no evidence for reflex ovulation contingent on somatosensory stimulation (*50*). Evolutionary enlargement of the primate neocortex has enabled the rapid assimilation and integration of information from a number of sensory channels simultaneously. Behavior does not come under the obligatory regulation of any one sense (*51*). It therefore seems implausible that humans might experience significant behavioral or endocrine regulation by pheromones. Nevertheless there have been claims for human pheromones (*52*) and for a functional human VNO (*53*). There is strong anatomical evidence for the foetal human VNO (*54*), and this has been recognized since the days of Jacobson. However, the molecular genetic studies suggest that human olfactory sensibilities are in decline [72% of human olfactory receptor genes were pseudogenes (*55*), and only pseudogenes have so far been identified for the human VNO receptors (*14*)] and that the human TRP2 is a pseudogene (*28*). Anatomical studies lend support to this viewpoint. A study of 564 adults has located the vomeronasal vestibule bilaterally in only 8% of subjects, whereas it was unilateral in 22% and

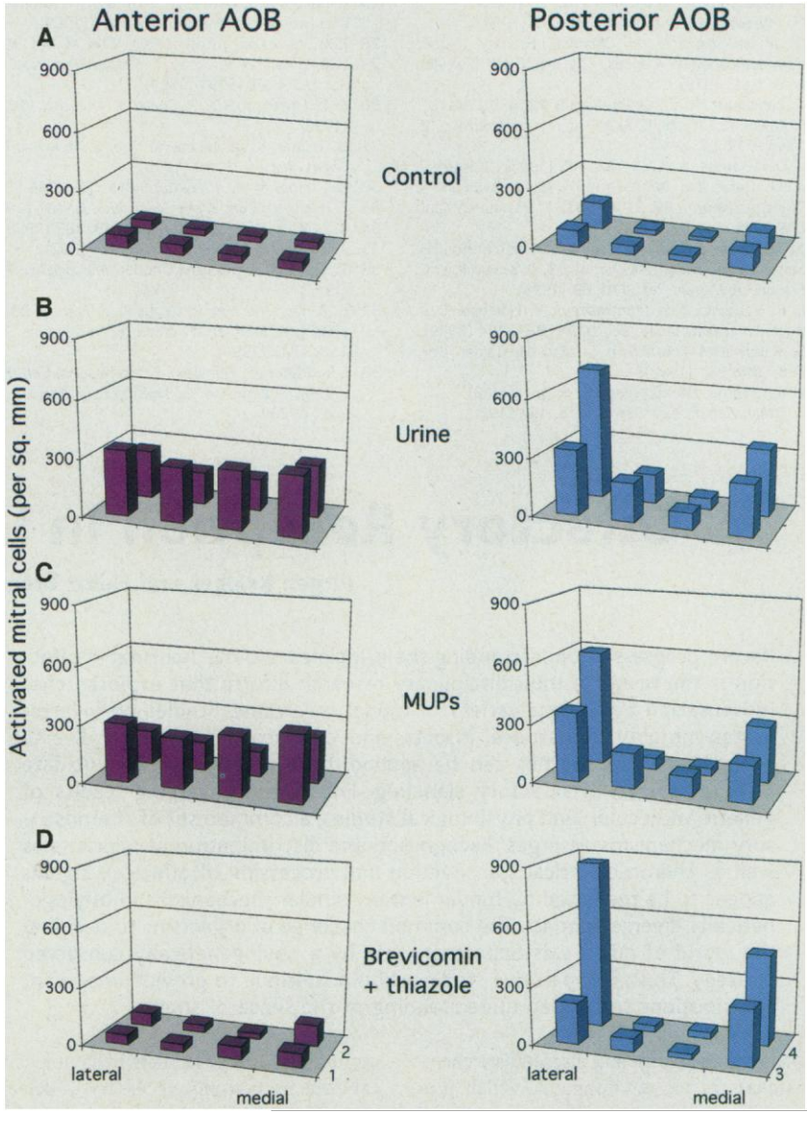

Fig. 4. Mitral cell activity (*egr* expression) in the anterior and posterior regions of the AOB of (**A**) control females that have been exposed to water and (**B**) females that have been exposed to male urine, (**C**) major urinary proteins (MUPs) stripped of ligand, or (**D**) a combination of the ligands that bind to MUPs (brevicomin and thiazole). Each set of histograms is based on coronal sections made rostro-caudally with six to eight mice in each treatment condition, and each column representing an octant of the anterior or posterior AOB. Urine and MUPs show similar patterns of activation in anterior and posterior AOB, whereas the ligands activate only posterior AOB. [Modified from (*46*)]

absent in 70%. Biopsy and autopsy investigations have failed to identify neurons in the adult human VNO or the presence of vomeronasal nerve bundles by using a wide variety of neural markers (*56*). Moreover, antibodies against the olfactory marker protein (OMP) have failed to reveal OMP-expressing cells in the human VNO, a finding supported by the absence of an AOB in humans (*57*). The overwhelming evidence would therefore not support a human VNO that is functional in any meaningful way.

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REVIEW

Olfactory Reception in Invertebrates

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Recent progress in understanding the principles and mechanisms in olfaction is the result of multidisciplinary research efforts that explored chemosensation by using a variety of model organisms. Studies on invertebrates, notably nematodes, insects, and crustaceans, to which diverse experimental approaches can be applied, have greatly helped elucidate various aspects of olfactory signaling. From the converging results of genetic, molecular, and physiological studies, a common set of chemosensory mechanisms emerges. Recognition and discrimination of odorants as well as chemo-electrical transduction and processing of olfactory signals appear to be mediated by fundamentally similar mechanisms in phylogenetically diverse animals. The common challenge of organisms to decipher the world of odors was apparently met by a phylogenetically conserved strategy. Thus, comparative studies should continue to provide important contributions toward an understanding of the sense of smell.

Animals can recognize and discriminate chemical signals in the environment, which provide essential information for survival and profoundly influence their behavior. Chemical cues are not only necessary to detect and assess food, mating partners, prey, and predators, but also for communication with other animals. Remarkable chemosensory abilities have been demonstrated in invertebrates, and in many ways, they offer ideal models for addressing basic questions of molecular recognition, chemo-electrical transduction, and processing of olfactory signals. Because most invertebrates rely on olfaction as the principal sensory modality, their olfactory systems have evolved to a level of extreme sensitivity and specificity (*1*). This enables them to identify minute concentrations of behaviorally relevant compounds. The detection of pheromones by the antennae of male moths is a prime example (*2*). Exploring the organization, development, and function of invertebrate olfactory sensory systems may help unravel fundamental principles of chemosensation and contribute to understanding of the more complex process of olfaction in higher organisms. Although invertebrate chemosensory systems display tremendous diversity across phyla, strong morphological similarities are found at the cellular level (*3*). In all olfactory systems, even in animals as phylogenetically diverse as flies, lobsters, or nematodes, specialized bipolar sensory neurons are employed for the detection of odorous compounds. The neurons extend a thin dendrite to the environmental interface and

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