CMLS, Cell. Mol. Life Sci. 58 (2001) 546–555
1420-682X/01/040546-10 $ 1.50 + 0.20/0


CMLS Cellular and Molecular Life Sciences

# The vomeronasal system

**P. A. Brennan**

Sub-Department of Animal Behaviour, University of Cambridge, High Street, Madingley, Cambridge CB3 8AA, (United Kingdom), Fax +44 195 421 0247, e-mail: pab23@cus.cam.ca.uk

**Abstract.** In addition to the main olfactory system, many vertebrates possess a vomeronasal system that conveys more specialized chemosensory information. Unlike the airborne, volatile stimuli detected by the main olfactory system, vomeronasal stimuli are typically proteins of the lipocalin family which bind small, volatile ligands. Despite the smaller number of vomeronasal receptor types, the projection patterns of the vomeronasal receptor neurons to multiple glomeruli in the accessory olfactory bulb appear to be more complicated than those of the main olfactory system. The vomeronasal system has a direct subneocortical projection to hypothalamic areas that mediates specific behavioural and hormonal responses to pheromonal stimuli. However, the integration and transmission of this information can be modulated by learning mechanisms. The aim of this article is to outline some of the functions of the vomeronasal system, and in particular to comment on recent advances in our understanding of how vomeronasal information is coded and processed.

**Key words.** Vomeronasal; pheromones; chemosensory; MUPs; accessory olfactory bulb; reproduction.

## Introduction

The vomeronasal system is often regarded as being specialized for mediating pheromonal communication. However, this can be misleading, as the term 'pheromone' is often used rather loosely [1]. The term was originally applied to certain conspecific chemical signals found in insects which fulfilled the definition as 'substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or developmental process' [2]. The difficulty in applying this term to higher vertebrates, and in particular to mammals, is that their behaviour has many determinants. In addition, there is a continuum of responses ranging from those that are reliably elicited by a conspecific chemosensory signal to those that merely increase the likelihood of a particular pattern of behaviour. For instance, odour cues that identify an individual may influence maternal behaviour, mate choice behaviour or social behaviour; however, these complex odour signatures are not normally classed as pheromones.

Even if we accept a narrow definition of which substances qualify as pheromones, their effects are not mediated exclusively via the vomeronasal system. For example, a pheromone produced on the ventrum of lactating rabbits elicits a highly specific and stereotyped nipple search behaviour in young rabbit pups that enables them to locate the nipples during suckling [3]. Yet lesioning the VNO does not disrupt the pheromonal effects, which are mediated by the main olfactory system [4]. Moreover, the vomeronasal system may respond to nonpheromonal stimuli. Indeed, although the vomeronasal system is important for reproductive behaviour in garter snakes, it is also vital for prey tracking and striking behaviour, which do not involve conspecific cues and are certainly not pheromones. Thus, the vomeronasal system does not mediate all pheromonal effects and may mediate some nonpheromonal effects. In comparison with the main olfactory system, which can respond to an enormous variety of odourants and flexibly link them to different responses, the vomeronasal system responds to a narrower range of stimuli which elicit relatively stereotyped responses.

There are species differences in the occurrence and elaboration of the vomeronasal system that depend on their behavioural ecology [5]. A vomeronasal system is present in most amphibia and reptiles, but absent in fish, crocodilians, marine turtles and exclusively marine mammals

including porpoises, dolphins, whales and manatees. This suggests that the vomeronasal form of communication is of little use in the marine environment. It also would appear that the vomeronasal system is of less use for airborne vertebrates as it is absent in birds and many bats, although there are a few bat species in which it is particularly well developed [6]. In addition to the physical factors of the environment that may limit its usefulness, the lack of flexibility of vomeronasal responses may limit its role in species with complex social systems. Thus, although there are well-developed VNOs in platyrrhine New World monkeys, the VNO is absent from adult catarrhine monkeys, such as macaques and baboons as well as gibbons and the great apes, i.e. gorillas, chimpanzees and orangutans [6].

Given the complexity of human society and relationships, and that VNOs appear to be absent in other apes, it may be expected that adult humans would not possess a VNO. A VNO does develop in early fetal life and is essential to guide the migration of developing lutenizing hormone releasing hormone (LHRH) neurons to hypothalamic areas. Indeed, lack of proper fetal development of the vomeronasal pathway leads to Kallman's syndrome with its attendant neuroendocrine deficits. However, the VNO was believed to degenerate in late fetal life so that only vestigial remnants could occasionally be found in adults. Recently, there has been a resurgence of interest in the question of whether a functional VNO persists in the adult [7, 8]. Although a bilateral, tubular epithelial structure exists in the adult human nasal septum, there are numerous histological differences from the typical VNO found in other mammalian species. The vomeronasal epithelium is relatively thin and is similar on lateral and medial surfaces, which are both covered in cilia typical of nonsensory respiratory epithelium [8]. Although there have been reports of finding bipolar cells with microvillar processes characteristic of vomeronasal receptor neurons (VRNs), no axons have been observed at their basal ends. Furthermore, olfactory marker protein (OMP), which is a characteristic feature of mature chemosensory neurons, has not been found in the adult human VNO [9]. A few bipolar neurons do stain for some neural markers, but they share these immunohistochemical characteristics with neuroendocrine cells scattered through human nonsensory, respiratory epithelium [10]. Convincing evidence of a central projection from the human VNO is also lacking in adults, although clearly present in the fetus. Finally, although an accessory olfactory bulb (AOB), which is the sole projection target of the VRNs, is clearly discernable in human fetuses, it is absent in adults [11]. Therefore, although there may be a VNO-like structure in adult humans, its function is likely to be different from the VNO of other mammals.

## Behavioural and physiological responses mediated by the vomeronasal system

Of the responses mediated by the vomeronasal system, those that influence reproduction in rodents have been most extensively studied. These can be divided into releaser pheromonal effects, which directly elicit a specific behaviour, and primer pheromonal effects, which change the endocrine state of an individual with consequent longer-term effects. One of the first mammalian pheromonal effects to be ascribed to the vomeronasal system was that caused by the vaginal fluid produced by sexually active female hamsters [12]. This hamster vaginal fluid (HVF) not only contains pheromonal components that attract males to lick it, but also conveys individual-specific information. Thus, preexposure to HVF of a certain female increases a male's subsequent investigation of that female compared with a novel female [13]. This recognition component of the behavioural response appears to be mediated by the vomeronasal systems, as it is abolished by lesions of the VNO but not by zinc sulphate lesions of the main olfactory epithelium [14]. This has been confirmed by the finding that male hamsters with VNO lesions are unable to distinguish between HVF of two individual females in a habituation-dishabituation test [1].

Pheromonal components of HVF increase testosterone levels in males and elicit mounting behaviour. Whereas lesions of the VNO abolish the HVF-induced testosterone rise, their effect on mounting behaviour is less dramatic. Although lesions of the VNO prevent mounting behaviour in sexually naive males, similar lesions in sexually experienced males have little effect [15]. Thus, the first mating experience seems to involve a learning process by which odour cues received via the main olfactory system become capable of eliciting full mounting behaviour. This occurs via links between the main olfactory system and vomeronasal pathway, at the level of the amygdala (fig. 1), which control mounting behaviour via an output to the medial hypothalamus [16]. Interestingly, exogenous, intraventricular administration of LHRH is able to compensate for VNO lesions to restore sexual behaviour in inexperienced males [17]. Thus, the vomeronasal system may mediate the pheromonal effects of HVF to elicit mounting behaviour through increased LHRH release in the medial preoptic area [16].

The vomeronasal system also mediates important primer pheromonal effects on reproduction [18]. Pheromones present in female mouse urine delay the onset of puberty in prepubertal females and suppress oestrous cyclicity in grouped females (Lee Boot effect) [19]. In contrast, pheromones present in male urine generally have oestrous-inducing effects on females, such as accelerating the onset of puberty (Vandenbergh effect) [20], inducing oestrous in anoestrous females (Whitten effect) [21] and

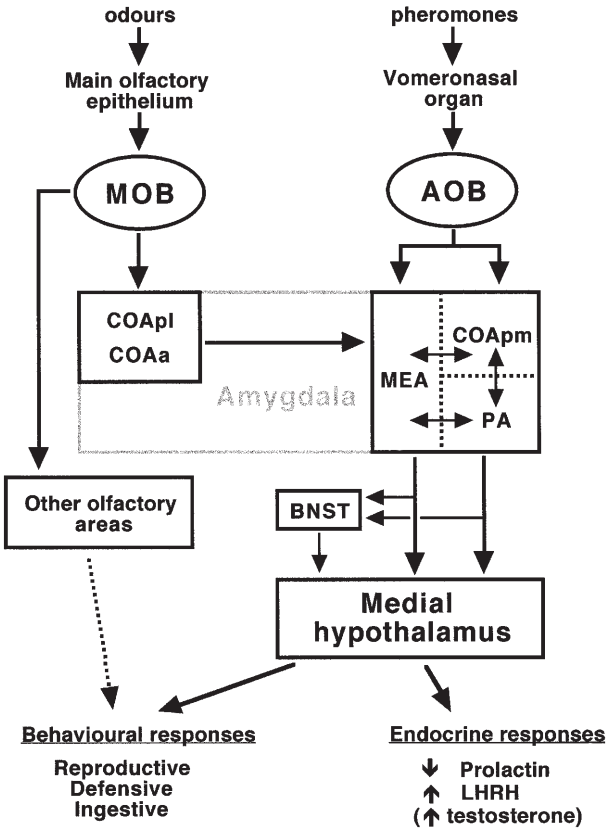


Figure 1. Pheromones stimulate receptors in the vomeronasal organ that project via the accessory olfactory bulb and corticomedial amygdala to medial hypothalamic nuclei. In rodents, this pathway is responsible for the specific endocrine and behavioural effects of pheromones. Information about odours is processed separately by the main olfactory system, but it can elicit the same behavioural responses in experienced animals via links with the vomeronasal pathway at the level of the amygdala. Abbreviations: AOB, accessory olfactory bulb; BNST, bed nucleus of the stria terminalis; COAa, COApl, COApm, anterior, posterior-lateral and posterior-medial parts of the cortical amygdala; LHRH, lutenizing hormone releasing hormone; MEA, medial amygdala; MOB, main olfactory bulb; PA, posterior amygdala.

preventing pregnancy in recently mated females (Bruce effect) [22, 23]. Lesions to the VNO or to the AOB of female mice abolish these effects [24]. Moreover, as well as conveying information about sexual identity, male urine also conveys individual-specific pheromonal information. Thus, female mice are able to distinguish between pheromones from different inbred strains of male in the context of the pregnancy block effect, and this recognition is also mediated by the vomeronasal system [25]. In addition to its important role in reproduction, the vomeronasal system has been implicated in territorial behaviour in male mice such as countermarking of urine deposits, and in the promotion of aggressive behaviour by pheromonal components of male urine [26].

## The structure and function of the VNO

The VNO was first described by Jacobson in 1813, although he believed that it had a secretory function. It is a blind-ended epithelial tube found bilaterally at the base of the nasal septum in most mammals [27], and is connected via the narrow vomeronasal duct to the nasal cavity or to the nasopalatine canal in ungulates and carnivores. In cross-section the VNO has a characteristic crescent shape with a thick pseudostratified sensory epithelium medially and a thinner, ciliated nonsensory epithelium laterally. Like the olfactory receptor neurons in the main olfactory epithelium, the VRNs have a limited life span and are continually being replaced from basal stem cells. These new neurons are particularly concentrated at the margins of the receptor epithelium where it borders the sensory epithelium [28].

As the VNO is a blind-ended, mucus-filled tube, the VRNs are isolated from the nasal airstream that conveys volatile odourants to the main olfactory epithelium. Therefore, stimulus access to the VNO is entirely dependent on a pumping mechanism, which is under autonomic control [29]. Electrical stimulation of the nasopalatine nerve causes a vasoconstriction of the vessels within the VNO capsule, which reduces the volume of blood in the cavernous tissue. The consequent lowering of intraluminal pressure, aided by the resilient bony casing and cartilaginous surrounding tissue, draws fluid in from the vicinity of the vomeronasal duct opening [30]. The pump is not activated continuously or linked to respiratory cycles, but rather is activated in situations of novelty associated with a investigation of the environment [29].

Specific behaviours are often observed that are associated with investigating vomeronasal stimuli and the delivery of those stimuli to the VNO. Snakes and lizards have a highly specialized tongue that flicks out to sample trail stimuli and delivers them to the area of the vomeronasal ducts in the roof of the mouth [31]. In many mammals, such as dogs and rodents, the licking and nuzzling of urine marks introduces the proteinaceous compounds into the nasal cavity from where they can be taken up into the VNO in the mucus. Other mammals, especially ungulates, have a specialized flehmen behaviour in which the head is lifted and the upper lip curled back. These specialized behaviours make it difficult to predict the pattern and timing of stimulus delivery to the vomeronasal epithelium, which has hampered in vivo electrophysiological recordings from the vomeronasal system in response to natural pheromonal stimuli.

## Neural pathways in the vomeronasal system

The distinction between the vomeronasal system and the main olfactory system is further reinforced when their

central projections are considered (fig. 1). VRNs project via the vomeronasal nerves to the AOB, which is located in the dorsocaudal part of the main olfactory bulb (MOB), although entirely separate from it functionally [32]. Olfactory information from the main olfactory system has access to neocortical processing through widespread projections from the MOB, including those to the orbitofrontal and insular cortex. In contrast, the AOB only projects subneocortically, with projections to the medial amygdala and the posterior medial part of the cortical amygdala [33]. From here projections terminate in the medial hypothalamus both directly and via the bed nucleus of the stria terminalis (BNST) [34]. Therefore, the vomeronasal system projects to the hypothalamic centres controlling both behavioural and neuroendocrine output [35].

The anatomical differences between the two olfactory systems reflect the different nature of the information that they process [36]. The main olfactory system has the complex role of discriminating the enormous variety of odours present in the environment, and must be able to associate these odours flexibly with different behavioural outcomes. In contrast, the accessory olfactory system has the simpler task of detecting a relatively limited range of species-specific pheromone molecules, and initiating relatively stereotyped behavioural and endocrine responses. Although both the MOB and AOB project to the amygdala, they terminate in adjacent, nonoverlapping areas [33]. The MOB projects to the anterior and posterior lateral parts of the cortical amygdala, and although there are both direct and indirect projections from these areas to the vomeronasal parts of the amygdala, there are no reciprocal connections. Therefore, it is likely that the accessory olfactory system has only indirect access to higher levels of olfactory processing, whereas the main olfactory system can influence the neuroendocrine output of the vomeronasal system.

## The nature of vomeronasal stimuli

Relatively little is known about the nature of vomeronasal stimuli, although physical contact with the stimulus appears to be necessary for pheromonal effects. The large influx of mucus into the VNO during pump activation carries with it many nonvolatile molecules, especially proteins. Indeed, one of the first vomeronasal stimuli to be identified was aphrodisin, a 17-kDa protein found in HVF that elicits mounting behaviour in sexually naive male hamsters [37]. More recently, a 20-kDa chemoattractant protein isolated from earthworm electric shock secretion has been found to act as a potent vomeronasal stimulus in the completely different context of prey striking behaviour in garter snakes [38].

Although the VNO appears to be specialized for detecting nonvolatile compounds, small, volatile compounds may also be transported into the organ in association with ligand-binding proteins such as the vomeromodulin produced by vomeronasal glands [39]. Such compounds are found at high concentration in male mouse urine and have been reported to possess pheromonal activity, including brevicomins, dihydrothiazoles, farnesones and heptanones [40]. Of these, a mixture of dehydo-*exo*-brevicomin and 2-(*sec*-butyl)-dihydrothiazole has been found to promote aggression in male mice, an effect that is dependent on the vomeronasal system [41]. However, this effect was only observed when the mixture of brevicomin and thiazole was administered in urine from castrated males. Therefore, although these compounds are not by themselves sufficient to elicit aggression, they may be an essential part of a larger pheromonal mixture. The same combination of 2,3-dihydro-*exo*-brevicomin and 2-*sec*-butyl-4,5-dihydro-thiazole has been reported to induce oestrous in grouped anoestrous females [42]. However, in this case, the compounds were effective when administered in water, suggesting that they are sufficient for this primer pheromonal effect.

In male mouse urine both brevicomin and thiazole are strongly bound to major urinary proteins (MUPs) [43]. These are approximately 20-kDa proteins that have a $\beta$-barrel structure enclosing a hydrophobic ligand-binding site [44] and belong to the lipocalin family of ligand binding proteins. This includes an $\alpha$-2-globulin which is found at high concentrations in rat urine, vomeromodulin produced by glands in the vomeronasal epithelium and the odourant-binding proteins present in the mucus covering the main olfactory epithelium. MUPs may be acting as a reservoir for volatile ligands, allowing their release over prolonged periods from dried urine marks. This would certainly be consistent with the finding that pheromonal activity is generally associated with the protein fraction of mouse urine [45, 46]. Another possibility is that the MUPs themselves function as pheromones. MUPs that had been stripped of their ligand have been tested for its ability to accelerate puberty in prepubertal female mice. Not only was the MUP effective in accelerating puberty, but the effect could also be elicited by the N-terminal hexapeptide, suggesting that the MUP proteins themselves possess pheromonal activity [47]. Another alternative is that the complex of MUP with bound ligand may be necessary to activate the receptors.

MUPs are highly polymorphic and are coded for by about 30 different genes and pseudogenes, of which an individual may express around 4–6 variants [48]. The variety of MUPs that are expressed varies across inbred strains, and it is likely that substantial interindividual differences exist within a wild population [49]. Thus, it is highly likely that MUPs convey the individuality of the pheromonal signal, either directly or via their bound ligands, and that this information can be used by female mice to dis-

criminate between males in the context of the pregnancy block effect.

## Transduction mechanisms

Although vomeronasal receptor neurons have low spontaneous activity, a current injection of only 1 or 2 pA is sufficient to fire a series of action potentials [50]. This sensitivity is due to a high input resistance and a membrane potential close to threshold of around −56 to −60 mV, which is maintained by the electrogenic $Na^+/K^+$ pump. Vomeronasal receptor neurons respond to maintained current injection with steady trains of action potentials with little sign of adaptation, in contrast to the rapid adaptation seen in olfactory receptor neurons [51]. This is consistent with the prolonged periods of exposure required to induce the endocrinological effects of rodent primer pheromones [52]. Additionally, a maintained level of activity in VRNs due to continuous pheromonal exposure could be subject to inhibitory interactions with other pheromonal stimuli at the level of the receptor neuron. In this respect, it is interesting that many of the responses of VRNs to putative pheromonal stimuli are inhibitory [53]

Vomeronasal receptor neurons show no response to cyclic nucleotide injection, indicating an absence of cyclic nucleotide-gated ion channels in the membrane [51]. Therefore, their transduction mechanisms differ from that of olfactory receptor neurons that involve a cyclic AMP (cAMP)-mediated opening of cation channels [54]. The G proteins expressed by VRNs are either $G_0$ or $G_{i\alpha 2}$ [55], both of which can activate the inositol trisphosphate ($IP_3$) second-messenger pathway. The involvement of this pathway in vomeronasal transduction is supported by the increases in $IP_3$ levels elicited by vomeronasal stimuli in VRNs from hamsters, pigs and garter snakes [38, 56, 57].

## Vomeronasal receptors

Molecular biological techniques have recently yielded valuable information about the putative pheromonal receptors. Two main classes of receptor termed V1R and V2R have been cloned from rodent VRNs [58–60]. They share little homology with olfactory receptors from the main olfactory epithelium and there is little homology between the two classes. The V1Rs exhibit significant variability in their transmembrane domains, which presumably constitute the ligand binding site [58]. In contrast, the V2Rs have a high degree of variability in a large extracellular N-terminal domain, which is likely to interact with completely different types of molecules. The two classes of receptors are also segregated in the vomeronasal epithelium. The V1R class of receptor is expressed by VRNs located more superficially near the lumen of the VNO, whereas the V2Rs are found in VRNs of the basal region [59].

Not only do the two classes of vomeronasal receptor have different structures, and presumably respond to different pheromonal signals, but there is also good evidence that they project separately to distinct subdivisions of the AOB [61]. The VRNs in the superficial region of the sensory epithelium, corresponding to the expression of V1Rs, express the G protein $G_{i\alpha 2}$ and project to the anterior part of the AOB. VRNs in the basal region of the epithelium, in which V2Rs are found, express $G_0$ and project to the posterior part of the AOB [55]. These subdivisions can be visualized using antibodies to cell surface proteins such as the adhesion molecule OCAM [62] and had previously been identified using monoclonal antibodies [63, 64] and lectin binding patterns [65, 66]. Voltage-sensitive dyes and population potential recordings have demonstrated that these subdivisions of the AOB are functionally distinct [67]. Activity evoked in either region, by selective stimulation of their vomeronasal nerve input, does not propagate across the anatomical boundary. Moreover, local field potential oscillations induced in the anterior subregion are more heavily damped than those in the posterior subregion, perhaps indicating differences in neural connectivity or pharmacology.

The occurrence of this subdivision in a range of species suggests that it is a general feature of the vomeronasal system, constituting separate pathways for the processing of distinct classes of pheromonal stimuli. This question has been investigated in mice using the expression of the immediate-early genes as markers of neuronal activity. Exposure to whole male urine increases the expression of c-fos messenger RNA (mRNA) in the AOB [68] with a significantly greater number of c-Fos-positive mitral cells in the anterior than the posterior region [69]. A similar anteroposterior distribution of expression of egr-1 has been found, both in response to whole male urine, and to the protein fraction of the urine that had been stripped of volatile ligands [70]. In contrast, a mixture of the ligands brevicomin and thiazole increased the number of mitral cells expressing egr-1 in the lateral and medial margins of the posterior AOB, but not in the anterior region. These findings suggest that the anterior AOB may be activated more strongly by MUPs and the posterior AOB by their ligands [70]. However, studies using immediate early gene expression do need to be treated with some caution: although the mixture of brevicomin and thiazole is physiologically effective and increased the number of AOB mitral cells expressing egr-1, it failed to increase the expression of c-fos mRNA in the AOB [68].

Studies of rat VRNs support the opposite conclusions to those based on the egr-1 immediate-early gene study. Volatile components of rat urine activated the $G_{i\alpha 2}$-expres-

sing VRNs that project to the anterior AOB, whereas the ligand-binding protein $\alpha$-2-globulin activated $G_0$-expressing VRNs that project to the posterior AOB [71]. These findings are more consistent with the structural differences between the two classes of receptors. Small molecules might be expected to bind more easily to the transmembrane binding site of the V1R type of receptor than proteins, whereas the proteins would be able to interact with the large extracellular domain on the V2R receptors.

## Information coding in the vomeronasal system

To understand the principles of sensory coding in the vomeronasal system, we need to know both about the specificity of individual receptor neurons and how that information is handled centrally. As yet the nature of vomeronasal stimuli is still unclear, and there is little information available regarding the response characteristics of VRNs. However, the first steps have been taken towards an understanding of how the input from the VRNs is organized at the level of the AOB. In two recent reports [72, 73], groups led by Peter Mombaerts and by Catharine Dulac and Richard Axel have visualized the projections of VRNs expressing a specific receptor. The three receptors that they analyzed were all from the V1R class that had cell bodies located in the superficial region of the vomeronasal sensory epithelium and projected exclusively to the anterior region of the AOB.

These projections appear to be very different from those of the main olfactory system in which neurons expressing a single receptor converge onto two out of approximately 2000 glomeruli in the MOB [74]. In the AOB, VRNs typically project to between 10 and 20 glomeruli out of a total of a few hundred. Furthermore, the complexity of the projection and the poorly defined glomerular structures in the AOB complicate the interpretation of these findings. Belluscio et al. claim that the pattern of projection is bilaterally symmetrical within an individual and although the detailed pattern of projections varies among individuals, receptors of a specific type consistently project to similar regions of the AOB [73]. In contrast, Rodriguez et al. emphasize the variability of the projection both between the AOBs of an individual and among different individuals [72]. It appears that the projection is considerably more variable than that seen in the MOB, but certainly not random. However, whether the complicated patterns of glomeruli are sufficiently organized to constitute a sensory map as claimed by Belluscio et al. requires further investigation.

The question of whether a glomerulus is innervated only by neurons containing a single receptor type has also been addressed by the two groups, who have again reached slightly different conclusions. They agree that there are small glomeruli that are exclusively innervated by neurons expressing a single receptor type. However, whereas Belluscio et al. identify large glomerular structures that receive segregated input from more than one receptor type, Rodriguez et al. report that small glomeruli that receive input from only one receptor type can form part of a higher-order organization with small glomeruli receiving input from other receptor types. The significance of these alternative interpretations depends on the level of integration of information from different receptor types in the AOB. Unlike the mitral cells in the MOB, which project a single primary dendrite to collect information from a single glomerulus and therefore receptor type, the mitral cells in the AOB, have a branched primary dendritic tree that projects to around 5–10 glomeruli [75]. This has implications for information processing in the AOB, and some of the main possibilities are shown in figure 2.

Although a mitral cell may collect information from several glomeruli, if they are all innervated by the same type of VRN, each mitral cell would process information from a given receptor type, a situation similar to that occurring in the MOB. Alternatively, each primary dendrite may project to a glomerulus receiving input from a different receptor type. Information would then be integrated mainly at the level of the mitral cells. Another alternative is that a significant amount of integration of information from different receptor types occurs at the glomerular level, either within large glomerular structures or via lateral connections of periglomerular cells. Each mitral cell primary dendrite would then receive a mixed pheromonal signal, thereby maximizing the number of combinations of inputs to a single mitral cell from different receptor types. This might be important in increasing the complexity of the signal generated by the AOB representing different pheromonal blends.

## Synaptic plasticity in the AOB and its implications for information processing

Olfactory bulb networks exhibit an extremely high level of plasticity due both to the continual addition of new inhibitory interneurons and to changes in synaptic strength of existing neural connections. This has made the olfactory bulb an ideal structure in which to investigate learning dependent neural changes, which have been studied in a variety of mammalian species and behavioural contexts [76]. Following pheromonal learning, in vivo microdialysis studies on freely behaving mice found an increased release of the inhibitory neurotransmitter GABA, in the AOB, in response to glutamate stimulation [77]. Moreover, the ratio of GABA levels to levels of the excitatory transmitters glutamate and aspartate also increased in the AOB, in response to the learned pheromonal stimulus. These findings suggest that pheromonal learning is asso-

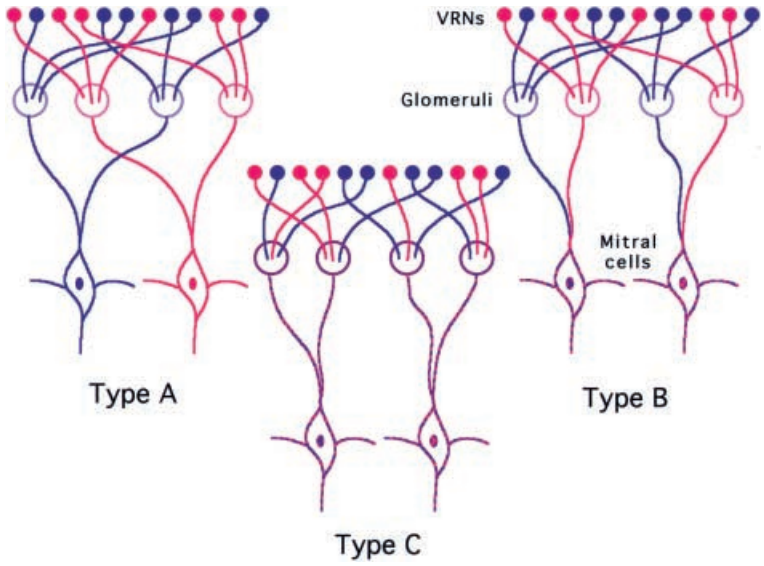


Figure 2. Schematic diagram showing possible connection patterns of vomeronasal receptor neuron (VRN) input to mitral cells. Connections of type A, in which mitral cells receive input from multiple glomeruli that, in turn, receive input from a single receptor type are similar to the connectivity in the main olfactory bulb. In type B, the specificity of input to individual glomeruli is maintained, but the mitral cells can selectively integrate this information from different primary dendrites, which receive input from glomeruli of different receptor types. In type C, large glomeruli may receive input from more than one receptor type. A significant amount of integration of information from different receptor types would therefore occur at the glomerular level. This mixed input to mitral cells might be important in efficiently representing the proportion of components found in complex pheromonal blends.

ciated with an increase in the inhibitory control of mitral cells in the AOB. This is evident in electron micrographs as increases in the length of the postsynaptic densities, of the excitatory part of the reciprocal synapses, from mitral to granule cells [78]. This occurs without alteration of the granule cell to mitral cell inhibitory part of the reciprocal synapses, or of synapses in the glomerular layer. These changes are hypothesized to increase the self-inhibition of mitral cells that respond to the mating male's pheromonal signal, disrupting it at the level of the AOB and thus preventing it from being transmitted centrally to induce pregnancy block (fig. 3) [79].

Similar increases in the inhibitory control of mitral cells occur in the MOB following odour learning, in the contexts of lamb odour recognition in sheep, neonatal odour conditioning in rats and appetitive odour conditioning in mice [80–82]. Thus, increases in the inhibitory gain of the reciprocal synapses between mitral and granule cells appear to be a general feature of odour learning [76]. The tightly coupled inhibitory control exerted at the reciprocal synapses could mediate either self-inhibition of active mitral cells or lateral inhibition of less active, neighbouring mitral cells. In the MOB, the mitral cells have extensive secondary dendritic trees that extend over large areas of the bulb. Thus, lateral inhibitory effects are likely to be predominant, integrating the glomerular information into a spatiotemporal response that is characteristic for a meaningful odour. However, in the AOB, the mitral cells have a very limited secondary dendritic tree, and the majority of the reciprocal synapses with granule cells are made on the extensive primary dendrites. These synapses are therefore ideally placed to control the transmission of information along individual primary dendrites from specific glomeruli to the mitral cell soma.

Pheromonal learning in mice depends on the association of mating and pheromonal exposure, and results in the increased inhibitory control of mitral cells in the AOB. However, exposure to pheromones without mating appears to have the opposite effect. This results in a slowly developing and sustained increase in glutamate release in the AOB, and an increased release of the excitatory transmitters aspartate and glutamate 2 days later, in response to the pheromones to which the females had been exposed [77]. This decreased inhibitory control of mitral cells is consistent with the priming effect of pheromonal preexposure to enhance the pheromonal acceleration of puberty in prepubertal female mice [83]. It is also consistent with the finding that the exposure of male hamsters to urine from female hamsters decreases the length of the postsynaptic densities of the excitatory, glutamatergic synapses from mitral to granule cells [84]. Such a 'wind-up' of mitral cell transmission may be important in maintaining a response to the pheromones during the long periods of exposure that are necessary for their primer pheromonal effects [52]. Taken together, these various lines of evidence suggest that synaptic plasticity of the reciprocal synapses between mitral and granule cells in the AOB may play a vital role in controlling the central transmission of the pheromonal signal. Different branches of the primary dendritic tree might be functioning indepen-

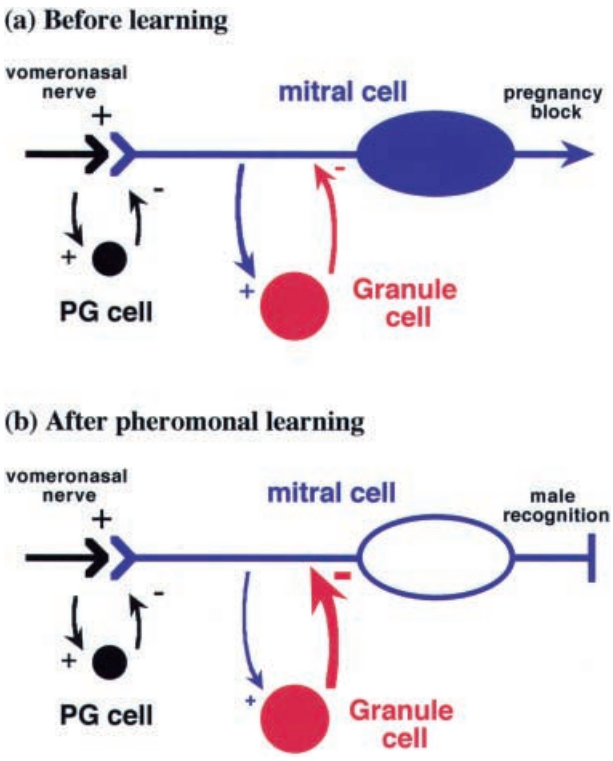


Figure 3. Schematic diagram showing the effect of pheromonal learning on the interactions between the main cell types in the accessory olfactory bulb in mice. Mitral cells receive excitatory input from the vomeronasal receptor neurons. Feedback inhibition from periglomerular (PG) cells at the glomerular level is likely to regulate the input to the mitral cells, whereas self-inhibition via granule cells probably has an important role in regulating mitral cell output. (*a*) Before learning, pheromonal stimulation excites a certain population of mitral cells. This leads to activation of the central vomeronasal pathway resulting in pregnancy block. (*b*) Following pheromonal learning, the strength of the excitatory synapses from mitral to granule cells is increased. This causes an enhancement of the self-inhibition of mitral cells, thereby disrupting the transmission of the pregnancy blocking signal and consequently resulting in male recognition.

dently exerting, selective control over the driving of mitral cell activity by glomerular input.

This article has only been able to touch on the important role played by the vomeronasal system in the biology of most vertebrates. However, despite its importance, there is still relatively little known about its function. This looks set to change as recent molecular biological investigations have provided new insights into the complexity of the system. Although the main olfactory system and vomeronasal systems both fulfil chemosensory roles, that of the vomeronasal system is considerably simpler, both in terms of the number of receptors used to detect the limited range of vomeronasal stimuli and the central connections to hypothalamic areas. This makes the vomeronasal system attractive for the investigation of olfactory function, and this will continue to require the application of a wide range of techniques. Although molecular biology has allowed us to visualize the first stages of vomeronasal processing, electrophysiological recordings from populations of neurons in freely behaving animals will be required before the picture becomes completely clear.

1 Johnston R. A. (1998) Pheromones, the vomeronasal system, and communication. Ann. N.Y. Acad. Sci. **855:** 333–348
2 Karlson P. and Lüscher M. (1959) Pheromones: a new term for a class of biologically active substances. Nature **183:** 55–56
3 Hudson R. and Distel H. (1986) Olfactory guidance of nipple-search behaviour in newborn rabbits. In: Ontogeny of Olfaction, pp. 243–254, Breipohl W. (ed.), Springer, New York
4 Hudson R. and Distel H. (1986) Pheromonal release of suckling in rabbits does not depend on the vomeronasal organ. Physiol. Behav. **37:** 123–129
5 Dawley E. M. (1998) Species, sex and seasonal differences in VNO size. Microsc. Res. Tech. **41:** 506–518
6 Bhatnagar K. P. and Meisami E. (1998) Vomeronasal organ in bats and primates: extremes of structural variability and its phylogenetic implications. Microsc. Res. Tech. **43:** 465–475
7 Monti-Bloch L., Jennings-White C. and Berliner D. L. (1998) The human vomeronasal system. Ann. N.Y. Acad. Sci. **855:** 373–389
8 Smith T. D., Siegel M. I., Burrows A. M., Mooney M. P. Burdi A. R., Fabrizio P. A. et al. (1998) Searching for the vomeronasal organ of adult humans: preliminary findings on location, structure and size. Microsc. Res. Tech. **41:** 483–491
9 Takami S., Getchel M. L., Chen Y., Monti-Bloch L., Berliner D. L., Stensaas L. J. et al. (1993) Vomeronasal epithelial cells in the adult human express neuron-specific molecules. Neuroreport **4:** 375–378
10 Johnson E. W. (1998) CaBPs and other immunohistochemical markers of the human vomeronasal system: a comparison with other mammals. Microsc. Res. Tech. **41:** 530–541
11 Meisami E. and Bhatnagar K. P. (1998) Structure and diversity in mammalian accessory olfactory bulb. Microsc. Res. Tech. **43:** 476–499
12 Powers J. B. and Winans S. S. (1975) Vomeronasal organ: critical role in mediating sexual behavior of the male hamster. Science **187:** 961–963
13 Steel E. (1984) Effect of the odour of vaginal secretion on non-copulatory behaviour of male hamsters (*Mesocricetus auratus*). Anim. Behav. **32:** 597–608
14 Steel E. and Keverne E. B. (1985) Effect of female odour on male hamsters mediated by the vomeronasal organ. Physiol. Behav. **35:** 195–200
15 Meredith M. (1986) Vomeronasal organ removal before sexual experience impairs male hamster mating behavior. Physiol. Behav. **36:** 737–743
16 Meredith M. (1998) Vomeronasal, olfactory, hormonal convergence in the brain. Ann. N. Y. Acad. Sci. **855:** 349–361
17 Fernandez-Fewell G. D. and Meredith M. (1995) Facilitation of mating behavior in male hamsters by LHRH and AcLHRH$^{5-10}$: interaction with the vomeronasal system. Physiol. Behav. **57:** 213–214
18 Keverne E. B. (1983) Pheromonal influences on the endocrine regulation of reproduction. Trends Neurosci. **6:** 381–384
19 Lee S. v. d. and Boot L. M. (1955) Spontaneous pseudopregnancy in mice. Acta Physiol. Pharmacol. Neurol. **4:** 442–443
20 Vandenbergh, J. (1969) Male odor accelerates female sexual maturation in mice. Endocrinology **84:** 658–660
21 Whitten W. (1956) Modification of the oestrous cycle of the mouse by external stimuli associated with the male. J. Endocrinol. **13:** 399–404
22 Bruce H. (1960) A block to pregnancy in the mouse caused by the proximity of strange males. J. Reprod. Fertil. **1:** 96–103

23 Brennan P. A. (1999) Bruce effect. In: Encyclopedia of Reproduction, vol. 1, pp. 433–438, Knobil E. and Neill J. D. (eds.), Academic Press, San Diego
24 Keverne E. B. (1983) The accessory olfactory system and its role in pheromonally mediated changes in prolactin. In: Olfaction and Endocrine Regulation, pp. 127–140, Breipohl, W. (ed.), IRL Press, London
25 Lloyd-Thomas A. and Keverne E. B. (1982) Role of the brain and accessory olfactory system in the block to pregnancy in mice. Neuroscience **7:** 907–913
26 Maruniak J. A., Wysocki C. J., and Taylor J. A. (1986) Mediation of male mouse urine marking and aggression by the vomeronasal organ. Physiol. Behav. **37:** 655–657
27 Døving K. B. and Trotier D. (1998) Structure and function of the vomeronasal organ. J. Exp. Biol. **21:** 2913–2925
28 Weiler E., McCulloch M. A. and Farbman A. I. (1999) Proliferation in the vomeronasal organ of the rat during postnatal development. Eur. J. Neurosci. **11:** 700–711
29 Meredith M. (1994) Chronic recording of vomeronasal pump activation in awake behaving hamsters. Physiol. Behav. **56:** 345–354
30 Salazar I. and Sánchez Quinteiro P. (1998) Supporting tissue and vasculature of the mammalian vomeronasal organ: the rat as a model. Micros. Res. Tech. **41:** 492–505
31 Burghardt G. M. and Pruitt C. H. (1975) Role of tongue and senses in feeding of naive and experienced garter snakes. Physiol. Behav. 14: 185-194
32 MacLeod N. K. and Reinhardt W. (1983) An electrophysiological study of the accessory olfactory bulb in the rabbit – I. Analysis of electrically evoked potential fields. Neuroscience **10:** 119–129
33 Scalia F. and Winans S. S. (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. J. Comp. Neurol. **161:** 31–56
34 Swanson L. W. and Petrovich G. D. (1998) What is the amygdala? Trends Neurosci. **21:** 323–331
35 Li C. S., Kaba H., Saito H. and Seto K. (1990) Neural mechanisms underlying the action of primer pheromones in mice. Neuroscience **36:** 773–778
36 Meredith M. (1991) Sensory processing in the main and accessory olfactory systems: comparisons and contrasts. J. Steroid Biochem. Molec. Biol. **39:** 601–614
37 Singer A. G. and Macrides F. (1990) Aphrodisin: pheromone or transducer? Chem. Senses **15:** 199–204
38 Wang D., Chen P., Liu W. M., Li C. S. and Halpern M. (1997) Chemosignal transduction in the vomeronasal organ of garter snakes: $Ca^{2+}$-dependent regulation of adenylate cyclase. Arch. Biochem. Biophys. **348:** 96–106
39 Miyawaki A., Matsushita F., Ryo Y. and Mikoshiba K. (1994) Possible pheromone-carrier function of two lipocalin proteins in the vomeronasal organ. EMBO J. **13:** 5835–5842
40 Novotny M., Jemiolo B. and Harvey S. (1990) Chemistry of rodent pheromones: molecular insights into chemical signaling in mammals. In: Chemical Signals in Vertebrates 5, pp. 1–22, Macdonald, D. W., Muller-Schwarze, D. and Natynczuk, S. E. (eds.), Oxford University Press, New York
41 Novotny M., Harvey S., Jemiolo B. and Alberts J. (1985) Synthetic pheromones that promote inter-male aggression in mice. Proc. Natl. Acad. Sci. USA **82:** 2059–2061
42 Jemiolo B., Harvey S. and Novotny M. (1986) Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. Proc. Natl. Acad. Sci. USA **83:** 4576–4579
43 Bacchini A., Gaetani E. and Cavaggioni A. (1991) Pheromone binding proteins of the mouse, Mus musculus. Experentia **48:** 419–421
44 Bocskei Z. et al. (1992) Pheromone binding to two rodent urinary proteins revealed by X-ray crystallography. Nature **360:** 186–188
45 Vandenbergh J. G., Whitsett J. M. and Lombardi J. R. (1975) Partial isolation of a pheromone accelerating puberty in female mice. J. Reprod. Fert. **43:** 515–523
46 Marchlewska-Koj A. (1981) Pregnancy block elicited by male urinary peptides in mice. J. Reprod. Fertil. **61:** 221–224
47 Mucignat-Caretta C., Caretta A. and Cavaggioni A. (1995) Acceleration of puberty onset in female mice bymale urinary proteins. J. Physiol. Lond. **486:** 517–522
48 Robertson D. H., Cox K. A., Gaskell S. J., Evershed R. P. and Beynon R. J. (1996) Molecular heterogeneity in the major urinary proteins of the house mouse *Mus musculus*. Biochem. J. **316:** 265–272
49 Robertson D. H. L., Hurst J. L., Bolgar M. S., Gaskell S. J. and Beynon R. J. (1997) Molecular heterogeneity of urinary proteins in wild house mouse populations. Rap. Comm. Mass Spec. **11:** 786–790
50 Trotier D., Døving K. B., Ore K. and Shalchiantabrizi C. (1998) Scanning electron microscopy and gramicidin patch clamp recordings of microvillous receptor neurons dissociated from the rat vomeronasal organ. Chem. Senses **23:** 49–57
51 Liman E. R. and Corey D. P. (1996) Electrophysiological characterization of chemosensory neurons from the mouse vomeronasal organ. J. Neurosci. **16:** 4625–4637
52 Rosser A. E., Remfry C. J. and Keverne E. B. (1989) Restricted exposure of mice to primer pheromones coincident with prolactin surges blocks pregnancy by changing hypothalamic dopamine release. J. Reprod. Fert. **87:** 553–559
53 Moss R. L., Flynn R. E., Shen X.-L., Dudley C. and Shi J. (1997) Urine-derived compound evokes membrane responses in mouse vomeronasal receptor neurons. J. Neurophys. **77:** 2856–2862
54 Shepherd G. M. (1994) Discrimination of molecular signals by the olfactory receptor neuron. Neuron **13:** 771–790
55 Berghard A. and Buck L. B. (1996) Sensory transduction in vomeronasal neurons: evidence for Gao, Gai2, and adenylyl cyclase II as major components of a pheromone signalling cascade. J. Neurosci. **16:** 909–918
56 Kroner C., Breer H., Singer A. G. and O'Connell R. J. (1996) Pheromone-induced second messenger signalling in the hamster vomeronasal organ. Neuroreport **7:** 2989–2992
57 Wekesa K. S. and Anholt R. R. H. (1997) Pheromone regulated production of inositol-(1,4,5)-trisphosphate in the mammalian vomeronasal organ. Endocrinology **138:** 3497–3504
58 Dulac C. and Axel R. (1995) A novel family of genes encoding putative pheromone receptors in mammals. Cell **83:** 195–206
59 Herrada G. and Dulac C. (1997) A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell **90:** 763–773
60 Matsunami H. and Buck L. B. (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell **90:** 775–784
61 Halpern M., Jia C. P. and Shapiro L. S. (1998) Segregated pathways in the vomeronasal system. Micros. Res. Tech. **41:** 519–529
62 Campenhausen von H., Yoshiharal Y. and Mori K. (1997) OCAM reveals segregated mitral/tufted cell pathways in developing accessory olfactory bulb. Neuroreport **8:** 2607–2612
63 Mori K., Imamura K., Fujita S. C. and Obuta K. (1987) Projections of two subclasses of vomeronasal nerve fibers to the accessory olfactory bulb in the rabbit. Neuroscience **20:** 259–278
64 Schwarting G. A., Drinkwater D. and Crandall J. E. (1994) A unique neuronal glycolipid defines rostrocaudal compartmentalization in the accessory olfactory system of rats. Dev. Brain Res. **78:** 191–200
65 Takami S., Graziadei P. P. C. and Ichikawa M. (1992) The differential staining patterns of two lectins in the accessory olfactory bulb of the rat. Brain Res. **598:** 337–342

66 Halpern M., Shapiro L. S. and Jia C. P. (1995) Differential localisation of G-proteins in the opposum vomeronasal system. Brain Res. **677:** 157–161
67 Sugai T., Sugitani M. and Onoda N. (1997) Subdivisions of the guinea pig accessory olfactory bulb revealed by the combined method with immunohistochemistry, electrophysiological, and optical recordings. Neuroscience **79:** 871–885
68 Guo J., Zhou A. and Moss R. L. (1997) Urine and urine-derived compounds induce c-fos mRNA expression in accessory olfactory bulb. Neuroreport **8:** 1679–1683
69 Halem H. A., Cherry J. A. and Baum M. J. (1998) Vomeronasal neuroepithelium and forebrain Fos responses to male pheromones in male and female mice. J. Neurobiol. **39:** 249–263
70 Brennan P. A., Schellinck H. M. and Keverne E. B. (1999) Patterns of expression of the immediate-early gene egr-1 in the accessory olfactory bulb of female mice exposed to pheromonal constituents of male urine. Neuroscience **90:** 1463–1470
71 Krieger J. Schmitt A., Lobell D., Gudermann T., Schultz G., Breer H. et al. (1999) Selective activation of G protein subtypes in the vomeronasal organ upon stimulation with urine-derived compounds. J. Biol. Chem. **274:** 4655–4662
72 Rodriguez I., Feinstein P. and Mombaerts P. (1999) Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. Cell **97:** 199–208
73 Belluscio L., Koentges G., Axel R. and Dulac C. (1999) A map of pheromone receptor activation in the mammalian brain. Cell **97:** 209–220
74 Mombaerts P., Wang F., Dulac C., Chao S. K., Newes A., Mendelsohn M. et al. (1996) Visualizing an olfactory sensory map. Cell **87:** 675–686
75 Mori K. (1987) Membrane and synaptic properties of identified neurons in the olfactory bulb. Prog. Neurobiol. **29:** 275–320
76 Brennan P. A. and Keverne E. B. (1997) Neural mechanisms of mammalian olfactory learning. Prog. Neurobiol. **51:** 457–481
77 Brennan P. A., Kendrick K. M. and Keverne E. B. (1995) Neurotransmitter release in the accessory olfactory bulb during and after the formation of an olfactory memory in mice. Neuroscience **69:** 1075–1086
78 Matsuoka M., Kaba H., Mori Y. and Ichikawa M. (1997) Synaptic plasticity in olfactory memory formation in female mice. Neuroreport **8:** 2501–2504
79 Brennan P., Kaba H. and Keverne E. B. (1990) Olfactory recognition: a simple memory system. **250:** 1223–1226
80 Kendrick K. M., Levy F. and Keverne E. B. (1992) Changes in sensory processing of olfactory signals induced by birth in sheep. Science **256:** 833–836
81 Wilson D. A., Sullivan R. M. and Leon M. (1987) Single-unit analysis of postnatal olfactory learning: modified olfactory bulb output response patterns to learned attractive odors. J. Neurosci. **7:** 3154–3162
82 Brennan P. A., Schellinck H. M., de la Riva C., Kendrick K. M. and Keverne E. B. (1998) Changes in neurotransmitter release in the main olfactory bulb following an olfactory conditioning procedure in mice. Neuroscience **87:** 583–590
83 Mucignat Caretta C., Caretta A. and Cavaggioni A. (1995) Pheromonally accelerated puberty is enhanced by previous experience of the same stimulus. Physiol. Behav. **57:** 901–903
84 Matsuoka M., Mori Y. and Ichikawa M. (1998) Morphological changes of synapses induced by urinary stimulation in the hamster accessory olfactory bulb. Synapse **28:** 160–166