
REVIEW

Human Vomeronasal Organ Function: A Critical Review of Best and Worst Cases

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Abstract

The human vomeronasal organ (VNO) has been the subject of some interest in the scientific literature and of considerable speculation in the popular science literature. A function for the human VNO has been both dismissed with ridicule and averred with conviction. This question of VNO function has been needlessly tied to the separate question of whether there is any place for pheromone communication among humans, a topic that is itself bogged down in conflicting definitions. This review is an attempt to weigh the evidence for and against human VNO function, to deconvolve that question from the question of pheromone communication and finally to provide a working definition of 'pheromone'. Further experimental work is required to resolve the conflicting evidence for and against human VNO function but chemical communication does appear to occur among humans. However, several examples reported in the literature do not meet the proposed definition for communication by pheromones: 'chemical substances released by one member of a species as communication with another member, to their mutual benefit'.

Introduction

The vomeronasal organ (VNO) is the peripheral sensory organ of the accessory olfactory system. The paired organs are located at the base of the nasal septum or in the roof of the mouth in most amphibia, reptiles and mammals. There are numerous examples of vomeronasal involvement in chemical communication, although pheromone communication is not the exclusive province of the vomeronasal system. The increase in serum luteinizing hormone and testosterone when male mice and hamsters are exposed to chemosensory stimuli from females appears to be absolutely dependent on vomeronasal integrity (Coquelin *et al.*, 1984; Pfeiffer and Johnston, 1994). Induction of uterine growth and estrus in female prairie voles normally resulting from exposure to males is also dependent on an intact VNO (Tubbiola and Wysocki, 1997). There are numerous other behaviors and physiological responses where both vomeronasal and olfactory inputs contribute (Wysocki and Meredith, 1987; Johnston, 1998) and some where the main olfactory system seems to be critical (see below). In some non-mammalian species, for example in snakes, vomeronasal chemoreception may be used for tracking prey (Halpern, 1987), which is unlikely to be a pheromone function. Whether the vomeronasal systems in mammals have any similar non-social communication functions has

not been thoroughly investigated. In humans there has been a long-standing dispute over whether there is a VNO at all in adults. Recent endoscopic and microscopic observations suggest that there is an organ on at least one side in most adults. This review enquires into its function.

Description: anatomical, developmental and genetic evidence

Structure

The existence of a VNO in the human embryo similar to the VNOs of other species is undisputed (Boehm and Gasser, 1993). It contains bipolar cells similar to the developing vomeronasal sensory neurons of other species and also generates luteinizing hormone releasing hormone (LHRH)-producing cells as in other species (Boehm *et al.*, 1994; Kajer and Fischer Hansen, 1996). These authors showed the structure becoming more simplified later in development. The latter were unable to find any VNO structure at later stages (19 weeks), although others have shown a simplified but clear VNO continuing to increase in size up to at least 30 weeks (Bohm and Gasser, 1993; Smith *et al.*, 1997). Numerous reports of a structure identified as the VNO in the nasal septum in adult humans agree that it is a blind

ending diverticulum in the septal mucosa opening via a depression (the VNO pit) into the nasal cavity ~2 cm in from the nostril. The location of this structure is consistent with the location of the VNO in embryos (Trotier *et al.*, 2000) and it has a similar simplified form, with no large blood vessels, cavernous sinuses or supporting cartilage. The structure is reported at least unilaterally in 90% or more of subjects in some reports or in 50% or fewer in other reports. Trotier *et al.* recently demonstrated that the endoscopic appearance of the VNO pit can vary, unequivocal on one inspection and invisible on a later inspection, or vice versa (Trotier *et al.*, 2000). The real percentage of individuals with at least one VNO pit may thus be underestimated in many studies. Trotier *et al.* estimate ~92% with some evidence of at least one VNO pit in subjects with no septal surgery examined multiple times, but a substantially lower number after septal surgery (Trotier *et al.*, 2000). Standard septal surgery may remove the VNOs and there are anecdotal reports of adverse effects of vomeronasal removal, but no systematic study. In histological studies in cadavers or in septal tissue removed during nasal surgery, several authors (Moran *et al.*, 1991; Johnson *et al.*, 1994; Trotier *et al.*, 2000) describe a blind ending tube lined on all sides by a pseudo-stratified epithelium and with associated submucosal glands. It seems highly likely that this structure is the adult human remnant of the vomeronasal organ. Use of the word organ in this context does not presuppose function.

Best case: The vast majority of human adults have a VNO.

Worst case: There is a diverticulum of the nasal epithelium which happens to be remarkably consistently located at the expected position of the VNO.

Opinion: There is an adult human VNO.

Microanatomy

The epithelium lining the human VNO is unlike that of VNOs in other species and unlike that of olfactory or respiratory epithelium in humans (Moran *et al.*, 1991; Stensaas *et al.*, 1991). There are many elongated cells presenting a microvillar surface to the lumen of the organ but most are not similar to microvillar vomeronasal sensory organs (VSNs) of other species. They have not been shown to have axons leaving the epithelium nor to make synaptic contact with axons in the epithelium, so if they are chemosensitive they have no obvious way of communication with the brain.

Two studies of the adult human vomeronasal epithelium have reported the presence of bipolar cells resembling the VSNs found in other species and in early human embryos. These cells contain marker substances characteristic of neural cells. Takami *et al.* and Trotier *et al.* found neuron-specific enolase (NSE) staining in these cells (Takami *et al.*, 1993; Trotier *et al.*, 2000). It is clear from both reports that the number of such cells is small: ~4 per

100 μ m epithelial surface (Takami *et al.*, 1993) or less (Trotier *et al.*, 2000). Neither found the olfactory marker protein (OMP) staining characteristic of VSNs of all other species studied. No one has been able to show that these VSN-like cells in the adult human VNO taper down to form axons at their basal ends. Axons are observed in the epithelium (Stensaas *et al.*, 1991), but not in continuity or in synaptic contact with epithelial cells. Axon bundles are reported in the submucosa (Stensaas *et al.*, 1991), but do not appear to arise from axon bundles penetrating the lamina propria in the same way as in vomeronasal epithelia of other species. Moreover, the fact that a few human VNO cells show a morphological resemblance to VSNs does not preclude chemosensitivity in other cell types. The human vomeronasal epithelium differs in appearance from both the sensory and non-sensory epithelia in the VNOs of other species and from nasal 'respiratory' epithelium (Moran *et al.*, 1991; Stensaas *et al.*, 1991). The function of the cells is not immediately obvious from their morphology. However, the absence of OMP and any reports of putative vomeronasal receptor genes (see below) means that any such cells are quite different from known VSNs in other species.

Best case: The human VNO contains cells resembling sensory neurons even though these do not show many of the other characteristics of VSNs in other species and no axons have been identified. (Speculative) Other cells might conceivably be chemosensitive, even though there is no evidence for this in the morphology or characteristic staining patterns of any other cell type.

Worst case: The human VNO is devoid of neurons showing the characteristics of VSNs in other species and devoid of other cells with clear axons leaving the vomeronasal epithelium.

Opinion: There are no obvious sensory neurons.

Putative receptor gene expression

Recent evidence (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirrindelli, 1997) suggests that mammalian species with functional VNOs express two families of genes (V1R and V2R) that appear to code for 'seven transmembrane domain' membrane proteins thought to be the chemoreceptor molecules themselves. These genes are expressed in VSNs and are similar in apparent transmembrane organization to olfactory receptor genes (Buck and Axel, 1991), but differ in much of their DNA sequence. These genes were labeled 'putative pheromone receptor genes', although at the time of their discovery the evidence that they might code for pheromone receptor molecules was tenuous. Their expression in the vomeronasal epithelium is no guarantee: some pheromones are clearly detected by the main olfactory system (see below) and possible non-pheromone functions of the vomeronasal system (as in snakes) have not been

investigated. Recently, Leinders-Zufall *et al.* showed physiological responses in mouse VSNs to substances reported to be pheromones in that species (Leinders-Zufall *et al.*, 2000). The responsive neurons were in the apical zone of the vomeronasal epithelium where most neurons appear to express members of the V1R class of putative vomeronasal receptor genes. This is the best evidence yet that some members of this gene family might be pheromone receptors. The neurons were extremely sensitive and highly selective, characteristics we have come to expect for pheromone receptor neurons in insects. Electrical responses to urine of VSNs (Holy *et al.*, 2000) provide some supporting evidence, but this report does not address the questions of which sensory neuron types respond nor which components of urine are stimulatory.

Genes similar to the vomeronasal receptor genes are also present in the human genome. Those found in initial searches through the genome are clearly pseudogenes (Dulac and Axel, 1995; Herrada and Dulac, 1997), i.e. they have defects in their sequence that would prevent transcription and translation of the expected transmembrane protein. Not all human sequences related to vomeronasal receptor genes have been investigated in detail, so this negative evidence should be viewed with some caution. Some 70% of known olfactory receptor genes have also been reported to be pseudogenes in humans (Rouquier *et al.*, 1998), although a lower percentage is reported in more recent reports (Lane *et al.*, 2000), and humans still have a useful and important sense of smell. In a recent paper Rodriguez *et al.* reported the discovery of a previously undetected human gene closely related to the V1R family in rodents (Rodriguez *et al.*, 2000). Whether it is expressed in human vomeronasal epithelium was not reported, but it is expressed in the main olfactory epithelium. From the argument above it should be clear that the location of its expression does not preclude a pheromone detector function. However, its relationship to animal vomeronasal genes is not good evidence for such a function and throws no light on the question of human vomeronasal function. If expression of one of these genes is detected in human vomeronasal epithelium it will be interesting to know whether it is expressed in cells resembling axonless VSNs or in one of the other types of cell. In either case, a renewed effort to determine whether there is any connection with the brain would be critical to any hypothesis about function.

Best case: The expression in human olfactory epithelium of a gene related to those expressed in VSNs in animals raises the possibility that other new genes may be discovered which are expressed in human vomeronasal cells. There is also a possibility that neurons located in the main olfactory epithelium in humans may have taken over functions assigned to VSNs in rodents.

Worst case: The receptor coded for by the expressed gene of the vomeronasal gene family could bind a regular odor in humans or a substance that is a pheromone in other species

but not in humans. There is no evidence that the protein product of the gene, if any, is expressed on the apical surface membrane in a location accessible to external stimuli.

Opinion: The newly discovered gene tells us nothing about human vomeronasal function. Calling these genes putative pheromone receptor genes is speculative.

Connectivity

In rodents and other species with well-developed VNOs the axons of VSNs pass in bundles to an accessory olfactory bulb (AOB) of characteristic structure. There is no trace of this structure in adult humans (Humphrey 1940; Meisami and Bhatnagar, 1998), although it is present in the fetus (Chuah and Zeng, 1987), and it is generally reported missing in rhesus monkeys and other old world primates (Wysocki, 1979; Stephan *et al.*, 1982). It is possible for an accessory bulb to go undetected or be misidentified. The AOB of the mustelid carnivores (ferret and polecat) has been described as absent (Jawlowski, 1956) or large (Dennis and Kerr, 1969), but recent work in ferrets shows a small AOB, somewhat differently placed than in rodents (Kelliher *et al.*, 1997) (K.R. Kelliher *et al.*, unpublished results). The stretching out that occurs during development in the olfactory bulbs and peduncles of higher primates might distort any small AOB that did exist, although a normal AOB is present in new world primates and prosimians (Evans and Schilling, 1995). An explicit search for such a structure in humans has not found it (Meisami and Bhatnagar, 1998).

The best candidates for VSNs, those expressing NSE, have not been traced in connectivity with axons and neither have any other cells of the human VNO. One characteristic marker of axon bundles, the S100 protein, expressed in glial cells surrounding axons, was not observed in or near the human VNO epithelium by Trotier *et al.* (Trotier *et al.*, 2000). It is not clear whether a few isolated axons might go unnoticed with this method. There are axons within the human VNO and Schwann cell-wrapped bundles of axons underlying it (Stensaas *et al.*, 1991; Jahnke and Merker, 2000), so it is somewhat surprising that Trotier *et al.* did not find S100 expression close to the VNO (Trotier *et al.*, 2000). Many of the axons in this region belong to other well-recognized systems of the nasal cavity, the trigeminal, autonomic and nervus terminalis systems. The trigeminal system includes somatosensory and general chemosensory nerves, most or all of which may be nociceptive (Thurauf *et al.*, 1993). Nerve bundles of the autonomic nervous system control blood vessels and glands. The nervus terminalis (Brookover, 1914; Pearson, 1941) characteristically connects the VNO and the brain in the fetus and clearly persists in human adults (Brookover, 1914). The nerve appears to be the pathway for migration of LHRH (GnRH) neurons into the brain from the olfactory/vomeronasal epithelium early in development, in humans as in other species

(Schwanzel-Fukuda and Pfaff, 1989; Ronklev and Resko, 1990; Boehm *et al.*, 1994). Its persistence in adults suggests some continuing function, as does its internal structure in species where it is most prominent (White and Meredith, 1995). There is no evidence that this nerve is chemosensory or that the human terminalis nerve carries the axons of VSNs (although the two do run together in most mammals), but it may innervate the vomeronasal epithelium (Witkin and Silverman, 1983; Wirsig and Leonard, 1986).

Best case: (Speculative) If there are VSNs in the human VNO their axons could make their way to the brain singly or in small bundles expressing undetectable levels of S100 protein. The equivalent of the AOB might be present if distorted during normal development so as to be unrecognizable as a separate structure.

Worst case: There is no evidence for nerve-axon connections between any possibly sensory cells in the VNO and the brain and no evidence for an AOB.

Opinion: This is one of the big obstacles to the hypothesis of human VNO function.

Positive evidence?

None of the speculations about vomeronasal chemosensory neurons would be worth much consideration if there were not some positive evidence for a non-olfactory, non-trigeminal chemosensory function located in the region of the human VNO. This evidence comes almost exclusively from the work of Monti-Bloch and colleagues. They report an electrophysiological response to application of small amounts of steroid chemicals confined to the VNO region. Because these studies are supported in part by corporations with a commercial interest in exploiting the findings, the results are widely discounted by the academic community. However, they should be evaluated on their merits. There is no serious error in methodology that is obvious from the published papers, so they have to be taken seriously. There is also evidence in these reports for a systemic physiological response to this stimulation and although anecdotal evidence suggests no conscious response in awake human subjects, there is evidence for an alteration of mood. The physiological evidence is critically evaluated in the next section; the behavioral evidence is considered later.

Physiology

If physiological responses are to be attributed to chemical stimulation of the VNO there must be confidence that the stimuli were indeed confined to the VNO. Since there is no independent criterion for chemicals that stimulate VSNs, the nature of the stimulus is not a guarantor of VNO stimulation. The only published attempts to record responses to stimuli applied selectively to the human VNO come from Monti-Bloch and colleagues. Three types of responses have been reported, local electrical responses, responses from

isolated cells and systemic responses. The first type of response is a local negative electrical potential, termed the 'electrovomerologram' (EVG) (Monti-Bloch and Grosser, 1991), recorded from the VNO pit region in awake human subjects. It is named by analogy with the electro-olfactogram (EOG) which can be recorded from the surface of the olfactory epithelium in response to odor stimulation (Ottoson, 1956; Getchell and Getchell, 1987). Stimuli tested for an EVG response included steroids claimed to be similar to chemicals extracted from human skin, including androstadienones and estratetraenyl compounds, as well as conventional odors. The steroids elicited clear EVG responses; the conventional odors did not. In both cases stimuli were delivered directly to the VNO pit through the inner of a pair of concentric tubes, the outer of which was used to scavenge excess stimulus to prevent spread to other areas of the nose. In control experiments the same stimulator was directed at sites successively further from the pit resulting in a decline of EVG amplitude to undetectable levels a short distance away (Monti-Bloch and Grosser, 1991). These results are interpreted as showing that chemosensitivity is restricted to the pit and that the stimulus is restricted to a small region near the tip of the stimulator. The same stimulator directed at the olfactory epithelium allowed conventional odors to elicit an EOG. Several steroids effective in generating an EVG from the VNO failed to produce an EOG response from the olfactory epithelium. The subjects generally reported no sensation from direct chemical stimulation of the VNO, even when an EVG was recorded, but they reported an odor sensation when an EOG was elicited. The authors concluded that the EVG was the summed receptor potential of many VSNs responding to the stimulus. There are problems with this interpretation (see below), but there does appear to be some process located in or near the VNO pit that produces, selectively, an electrical response to small quantities of some chemicals. 'Vomeropherin' has been suggested as a name for chemicals that elicit this response and as a general term for substances that stimulate the VNO in any species (Berliner *et al.*, 1996). So far, there are no other distinguishing features for such chemicals.

As a second type of response, Monti Bloch *et al.* have also reported preliminary evidence that bipolar cells aspirated from the human VNO pit show an electrical responses to some 'vomeropherins' (Monti-Bloch *et al.*, 1998b). These are the EVG-eliciting steroids related to skin chemicals this group has proposed to be human pheromones. These experiments have not been published in a fully refereed report. In view of the extreme sparseness of NSE-expressing human vomeronasal bipolar cells, it seems unlikely that these are the cells involved. If this initial report is confirmed it may throw light on other cells contributing to EVG responses. However, as discussed above, any local VNO response must be communicated to the brain before a sensory communication pathway is established.

Although no anatomical connection has been demon-

strated, Monti-Bloch *et al.* deduce a physiological connection with the brain because stimulus delivery to the VNO pit elicited several systemic responses (Monti-Bloch and Grosser, 1991, 1998a,b). These include changes in blood pressure and heart rate, small but significant changes in hormonal levels (Monti-Bloch *et al.*, 1998a) and some changes in mood (Grosser *et al.*, 2000). It is important to note that these systemic responses were obtained with the same stimulator used for EVG recordings, which confines the stimulus to the VNO pit. Other studies (Berliner *et al.*, 1996) used a different type of stimulator which was not described in detail and for which there were no control experiments to determine stimulus spread. Thus, it is not clear in these experiments that stimuli were confined to the VNO region. Furthermore, repeated stimulus delivery over a prolonged period would make low level stimulation of other nasal sensory systems or systemic uptake of stimulus chemicals more likely. The hormonal changes elicited by steroid chemicals in these studies are not evidence for a physiological connection between the VNO region and the brain and provide no evidence for VNO function.

Physiological mechanisms

Sources of the EVG

The slow negative potential recorded from the VNO pit is claimed to be the summed potentials generated by many sensory neurons responding to chemical stimulation. For the EOG, a similar negative potential recorded from the olfactory epithelium, this is a reasonable explanation. There are hundreds to thousands of olfactory sensory neurons close to the electrode, each contributing a minute amount of current. If the current generators in the human vomeronasal system are the NSE-positive bipolar cells (their lack of demonstrable axons does not disqualify them as local current generators) it is difficult to explain the size of the recorded EVG.

Comparison with the EOG

In olfactory sensory neurons transduction channels open in response to odors producing an inward flow of positive charge into the apical ends of the cells. There is an equal flow of charge out of these cells in the depths of the epithelium. The electrical circuit is completed by current flowing extracellularly from the depths to the surface. The voltage drop along this current path through the extracellular resistance produces a potential difference, recordable extracellularly, between the surface (negative) and the depths (positive). Each responding cell produces a minute current and therefore a minute potential difference, but many cells all oriented in the same direction and activated together sum their currents and generate a correspondingly higher potential difference between the depths and surface. A conventional EOG surface electrode records part of this potential difference due to the small current flowing on a long pathway through the conductive

tissues of the head and past the reference electrode of the recording circuit. However, most of the current passes directly through the thickness of the epithelium, through extracellular space and inactive cells. In the fluid-filled VNO current can easily pass from a region of active cells to an inactive region. Where there are very few active cells, especially if they are widely spaced, there are many transepithelial pathways for the current. Resistance is low and little potential is developed. In the case of the human VNO as few as one putative sensory neuron per section has been reported (Trotier *et al.*, 2000), although they apparently did not examine every section. The precise location of the recording electrode in EVG recording experiments is not well described, but its effective recording volume is likely to be biased towards the regions close to the opening (VNO pit). Unless there were a previously unobserved dense accumulation of the bipolar cells close to the opening of the organ, the probability of recording a detectable 'EVG' from these cells is very small.

Potential artifacts

Alternative explanations for a chemically selective EVG electrical response include physicochemical artifacts, non-neural biological potentials, such as secretory or vasomotor responses, and, finally, other nerve cells or nerve fibers.

Physicochemical artifacts. These can easily be generated in a system where a bare metal electrode is used to record mucosal surface potentials. A steady DC junction potential due to polarization develops at a bare metal electrode in contact with the mucus surface of the epithelium. Any relative movement, for example by pressure transients during stimulation with a chemical vapor, will change the resistance between electrode and mucus, causing a greater or lesser proportion of the junction potential to be seen by the recording system. This change would appear as a stimulus-dependent electrical signal. However, the reported EVG recordings used a 'non-polarizable' silver/silver chloride electrode (Monti-Bloch and Grosser, 1991; Monti-Bloch *et al.*, 1998b), which should generate almost no junction potential. Artifacts resulting from changes in electrode-mucus coupling could be produced if DC potentials were to arise elsewhere in the recording circuit. However, these kinds of mechanical artifact would not generally depend on the chemical species of the stimulus, whereas the amplitude and time course of the EVG recordings are dependent on the chemical used as stimulus (Monti-Bloch and Grosser, 1991). Different potentials could be recorded for different stimuli if the coupling between electrode and mucosa were to change between stimuli, for example when the experimenter adjusted the electrode position or if there were drying of the mucosa with the passage of time. However, it is difficult to imagine that these kinds of changes could produce consistent differences between chemicals by chance, especially if stimuli were repeated in random order, as they should be for such an experiment. The published reports do

not give enough detail to judge whether this was done. Chemical species-dependent electrical artifacts can also occur in two other circumstances: if the stimulus chemicals adsorb on the metal electrodes creating transient surface potentials or if the conductive properties of some stimuli change the electrical resistance of the surrounding tissue. In EOG recordings a non-metallic agar/saline bridge can be used to avoid the former problem, but its larger size may have precluded its use for EVG recordings. In any case, the very small amounts of the chemicals used in the published EVG experiments would not be expected to have large effects of these kinds. Thus, in general, physicochemical artifacts seem unlikely as an explanation for the published EVG recordings.

Biological non-neural potentials. These have several possible sources. Secretory potentials are generated when gland cells secrete their contents. This may occur in response to local irritation, to a neural response, that then activates the gland, or, conceivably, through receptor molecules expressed on the surface of the gland cells themselves. There are many glands around the human VNO and many of those empty into the VNO lumen (Trotier *et al.*, 2000). Secretory potentials can contribute to the EOG recorded from the olfactory mucosa (Okano and Takagi, 1974) and may contribute to the EVG. Blood vessel dilation may also generate a potential from smooth muscle action or may modulate a pre-existing potential due to changes in tissue resistance. Some chemicals entering the nose elicit an immune response from mast cells and other cells in the mucosa (Suzuki *et al.*, 1999). Other substances may trigger metabolic breakdown processes (Gu *et al.*, 1999). Either of these processes could elicit mucus secretion or dilation of local blood vessels, due to the release of cytokines (short-range extracellular messenger molecules) from the activated cells. Stimulus chemicals that activate nociceptive nerve endings also set off a series of local reactions due to the release of substance P and other cytokines from the nerve endings (Suzuki *et al.*, 1999). The effects include secretion and blood vessel dilation. Nasal mucosa tends to be rich in all these mechanisms.

The EVG is reported (again without experimental details) not to be eliminated by topical lidocaine, a local anesthetic, or atropine, an autonomic cholinergic antagonist (Monti-Bloch *et al.*, 1998b). None of the processes described above necessarily involves nerve action potentials, so would not be eliminated by blocking nerve transmission with local anesthetics. Atropine would be expected to block some reflex secretory responses and some vasodilation but many autonomic functions, including vasodilation in the VNO (hamster) (Meredith and O'Connell, 1979), are not sensitive to atropine. Any potentials generated by any of these mechanisms would have to be fairly rapid to be responsible for the observed EVGs. This (and the EVG insensitivity to local anesthetics) would probably rule out a reflex secretion or vasomotor response that depended on transmission to the

CNS and back. Reflex blood flow changes in response to nasal irritants are clearly too slow (see below). Reflex changes due to cytokine release are still a possibility.

Neural responses. Irritating chemicals that stimulate the chemoreceptor nerve endings of the nasal trigeminal system produce a neurogram potential, detectable over wide areas of the nasal septum, that correlates with pain sensations (Kobal, 1985; Hummel *et al.*, 1996). The potential is strongly reduced by local anesthetics, suggesting the involvement of voltage-gated sodium channels, and (in rats) by capsaicin, suggesting the involvement of small, probably nociceptive, nerve endings. The potential clearly precedes changes in blood flow (Thurauf *et al.*, 1993). Whether this potential is generated by propagation of action potentials, by depolarization of the nerve endings, or is the consequence of rapid local cytokine action is not clear. Whether a similar potential contributes to the EVG is not known, although any contribution from action potential generation (or other voltage-gated sodium channel function) seems ruled out by the insensitivity of the EVG to local anesthetics. The other neural system in this region of the nose that is a candidate for the source of the EVG is the nervus terminalis. The terminalis system is concentrated in the VNO region and has been suggested to be chemosensory, but has not been demonstrated to be so (Meredith and White, 1987; Fujita *et al.*, 1991). There is a reasonably high density of unmyelinated axons in the mucosa below and near the human VNO (Stensaas *et al.*, 1991; Jahnke and Merker, 2000), some of which could be the unmyelinated terminal branches of trigeminal nerve fibers or terminalis fibers, which are also generally unmyelinated. Depolarization of nerve fibers, especially very fine fibers, generates little extracellular potential. If fibers were in high density and all oriented in the same direction they might be capable of producing a potential detectable at the surface of the mucosa. Bundles containing up to 200 nerve fibers have been reported in the nasal mucosa, but these are not limited to the region of the VNO (Cauna *et al.*, 1969) and are most likely trigeminal endings. More than one such bundle might be necessary to generate a detectable potential, especially if the fibers did not all respond together. The overall density of these bundles per unit area of the mucosa was not reported by Cauna *et al.* and their chemical sensitivity, if any, is completely unknown. In general, nerve fiber endings seem unlikely as generators of a potential like the EVG. However, the trigeminal response to irritants shows that a system whose only peripheral components appear to be free nerve endings can generate a surface potential, although not necessarily by summation of individual nerve potentials alone. Peripheral nociceptive nerve endings that are sensitive to capsaicin, as the trigeminal potential is, are known to release substance P, prostaglandins and possibly other cytokines (Devor, 1991). The actions of these substances on surrounding tissues might contribute to the observed

response. If the EVG were found to be generated by some of the nerve endings visible in the mucosa a process of that sort would also have to be considered for the EVG potential.

Summary: electrical responses

It is clear that chemical species-dependent potentials could be generated in the neighborhood of the VNO by non-vomeronasal mechanisms. Some of these are ruled out by the nature of the EVG response or by the controls in the published experiments, although some important controls are not described in detail. Trigeminal nerve endings and the components of the immune system are distributed throughout the nose, so responses from these systems should not be limited to the region of the VNO. Glands are localized in the nose, including in the VNO (Stensaas *et al.*, 1991; Trotier *et al.*, 2000). Electroneurogram potentials similar to those from the trigeminal system could also appear more localized if there were a concentration of nerve endings in or near the VNO. Contributions from the trigeminal potential itself seem unlikely because it has a different susceptibility to local anesthetics and because the trigeminal system certainly responds to irritating chemicals over a wider area. Nervus terminalis endings are localized to the VNO, but their chemosensitivity is questionable. The report that local anesthetics fail to block the EVG indicates that nerve transmission is not involved, which rules out CNS reflexes. A local response mediated by cytokines is not ruled out. The other possibility is a direct response from cells expressing receptors for the effective chemicals, whether VNO sensory neurons, trigeminal or terminalis nerve endings, non-neural secretory cells or others. Any cellular components capable of generating a detectable potential would have to be clustered and have a common orientation for their individual potentials to sum. VNO sensory neurons, if these are limited to the NSE-expressing bipolar cells, are unlikely candidates because of their sparseness, even if one believes that these cells are VSNs.

Importance of the EVG response

Whatever the source, the reported selectivity of the EVG response is startling. It represents information that, if conveyed to the CNS, could serve a communication function. If the EVG is generated by primary sensory neurons or afferent nerve terminals, the connection pathway to the CNS is obvious and a contribution to chemical communication is likely. If the EVG is generated by secretory cells or other purely peripheral cells the CNS connection is not clear and a contribution to chemical communication more dubious. In either case, EVGs are probably not generated directly by the bipolar cells that express NSE. Perhaps other cells in the human VNO are VSNs with the appropriate sensitivity and geometry, but, if so, they are yet unrecognized.

Best case: The local electrical response is from VNO region chemosensory cells, but these are unlikely to be the too-sparse bipolar cells. Systemic responses to stimulation

restricted to the VNO pit constitute physiological evidence for a chemosensory function in this region

Worst case: (Speculative) The local response is an artifact, albeit surprisingly dependent on the nature of the stimulus, perhaps because of electrode movement between stimulations. Alternatively, the response could be from non-chemosensory cells with no connections to the brain. Systemic responses could be due to leakage of stimuli to the olfactory area.

Opinion: The EVG is the best evidence for a selective chemosensory process in the VNO region. Systemic responses to restricted VNO region stimulation are an important stumbling block for the hypothesis that there is no special chemosensitivity in this region.

Function: evidence from chemical communication?

There is fairly clear evidence for chemical communication among humans. The most notable example is a trend towards synchronization of menstrual cycles in women who live together (McClintock, 1971). Stern and McClintock have recently deduced the presence of two substances that can mediate this response when extracts of skin secretions are placed on the upper lip (Stern and McClintock, 1998). Thus, the signals are most likely to be airborne chemicals. The trend towards synchronization arises from either shortening or lengthening of the cycle by secretions produced at different phases of the donor's cycle [but see the comment by Whitten (Whitten, 1999)]. The substances involved are unknown and although the effect does appear to be chemosensory, there is no evidence that it is due to vomeronasal sensory input. Jacob and McClintock have also recently reported a human behavioral response to odor; changes in mood elicited by androstadienone and 1,3,5(10)16 estratetraen-3-ol (Jacob and McClintock, 2000). These are substances that elicit sexually dimorphic EVGs and are related to skin chemicals claimed to be human pheromones. Jacob and McClintock report the maintenance of a more positive mood in women in the presence of androstadienone under circumstances where control subjects showed an increasingly negative mood. The response cannot be attributed to the vomeronasal system because the stimuli were placed on the upper lip, not confined to the VNO. Grosser *et al.* also report significantly less negative mood in subjects exposed to androstadienone than in control subjects (Grosser *et al.*, 2000). In their experiments androstadienone was applied directly to the VNO, a much better case for vomeronasal mediation. However, as with the EVG, responses due to stimulation in the region of the VNO are not necessarily mediated by VSNs.

Whether any of these findings are evidence for human pheromones is a different question. None of them meet the test for pheromone communication proposed below, i.e.

evidence that the communication is beneficial (in the evolutionary sense) to both sender and receiver. The subjects in these studies had no conscious perception of odor stimulation, which could be a feature of vomeronasal input although not a *sine qua non* for pheromonal communication. The suggestion that vomeronasal input might be unconscious (Lloyd-Thomas and Keverne, 1982) comes in part from observations of vomeronasal system connections in the rodent brain. There are close connections with the amygdala and limbic system (Halpern, 1987; Meredith, 1991), the seat of emotional, hormonal and autonomic control, but there are only indirect connections with the cerebral cortex, generally considered to be the site of consciousness. The main olfactory system in general has good connections with cerebral cortex, but also has connections to the amygdala. In hamsters pheromonal information from the main olfactory system in sexually experienced animals appears to be transferred to the vomeronasal pathway at the amygdala (Meredith, 1998). In this case the olfactory information appears to be a back-up for a primary vomeronasal communication system. However, in the cases where main olfactory input is the only important information on pheromones we still have no idea whether information about main olfactory pheromones has access to the cortex or is routed through the amygdala and basal forebrain. Thus, a chemosensory communication that does not engage consciousness, if it could be proven, is not diagnostic for vomeronasal participation. A chemosensory response in the human brain without any conscious perception of stimulation has been identified by fMRI using another 'vomopherin' steroid, estra-1,3,5(10) tetraen-3-yl acetate, related to substances extracted from human skin (Sobel *et al.*, 1999). Vomeronasal involvement in this response is unknown, since the stimulus was not confined to the organ.

Other examples of potential chemosensory communication are discussed by Preti and Wysocki in a comprehensive review (Preti and Wysocki, 1999). They conclude that chemical communication does occur and are willing to call the chemical mediators pheromones in some cases. Preti and Wysocki's conclusions are based on specific examples, but a similar conclusion would not be outrageous on basic principles. Intra-specific chemical communication, some of which is vomeronasal and some olfactory, is a common feature in land mammals. Higher primates have highly developed visual systems and reduced olfactory systems, but still use olfactory information. It would seem surprising if all olfactory/chemosensory communication were lost. The fact that chemical communication does not seem to be a strong determinant of human behavior is not a good logical argument for dismissing vomeronasal function, as seems to be implied by Keverne (Keverne, 1999), anymore than it is for dismissing olfactory function. Sensory input of any kind in humans, unless signaling imminent danger, is often subordinate to experiential and cultural factors. Chemical

communication does appear to persist despite its apparently minor impact. Stoddart has proposed that there might be evolutionary pressure for loss of human vomeronasal function (Stoddart, 1991). He speculates that it was important for males in early hominid groups *not* to be able to detect the time of ovulation in females. Whatever its anthropological merit, this argument is logically circular in the context of an evaluation of VNO function because it starts with the premise that there is no human VNO. It also assumes that detection of 'pheromones' signaling reproductive state would be a vomeronasal function.

Among species where some chemical communication can be assigned to the vomeronasal sensory pathway there are a number of examples where the signals appear to be non-volatile and to be transmitted by direct contact between receiver and stimulus source (Meredith, 1983; Clancy *et al.*, 1984). However, there is no requirement that vomeronasal chemoreceptors be stimulated only by non-volatile chemicals. Nor would the demonstration of a non-volatile chemical signal be any assurance that the vomeronasal system were involved.

Best/worst cases: There is nothing to be learned about vomeronasal function, whether in humans or other species, from the existence of chemical communication *per se* or from its features, such as involvement of volatile versus non-volatile chemicals or the access of information to consciousness. There are other sensory systems that could be involved.

Pheromones

What is a pheromone and is it a well-defined, scientifically useful concept? The term pheromone was coined to describe a chemical substance which carries a message about the physiological or behavioral state of an insect to members of its own species, resulting in 'a specific reaction, for example a definite behaviour or a developmental process' (Karlson and Luscher, 1959). It is clear in the original description, and in a later more extensive review of examples (Karlson and Butenandt, 1959), that this was to be real communication, beneficial to the sender and, by implication, to the receiver. Karlson and Luscher state: 'the organism . . . creates for itself a means of communication . . .' (Karlson and Luscher, 1959). We can be sure that the authors did not mean that the individual organism created this capacity, but that it was established and maintained by natural selection. This would require that the communication contributed to evolutionary 'fitness' for both sender and receiver. If this mutual benefit requirement is included as an explicit part of the definition (Rutowski, 1981; Meredith, 1983), the application of the term becomes more restricted but more scientifically useful. Many examples of 'a specific reaction' to biological chemicals are then excluded from the category of 'pheromone communications'. Among these non-pheromone responses are intra-specific predation and

chemical defense, where there is clear benefit either only to the receiver or only to the sender. Inter-specific communication could be mutually beneficial, for example where chemosensory information about defense chemicals benefits the receiver by allowing avoidance. Nevertheless, there seems to be some advantage to *our* communication in arbitrarily limiting the term pheromone to intra-specific communication.

Following Karlson and Luscher's suggestion that responses could be behavioral or developmental, later authors have classified pheromone communications into two types: priming pheromones and releasing or signaling pheromones.

Priming pheromones produce a change of state in the receiver, usually a change in hormonal secretion that primes the animal for a later response. Examples include the acceleration of puberty in immature female mice that brings them into reproductive condition in the presence of chemical signals from mature males (Vandenberg, 1983). In this case the mutual benefit is clear, and a good case can be made for many other priming pheromonal communications in mice. The mutual suppression of estrus in group housed females (the 'Lee-Boot Effect') (van der Lee and Boot, 1955) conserves the energy normally put into cycling when there is no possibility of pregnancy. A suppression of estrus also occurs in fasting females where energy conservation is essential (Wade and Schneider, 1992). In the presence of male stimuli, group housed females return to estrus cycling (the 'Whitten Effect') (Whitten, 1959), clearly a mutually beneficial response. Reproductive suppression in subordinate females, as may occur in some primate species (Barrett *et al.*, 1993), may also involve a conservation of metabolic effort until more favorable circumstances arise. In cases where the subordinate and dominant females are genetically related there might be some increase in inclusive fitness (inclusive fitness takes into account an individual's contribution to the reproductive success of related individuals that carry some of the same genes).

The other class of pheromones, releasing pheromones, were originally considered to release a stereotyped behavioral pattern that required no further information for its completion. This concept seemed inappropriate for mammals, where responses are often modified by experience or other contingencies, and behavioral responses are now said to be elicited by 'signaling' pheromones (Bronson, 1971, 1976; Albone, 1984).

Preti and Wysocki examined reports of human pheromone communication. They concluded that there is evidence for priming pheromones in humans, including the data on menstrual cycle shifts (although the latter do not clearly meet the mutual benefit criterion proposed here) (Preti and Wysocki, 1999). They did not find solid evidence for signaling pheromones but they point out that mammalian, and especially human, behavior is influenced by many factors. An immediate unvarying response to any stimulus should

not be expected. Thus, signaling pheromones might communicate information that alters an individual's probability of responding without necessarily evoking an immediate observable response. Perhaps we don't need to distinguish categorically between priming and signaling communications: both are essentially informational. Furthermore, if we concentrate on pheromone communication rather than pheromone chemicals, we avoid definitional problems associated with chemicals that have different meanings in different contexts or to different individuals, for example mature versus immature or male versus female. The fact that the same chemicals may be used by different species, whether or not in different combinations or different circumstances, is also not a problem.

It can be argued (Beauchamp *et al.*, 1976) that there is no need for a special term for mutually beneficial chemical communication, but, as emphasized by Karlson and Luscher (Karlson and Luscher, 1959), some distinction between communication and a casual use of chemosensory information does seem a useful distinction. The term pheromone is not going to disappear so long as it holds the public fascination. Its use for a class of chemicals that communicate information seems reasonable, but the definition is important if the term is to be useful in scientific discourse. Too rigid a definition can make its applicability to real situations so limited that it is useless. We know that even archetypal insect pheromones are not unique chemicals used by single species, as supposed in some definitions [see discussions in Beauchamp *et al.* and Albone (Beauchamp *et al.*, 1976; Albone, 1984)]. Similarly, too broad a definition devalues the term and also makes it useless.

The essence of the concept is that a particular chemical or complex of chemicals communicates meaning and, thus, must be identified. Non-specialist functions of mammalian olfactory systems may involve a simple association between a complex of chemicals and an external situation, permitting later recognition of similar situations. Particular chemicals may be associated with particular objects, but there may be no necessity for the chemicals to be identified, and the associations can be reassigned. This mechanism is less suitable for communication where the messages have special meanings. Preprogrammed meaning may be assigned to odors in other contexts, especially in invertebrates, where individuals may be adapted to finding and consuming host plants using specialized receptors (Rostelien *et al.*, 2000). These are not pheromone communications because they are not mutually beneficial and not intra-specific. Odor communication between flowers and pollinating insects is mutually beneficial, but I would not label it pheromonal because it occurs across species, even though its evolutionary mechanisms may be similar to those maintaining intra-specific mutually beneficial communication.

The mutual benefit criterion for pheromone communication does not exclude learned responses, especially the imprinting type, where meaning is assigned in some special

circumstance. It does imply that meaning is not infinitely reassignable; that it is not just an association even though there are instances where arbitrary odors can be substituted for preprogrammed stimuli. For example, newborn rabbits exposed to a commercial perfume in association with their first feeding can use the odor as information to elicit the nipple search behavior normally elicited by the mother's nipple pheromone (Hudson, 1985). In this case the chemical is not a pheromone although a response normally elicited by pheromonal communication has been linked to it by conditioning. The response to the natural pheromone does not require conditioning. The plasticity of the mammalian nervous system in the assignment of input/output routing extends to normally stereotyped relationships such as these responses or the eye blink, which is normally elicited by an air puff but can be conditioned to a tone.

The mutual benefit criterion for pheromones also does not exclude emotional (mood) changes as a valid response, even if these do not immediately affect overt behavior. We know that in humans mood can affect future behavior (a sign of information transfer) and reliable biases in behavior could have evolutionary consequences. On the other hand, a change of mood on exposure to a human-derived chemical (Grosser *et al.*, 2000; Jacob and McClintock, 2000) does not adequately define a pheromone. There are many biological chemicals that can be expected to evoke behavioral and mood changes. Some of these responses, such as avoidance and disgust with fecal and body odors, may be culturally determined. Some benefit to the receiver in avoiding parasite transmission may be associated with avoidance of fecal odors, but a similar benefit with respect to general body odors is less likely, and a benefit to the sender in either case seems doubtful if no definite message is transmitted.

Identifying mutual benefit in a given case is not always easy, but the criterion provides a conceptual framework for understanding the establishment of a chemical communication. If there is no communication there seems no reason to use a special term. Where a mutual advantage does not seem reasonable, communication is suspect.

Whatever the definition of pheromone, there is no evidence that pheromones are necessarily detected by the VNO. Several recent examples in animals with well-developed VNOs make this clear. The response of newborn rabbits to the mother's nipple (Hudson and Distel, 1986), referred to above, and the standing response of a receptive female pig to the male's pheromone (Dorries *et al.*, 1997) both depend on the main olfactory system. The recognition of newborn lambs by ewes also appears to depend on the main olfactory system (Levy *et al.*, 1995), although a vomeronasal contribution has also been reported (Booth and Katz, 2000). Thus, even if an authentic pheromone response were to be documented in humans, that would not be evidence for a functional VNO.

Furthermore, one of the prime examples of main olfactory pheromones, nipple search behavior in rabbits,

appears not to be learned, although the same response pattern can be conditioned to arbitrary odors. A ewe's recognition of her lamb is learned during the first few hours after parturition. The recognition of a mate's pheromone signature in the pregnancy block or 'Bruce effect' in mice also appears to be learned, but this is a vomeronasal process. It may well be in both of these cases that the learning involved is imprinting of a particular combination from a limited set of signals. Nevertheless, we cannot use the preprogrammed unlearned nature of a response to a chemical signal as diagnostic of vomeronasal involvement.

Best case: The existence of a functional VNO in humans would not be ruled out either by the presence or the absence of pheromone communication in humans nor, if present, by any of its features, such as learned versus unlearned responses.

Worst case: Vomeronasal function is not necessary to explain any aspect of chemical communication in humans, nor is it necessary for pheromonal communication.

Opinion: The term 'pheromone' is useful if defined in the context of mutually beneficial pheromonal communication. Chemical communication occurs in humans. Whether it is pheromonal in this sense remains to be established. The presence or absence of pheromones and pheromonal communication is independent of the existence and/or functionality of a human VNO.

Summary: evidence for human vomeronasal function

Best case: VNO is a minor but not insignificant contributor to human communication. More work by independent groups is needed to confirm the reported electrical and hormonal responses. The expression of a vomeronasal-type receptor gene in humans raises the possibility that such genes may underlie chemosensitivity in the vomeronasal region.

Worst case: The VNO is absent or if present is not chemosensitive nor necessarily functional in communication. The evidence for chemosensitivity is poorly documented and has not all been subject to effective peer review. The evidence for a communication function could be artifactual.

Opinion: The EVG constitutes evidence for a selective and sensitive response to human-derived chemicals located in the region of the VNO. Systemic autonomic responses and emotional changes elicited by stimulation in this region suggest some chemosensitivity, even though the anatomical substrate is difficult to demonstrate and seems unlikely to be conventional VSNs. If we didn't have the positive evidence from EVG, autonomic and psychological responses, reasonable scientific judgment would assign the role of detecting human-derived chemicals that might be involved in chemical communication to the main olfactory system. However,

ignoring the evidence for vomeronasal function because most of it comes with commercial baggage is not a rational scientific response in the absence of evidence for error, bias or fraud. An independent investigation is required to test the findings and assumptions of the original reports, with the appropriate controls and a full description of experimental details. This cannot be done within the pages of this or any journal. It requires laboratory time.

Acknowledgements

The author thanks *Chemical Senses* editor Robyn Hudson for suggesting the topic and colleagues too numerous to mention for stimulating discussions on this topic. In addition, I thank two anonymous *Chemical Senses* reviewers for helpful suggestions on improving the manuscript. This work was supported by a grant from the NIDCD (DC-00906).

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Accepted November 29, 2000