Molecular aspects of pheromonal communication via the vomeronasal organ of mammals

Roberto Tirindelli, Carla Mucignat-Caretta and Nicholas J.P. Ryba

Recently, two large multigene families of putative G-protein-linked receptors that are expressed in distinct subpopulations of neurones in the vomeronasal organ have been identified. These receptors probably mediate pheromone detection. The most surprising aspects of these findings are that there are so many receptors of two very different classes and that the receptors are unrelated to their counterparts in the main olfactory epithelium. This suggests that many active ligands are likely to exert effects through the vomeronasal organ. Parallel experiments addressing the nature of these ligands indicate a role for some proteins, as well as small molecules, as functional mammalian pheromones. In combination, these results begin to suggest a molecular basis for mammalian pheromone signalling.

Trends Neurosci. (1998) 21, 482–486

Roberto Tirindelli is at the Istituto di Fisiologia Umana, Università di Parma, Via Volturno 39, I-43100 Parma, Italy, Carla Mucignat-Caretta is at the Istituto di Fisiologia Umana, Università di Padova, Padua, Italy, and Nicholas J.P. Ryba is at the Taste and Smell Unit, Oral Infection and Immunity Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892-1188, USA.

PHEROMONES are used for intraspecific communication almost throughout biology; for example, in yeast, specific peptides are secreted and result in a stereotyped mating response. Many insects have developed exquisitely sensitive systems that use volatile pheromones to attract and find mates. In mammals, pheromonal communication also provides a range of social and sexual information using a complex signal emission programme and a dedicated detection system – the accessory olfactory system[1,2]. Some of these chemical signals, or pheromones, activate receptor neurones in the vomeronasal organ (VNO) of Jacobson (Fig. 1).

The main evidence that the VNO functions in mammalian pheromone detection comes from studies involving lesion experiments in rodents, where removal of the VNO always results in impairment of reproductive behaviour[3]. Several unrelated molecules have been suggested as candidate mammalian pheromones[4–8]. However, perhaps the most interesting and conclusive results are reports that proteins act as pheromones[9,10]. Blends of these active molecules are contained in urine, sexual secretions and specialized glands, and it is possible that specific mixtures rather than individual components are necessary to elicit the behavioural responses conveyed by pheromones. How is this chemical information processed by the transduction machinery of the VNO? Recent molecular breakthroughs could help provide important mechanistic clues to account for some aspects of pheromonal communication.

The VNO is a specialized structure for pheromone detection

The main olfactory epithelium (MOE) covers a vast surface of the nasal cavities and contains specialized bipolar neurones that are responsible for the detection of volatile odorants (Fig. 1). In most mammals the MOE is probably vital, especially during the early stages of postnatal development, as a means of maternal recognition by the pups[11]. In humans, smell is considered the most evocative of senses, underlining its central connections to cognitive centres of the brain. In terrestrial mammals, the separate chemosensory epithelium of the VNO typically lines the ventromedial face of a blind-ended tubular canal lying at the base of the nasal septum[12]. In contrast to the MOE, the sensory epithelium of the mammalian VNO generally offers no direct communication to airborne odorants. A narrow fluid-filled duct provides access from the base of the nasal cavity in rodents and some primates, or the nasopalatine canal in ungulates, new-world primates and carnivores. In catarrhine monkeys, the VNO is vestigial, with no obvious thick sensory epithelium[13].

Access to the VNO has been reported to be controlled by an autonomic vascular pump[14]. The cavernous tissue of the VNO undergoes cyclical swelling and emptying, which draws fluid into the duct. Thus, pheromones, dissolved in the fluid, reach the receptor cells. Novel stimuli, for example odorants and visual stimuli, activate this vascular pump[14].

After crossing the cribriform plate, axons of the VNO and MOE take different routes[15]. MOE neurones project to the olfactory bulb and synapse with mitral cells in the glomeruli. Nerve fibres from the VNO converge on the smaller accessory olfactory bulb (AOB). The internal connections are similar in the AOB and the main olfactory bulb. However, the secondary projections of the MOE and VNO are to separate areas of the CNS. The main connections of the main olfactory bulb are towards the olfactory cortex. In contrast, the AOB projects to hypothalamic areas of the brain that are involved in hormonal and reproductive functions[16]. Thus, in essence, pheromonal cues probably trigger signals that go to brain structures that are well positioned to control sexual and reproductive behaviour and endocrine status via the pituitary–gonadal axis.

In humans there is no discernible AOB, and the question of whether humans possess a functional VNO

PII: S0166-2236(98)01274-0

remains open. However, there are reports of what appear to be bipolar receptor neurones in the human VNO (Ref. 17). Moreover, it is now well documented that the synchronization of menstrual cycles among women who live together involves chemical signals[18,19]. What is lacking is proof that this is mediated through the VNO.

Some pheromonal proteins drive sexual responses

The urine of male rodents contains a strikingly high concentration of proteins. This, coupled with the observation that physical contact with the protein fraction of male mouse urine accelerated the onset of puberty in female mice (Vandenbergh effect; see Box 1)[20], raised the question of whether proteins act as pheromones. As major urinary proteins (MUPs) – a family of related proteins – are the most abundant proteins in male mouse urine, they became plausible candidate pheromones.

MUPs are lipocalins, a superfamily of related proteins[21], some of which are known to bind and transport hydrophobic ligands. Despite poor sequence conservation, all these proteins possess a binding pocket modelled to harbour small lipophilic molecules[22]. In MUPs (Fig. 2), this binding pocket is naturally filled with the small volatile molecules brevicomin and 2-*sec*-butyl-thiazoline[7,8]. MUPs purified from urine still contain their endogenous ligands, posing the question of which molecules elicit behavioural effects. Surprisingly, when the endogenous ligands were removed and the proteins alone were tested, it appeared that the MUPs, rather than their volatile ligands, were responsible for the Vandenbergh effect[9]. Still more surprising was the finding that an N-terminal peptide corresponding to a single MUP could also mimic the effect[9] (Fig. 2). Thus, there is compelling evidence that lipocalins function not only as carriers of pheromones in mice but modulate VNO function themselves. In hamsters, another lipocalin also plays a role as a pheromone or carrier. Aphrodisin, a lipocalin secreted by vaginal glands, strongly stimulates mating behaviour in males[10]. It is also conceivable that the highly expressed lipocalin, vomeromodulin, found in the mucus of the VNO is involved in the transport of some volatile pheromones[23]. The question is whether the lipocalin system operates via the VNO. It is well established that VNO ablation abolishes the Vandenbergh effect[24]. However, the molecular consequences of lipocalin stimulation of the accessory olfactory system have been observed only recently. Exposure of hamsters to aphrodisin stimulates production of the signalling molecule inositol (1,4,5)-trisphosphate in the AOB (Ref. 25), and exposure of mice to MUPs results in expression of the immediate early gene c-*fos* (Ref. 26).

Putative pheromone receptors in the VNO

Over the past decade, study of the main olfactory system has suggested a molecular basis for odorant detection and discrimination. Here, many odorants stimulate the production of cAMP through a G-protein-coupled signal transduction pathway[27–29]. Molecular approaches have also hinted at how odour information can be encoded and transmitted through the MOE to higher centres. A family of approximately

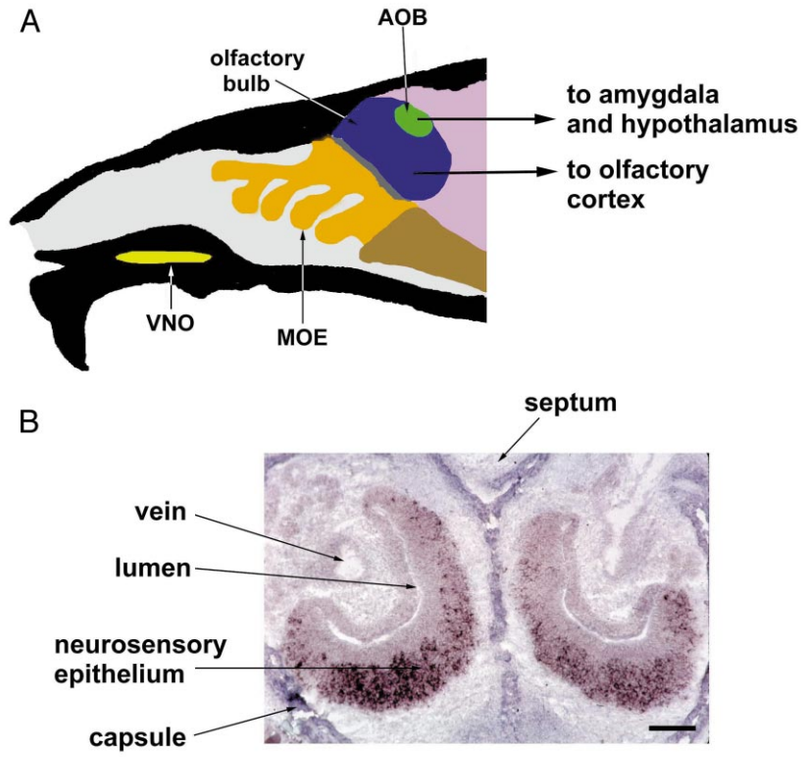

Fig. 1. ***The vomeronasal organ (VNO).*** **(A)** *Schematic diagram showing the location of the VNO and accessory olfactory bulb in rodents.* **(B)** In situ *hybridization with olfactory marker protein shows details of the VNO in a coronal section through the nasal cavity of a neonatal rat. The stained cells are mature vomeronasal neurones. Scale bar, 250 μm.*

Box 1. VNO-dependent pheromone responses

Pheromones can be classified into two groups, according to the timing or duration of their evoked responses. Releaser (or signalling) pheromones act as attractants or repellents and induce relatively fast behavioural responses, such as sexual activity, parental care and aggression. Primer pheromones elicit a sequence of slower physiological events that eventually influence specific aspects of reproduction.

Well-defined responses that have been demonstrated to involve the VNO include:

- **The Lee–Boot effect**[a]. Grouping several female mice in a cage results in suppression or modification of their oestrous cycles.
- **The Vandenbergh effect**[b]. Onset of puberty in young female mice can be accelerated by pheromones, most likely nonvolatile molecules contained in the urine of adult males.
- **The Bruce effect**[c]. The physical presence, or the urine, of a male mouse of a different strain from the 'stud' can preclude the implantation of fertilized eggs in recently mated females.
- **The Whitten effect**[d]. The induction of synchronized oestrous by urinary cues of conspecific male mice in females with group-dependent oestrous suppression.

References

a Van der Lee, S. and Boot, L.M. (1955) *Acta Physiol. Pharmacol. Neerl.* 4, 442–443
b Vandenbergh, J.G. (1969) *Endocrinology* 84, 658–660
c Bruce, H.M. (1960) *J. Reprod. Fertil.* 1, 96–103
d Whitten, W.K. (1966) *Adv. Reprod. Physiol.* 1, 155–177

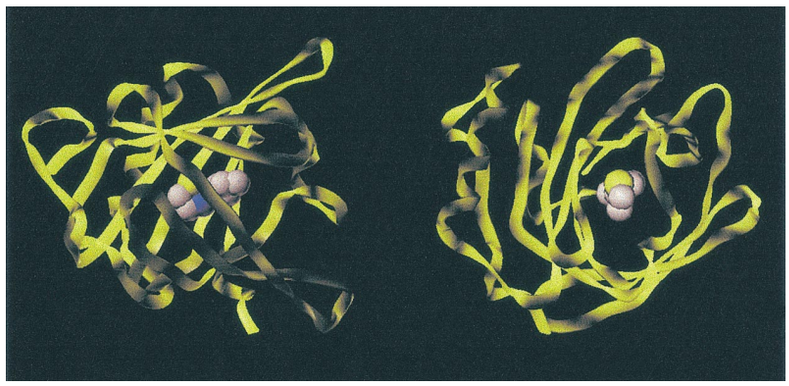

Fig. 2. ***Peptide backbone of a major urinary protein (MUP) with a bound pheromone.*** *The peptide backbone is shown as a yellow ribbon. The barrel-like structure formed by eight β sheets, typical of lipocalins, and a short α helix are visible. In the left image, the N-terminus, a presumptive receptor-binding region, is in the lower part of the molecule. The right-hand image is taken facing the open end of the hydrophobic pocket of the barrel structure. The natural MUP pheromone, 2-sec-butyl-thiazoline, is shown within the pocket.* The images were obtained from X-ray diffraction data (Protein Data Bank, Brookhaven National Laboratory).

1000 genes coding for odorant receptors with seven transmembrane helices was discovered[30]. Individual olfactory neurones express a single receptor[31] that recognizes a restricted range of ligands[32]. These receptors are quite broadly distributed in the MOE, but there is a striking convergence of all neurones expressing one type of putative receptor to one or a few glomeruli[33,34]. This supports a model in which discrimination of odours is achieved by detection of spatial patterns of activation of the olfactory bulb[35–37].

It appears likely that the VNO and the accessory olfactory system use a broadly similar mechanism for distinguishing pheromonal cues. However, attempts to identify pheromone receptors on the basis of their homology with the olfactory receptors were unsuccessful, stressing the most fundamental difference between odour and pheromone reception, namely that the receptors are at best very distantly related to one another. A novel molecular approach was developed that relied on the simple assumption that VNO neurones express a single receptor. The hypothesis proved to be correct[38]. In elegant experiments, two cDNA libraries were constructed using mRNA isolated from single VNO neurones as starting material. Differential screening of the two libraries identified a clone, VN1, that was expressed at a high level in only one of the neurones. Further analysis revealed that this clone was expressed in a subpopulation of VNO neurones. Related full-length cDNA clones encoding seven transmembrane domain receptors were isolated, and experiments indicated that roughly 40 related genes were expressed in the VNO. These receptors had 50–90% sequence identity with one another but no significant homology with other proteins, including G-protein-coupled receptors. Like olfactory receptors, these VNO receptors exhibit high sequence divergence within the transmembrane domains[30,38]. These interesting observations provide insight into two important issues. First, ligand-binding domains in V1Rs and olfactory receptors are likely to be within the transmembrane domain of the receptors. Second, if V1Rs bind pheromones, the diversity of receptors indicates the existence of a reasonably large number of active molecules.

Another intriguing aspect of this work was the finding that neurones expressing these receptors were located essentially in only the apical half of the VNO. Several groups found that two G-protein subunits, $G\alpha_{i2}$ and $G\alpha_o$, were differentially expressed in subpopulations of VNO neurones[38–41]. Thus, it appeared that the family of new receptors might be restricted to the $G\alpha_{i2}$-expressing neurones and that other receptors might be present in the $G\alpha_o$-expressing neurones. This stimulated the search for new receptors. The efforts of three research groups recently ended with the discovery of a second large multigene family of G-protein-coupled vomeronasal receptors that are primarily expressed in the basal half of the VNO (Refs 42–44). These new receptors, which we named V2Rs to distinguish them from the unrelated first family of VNO receptors (V1Rs), comprise a family of as many as 100 genes that share similarity with the parathyroid Ca^{2+}-sensing receptor[45] and the metabotropic glutamate receptors[46]. The only similarity between V2Rs, olfactory receptors and V1Rs is that all three classes are predicted to have seven membrane-spanning helices[30,38,42–44] (Fig. 3). V2Rs are distinguished by their long N-terminal extracellular domains, which precede the seven transmembrane helices. Ca^{2+} and glutamate have been suggested to bind to their respective receptors through the extracellular domain[46,47] and, as might be expected if V2Rs bind multiple distinct ligands, V2Rs appear to be more divergent in this region than in their transmembrane domains[42–44].

Do these two families of receptors function as pheromone receptors? Comparison of their expression patterns with those of the odorant receptors indicates that this is likely. First, just as odorant receptors are expressed in small subpopulations of MOE neurones, so individual V1Rs and V2Rs are expressed in 0.5–3% of the VNO neurones[38,42–44]. As is also the case for the odorant receptors, the subpopulations of neurones expressing any one receptor do not appear to overlap with those expressing other receptors[31,38,42–44]. Moreover, the expression of V1Rs and V2Rs is

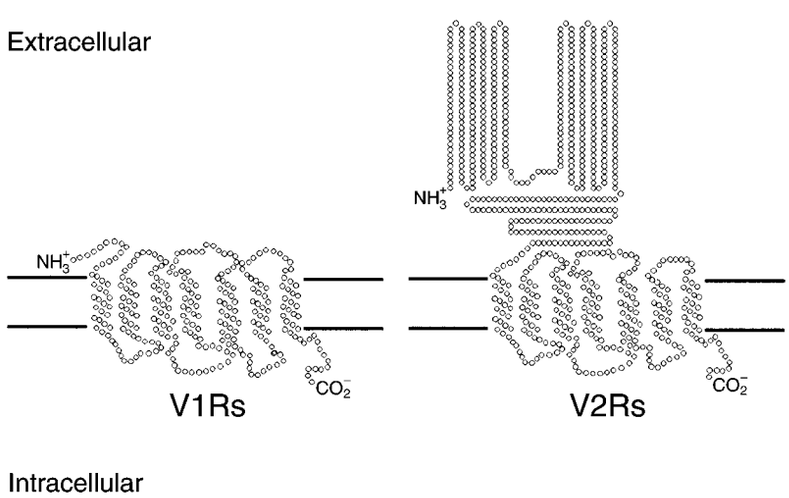

Fig. 3. ***Proposed topology of vomeronasal organ receptors.*** *V1Rs and V2Rs share seven putative transmembrane helices that are typical of G-protein-linked receptors. However, the V1Rs do not contain other structural features that are typical of the known G-protein-linked receptors. The V2Rs contain a large N-terminal extracellular domain that is related to the Ca^{2+}-sensing receptor and metabotropic glutamate receptors, but not to other G-protein-linked receptors. This domain is probably involved in ligand binding.*

restricted to sensory neurones in the VNO, and they are not expressed either in the MOE or in other neural or nonneural tissues[38,42–44]. These properties are consistent with both V1Rs and V2Rs functioning as pheromone receptors. However, it took seven years to demonstrate unambiguously that one particular odorant receptor mediates a specific response[32]. Therefore, even though V1Rs and V2Rs are good candidate pheromone receptors, it is likely that they will also remain putative pheromone receptors for some time. As yet there are no clues as to whether V1Rs and V2Rs bind distinct classes of ligands. However, given that compounds that have been reported to be pheromones seem to be either hydrophobic small volatile molecules or proteins, it is conceivable that V1Rs bind one class of ligand and V2Rs the other.

One fascinating observation was that one V2R displays some degree of sexual dimorphism[42]. However, other V1Rs and V2Rs are expressed equally in female and male rodents[38,42–44]. Thus, it is unlikely that the sexual dimorphism of most pheromone responses stems from distinct signal reception, but rather from sexual differences in the accessory olfactory system and the CNS that are mainly mediated by gonadal hormones[48]. It is tempting to speculate that pheromonal cues stimulate the same vomeronasal receptors in males and females but that most exert distinct responses because of differences in the effector systems that are sex-specific. This would also explain why pheromonal stimulation varies with timing, probably reflecting the hormonal status of the animal[2,16]. The coding of pheromonal information must also be elementary in the VNO since stimuli conveyed by different activated receptors converge and control the release of a small number of sex hormones[16]. These, in turn, are likely to elicit a limited number of behaviours and ultimately control a single event – reproduction.

Another aspect of the VNO is the layered expression pattern of signalling molecules. This is true for $G\alpha_{i2}$ and $G\alpha_o$, as well as for the receptors (Fig. 4). Indeed, careful examination of the expression pattern of the V2Rs revealed that there are several distinct layers of neurones that express particular subsets of receptors[42,44]. Moreover, the expression of some V2Rs even extends into what appears to be the $G\alpha_{i2}$ zone[42,44]. In many respects, these layers of receptor expression are reminiscent of the four zones of receptor expression that have been reported in the MOE (Ref. 34). These zones in the MOE also appear to be present in the olfactory bulb, and at least the $G\alpha_{i2}$ and $G\alpha_o$ layers of the VNO project to distinct and contiguous regions of the AOB. One major difference is that, whereas the zones of the MOE appear to be defined already at the earliest stages of embryonic development, the layers of the VNO develop only during the first few postnatal weeks[41,42] (N.J.P. Ryba and R. Tirindelli, unpublished observations).

A major question is, do VNO neurones expressing particular receptors focus on specific sites in the AOB? One hint that this might be the case is that neurones expressing $G\alpha_{i2}$ all project to the rostral part of the AOB, and those expressing $G\alpha_o$ project to the caudal region of the AOB (Ref. 40). Also, it was recently shown that a new neural cell-adhesion molecule shows a zone-specific expression similar to that of $G\alpha_o$ in the VNO (Ref. 49). However, unlike receptors in the MOE (Ref. 33), we have been unable to demonstrate focusing by *in situ* hybridization (N.J.P. Ryba and R. Tirindelli, unpublished observations). There are several technical reasons why this might be the case, including the smaller size of AOB glomeruli and the longer distance between the VNO and the AOB, which might reduce signal strength. However, it may also be that focusing in the AOB is not as precise as in the MOE, and that some level of signal integration occurs in the AOB. Clearly, just as in the case of the MOE, where the nature of the expressed olfactory receptor plays a role in controlling how focusing occurs[50], major questions as to how the expression of receptors is controlled and coupled to neuronal targeting need to be answered for the VNO. There is some hope that the simpler VNO system may be more amenable to study than the MOE. First, the scale of the problem is somewhat simpler. Second, there are two zones that appear to be distinct in terms of several markers in addition to the multiple layers of receptors. Third, the zonal organization in the VNO appears to be a dynamic process that occurs during the first weeks of life[41,42] rather than one that originates at the earliest stage of development[51].

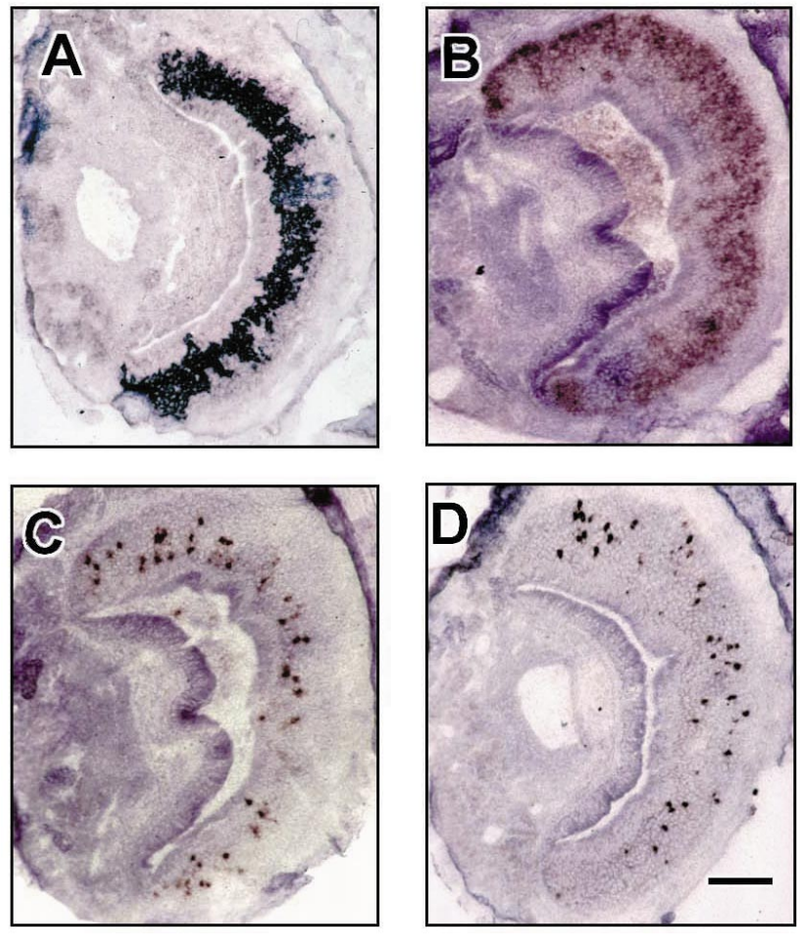

Fig. 4. In situ *hybridization in the rat vomeronasal reveals distinct expression patterns for G-protein subunits and vomeronasal organ receptors (V1Rs and V2Rs).* **(A)** *$G\alpha_{i2}$ is highly expressed in most neurones located in the apical half of the vomeronasal neuroepithelium.* **(B)** *$G\alpha_o$ is expressed in most neurones in the basal half of the neuroepithelium; its expression does not overlap with that of $G\alpha_{i2}$.* **(C)** *A V1R is expressed in a small subpopulation of neurones primarily in the $G\alpha_{i2}$-positive apical half of the sensory neuroepithelium.* **(D)** *Like the V1R, a V2R is expressed in a small subpopulation of neurones; however, these neurones are primarily in the $G\alpha_o$-positive basal half of the sensory neuroepithelium. Scale bar, 200 μm.* Reproduced from Ref. 44 with permission of Cell Press.

The presence of two families of unrelated receptors is, at present, the most intriguing aspect of VNO transduction. Pheromone responses are elicited by complex blends of molecules whose nature is still under investigation. Recent findings, however, suggest that at least one class of protein pheromones, MUPs, reinforce their behavioural effects if presented in a urinary context (C. Mucignat, unpublished observations). Since rodent urine also contains volatile lipophilic molecules[4], it seems that two different classes of receptors might be engaged simultaneously to elicit maximal behavioural effect.

The large multigene families of VNO receptors have presumably arisen by gene duplication followed by accumulation of mutations. Pheromonal responses are species- or even strain-specific. Thus, speciation probably requires changes in the systems responsible for conspecific discrimination. It is tempting to suggest that the large number of V2R pseudogenes that have been reported[43] and the large number of MUP pseudogenes[52] are remnants of processes that were involved in rapid species radiation during the course of evolution.

Receptor-coupled transduction cascade: an unresolved puzzle

The most obvious question raised by these observations is whether a direct correspondence exists between G-protein segregation and receptor family localization in the VNO. If this is the case, and the two classes of receptor recognize distinct classes of ligand, this would suggest that a degree of pheromone coding is segregated in the VNO. Moreover, it might well suggest that two distinct transduction pathways are used by the two classes of receptor. Evidence in favour of this stems from work with single cells[42,43]. However, at least some V2Rs seem to infringe the $G\alpha_o$–$G\alpha_{i2}$ partitioning rule and are expressed in the $G\alpha_{i2}$ zone[42,44]. It remains to be seen if these V2Rs are expressed in atypically localized $G\alpha_o$-expressing cells or whether some V2Rs are also present in $G\alpha_{i2}$-expressing cells.

Molecular studies have revealed more about what are not likely to be signalling pathways in the VNO than what are[53,54]. However, both adenyl-cyclase II (ACII) (Ref. 38) and one subunit of the cyclic nucleotide gated channel (oCNC2) are expressed in VNO epithelium[55,56]. ACII is stimulated by $G\alpha_s$ and this stimulation is potentiated by G-protein $\beta\gamma$ subunits[57]. However, $G\alpha_s$ is not highly expressed in the VNO (Ref. 58), and it is unclear whether ACII can play a role in pheromone signalling. Similarly, although the finding of oCNC2 in the VNO is intriguing, it does not form a channel when expressed in heterologous cells. Thus, one area that will be a focus for further investigation will be understanding how the two classes of vomeronasal receptors are coupled to generation of nerve responses.

In conclusion, although recent results strongly suggest that pheromone signalling shares many aspects of coding with the sense of smell, the surprising finding is that the molecular details differ so dramatically. To demonstrate conclusively that this is the case, it will be necessary to show that specific pheromones bind and activate particular receptors and signalling pathways. However, even in the absence of this definitive proof, further details of how pheromone coding works will be obtained by examining the projections of vomeronasal neurones expressing specific receptors to the AOB.

Acknowledgements
We are grateful to A. Cavaggioni, S. Ottonello and S. Petrucco for advice and comments on the manuscript. We also thank all members of R.T. lab. for helpful discussion, and R. Percudani and G. Sartor for computer assistance.

Selected references

1 **Halpern, M.** (1987) *Annu. Rev. Neurosci.* 10, 325–365
2 **Meredith, M. and Fernandez-Fewell, G.** (1994) *Psychoneuroendocrinology* 19, 657–672
3 **Wysoki, C.J. and Lepri, J.J.** (1991) *J. Steroid Biochem. Mol. Biol.* 39, 661–669
4 **Schwende, F.J. *et al.*** (1986) *J. Chem. Ecol.* 122, 277–296
5 **Beauchamp, G.K. *et al.*** (1980) in *Chemical Signals in Vertebrates and Aquatic Invertebrates* (Muller-Schwarze, D. and Silverstein, R.M., eds), pp. 327–379, Plenum Press
6 **Marchlewska-Koj, A.** (1980) in *Chemical Signals in Vertebrates and Aquatic Invertebrates* (Muller-Schwarze, D. and Silverstein, R.M., eds), pp. 413–414, Plenum Press
7 **Bacchini, A., Gaetani, E. and Cavaggioni, A.** (1992) *Experientia* 48, 419–421
8 **Robertson, D.H.L., Beynon, R.J. and Evershed, R.P.** (1993) *J. Chem. Ecol.* 19, 1405–1414
9 **Mucignat-Caretta, C., Caretta, A. and Cavaggioni, A.** (1995) *J. Physiol.* 486, 517–522
10 **Singer, A.G. *et al.*** (1988) *Biol. Reprod.* 38, 193–199
11 **Brunet, L.J., Gold, G.H. and Ngai, J.** (1996) *Neuron* 17, 681–683
12 **Barber, P. and Raisman, G.** (1974) *Brain Res.* 81, 21–30
13 **Halpern, M. and Kubie, J.L.** (1984) *Trends Neurosci.* 7, 472–477
14 **Meredith, M.** (1994) *Physiol. Behav.* 56, 345–354
15 **Scalia, F. and Winans, S.S.** (1975) *J. Comp. Neurol.* 161, 31–56
16 **Keverne, E.B.** (1983) *Trends Neurosci.* 6, 381–384
17 **Stensaas, L.J. *et al.*** (1991) *J. Steroid Biochem. Mol. Biol.* 39, 553–560
18 **McClintock, M.** (1991) *Nature* 229, 244–245
19 **Stern, K. and McClintock, M.K.** (1998) *Nature* 392, 177–179
20 **Vandenbergh, J.G. *et al.*** (1976) *Biol. Reprod.* 15, 260–265
21 **Flower, D.R. *et al.*** (1993) *Protein Sci.* 2, 753–761
22 **Bocskey, Z. *et al.*** (1992) *Nature* 360, 186–188
23 **Khew-Goodal, Y. *et al.*** (1991) *FASEB J.* 5, 2976–2982
24 **Kaneko, N. *et al.*** (1980) *Biol. Reprod.* 22, 873–878
25 **Kronek, C.** (1996) *NeuroReport* 7, 2989–2992
26 **Guo, J., Zhou, A. and Moss, R.L.** (1997) *NeuroReport* 8, 1679–1683
27 **Jones, D.T. and Reed, R.R.** (1989) *Science* 244, 790–795
28 **Dhallan, R.S. *et al.*** (1990) *Nature* 347, 184–187
29 **Bakalyar, H.A. and Reed, R.R.** (1990) *Science* 250, 1403–1406
30 **Buck, L.B. and Axel, R.** (1991) *Cell* 65,175–187
31 **Chess, A. *et al.*** (1994) *Cell* 78, 823–834
32 **Zhao, H. *et al.*** (1998) *Science* 279, 237–242
33 **Vassar, R. *et al.*** (1994) *Cell* 79, 981–991
34 **Ressler, K.J., Sullivan, S.L. and Buck, L.B.** (1994) *Cell* 79, 1245–1255
35 **Shepherd, G.M.** (1994) *Neuron* 13, 771–790
36 **Axel, R.** (1995) *Sci. Am.* 1273, 154–159
37 **Buck, L.B.** (1996) *Annu. Rev. Neurosci.* 19, 517–544
38 **Dulac, C. and Axel, R.** (1995) *Cell* 83, 195–206
39 **Halpern, M., Shapiro, L.S. and Jia, C.** (1995) *Brain Res.* 677, 157–161
40 **Jia, C. and Halpern, M.** (1996) *Brain Res.* 719, 117–128
41 **Berghard, A. and Buck, L.** (1996) *J. Neurosci.* 16, 909–919
42 **Herrada, G. and Dulac, C.** (1997) *Cell* 90, 763–773
43 **Matsunami, H. and Buck, L.B.** (1997) *Cell* 90, 775–762
44 **Ryba, N.J.P. and Tirindelli, R.** (1997) *Neuron* 19, 371–379
45 **Brown, E.M. *et al.*** (1993) *Nature* 366, 575–580
46 **Nakanishi, S.** (1992) *Science* 258, 597–603
47 **O'Hara, P.J. *et al.*** (1993) *Neuron* 11, 41–52
48 **Simerly, R.B.** (1990) *Trends Neurosci.* 13, 104–110
49 **Yoshihara, Y. *et al.*** (1997) *J. Neurosci.* 17, 5830–5842
50 **Mombaerts, P. *et al.*** (1996) *Cell* 87, 675–686
51 **Sullivan, S.L. *et al.*** (1995) *Neuron* 15, 779–789
52 **Clark, A.J. *et al.*** (1985) *EMBO J.* 4, 3159–3165
53 **Wu, Y., Tirindelli, R. and Ryba, N.J.P.** (1996) *Biochem. Biophys. Res. Commun.* 220, 900–904
54 **Berghard, A., Buck, L.B. and Liman, E.** (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 2365–2369
55 **Liman, E.R. and Buck, L.B.** (1994) *Neuron* 13, 611–621
56 **Bradley, J. *et al.*** (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 8890–8894
57 **Tang, W.J. and Gilman, A.G.** (1991) *Science* 254, 1500–1503
58 **Tirindelli, R. and Ryba, N.J.P.** (1996) *Eur. J. Neurosci.* 8, 2388–2398