Mammalian social odours: attraction and individual recognition

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Mammalian social systems rely on signals passed between individuals conveying information including sex, reproductive status, individual identity, ownership, competitive ability and health status. Many of these signals take the form of complex mixtures of molecules sensed by chemosensory systems and have important influences on a variety of behaviours that are vital for reproductive success, such as parent–offspring attachment, mate choice and territorial marking. This article aims to review the nature of these chemosensory cues and the neural pathways mediating their physiological and behavioural effects. Despite the complexities of mammalian societies, there are instances where single molecules can act as classical pheromones attracting interest and approach behaviour. Chemosignals with relatively high volatility can be used to signal at a distance and are sensed by the main olfactory system. Most mammals also possess a vomeronasal system, which is specialized to detect relatively non-volatile chemosensory cues following direct contact. Single attractant molecules are sensed by highly specific receptors using a labelled line pathway. These act alongside more complex mixtures of signals that are required to signal individual identity. There are multiple sources of such individuality chemosignals, based on the highly polymorphic genes of the major histocompatibility complex (MHC) or lipocalins such as the mouse major urinary proteins. The individual profile of volatile components that make up an individual odour signature can be sensed by the main olfactory system, as the pattern of activity across an array of broadly tuned receptor types. In addition, the vomeronasal system can respond highly selectively to non-volatile peptide ligands associated with the MHC, acting at the V2r class of vomeronasal receptor.

The ability to recognize individuals or their genetic relatedness plays an important role in mammalian social behaviour. Thus robust systems for olfactory learning and recognition of chemosensory individuality have evolved, often associated with major life events, such as mating, parturition or neonatal development. These forms of learning share common features, such as increased noradrenaline evoked by somatosensory stimulation, which results in neural changes at the level of the olfactory bulb. In the main olfactory bulb, these changes are likely to refine the pattern of activity in response to the learned odour, enhancing its discrimination from those of similar odours. In the accessory olfactory bulb, memory formation is hypothesized to involve a selective inhibition, which disrupts the transmission of the learned chemosignal from the mating male. Information from the main olfactory and vomeronasal systems is integrated at the level of the corticomedial amygdala, which forms the most important pathway by which social odours mediate their behavioural and physiological effects. Recent evidence suggests that this region may also play an important role in the learning and recognition of social chemosignals.

Keywords: amygdala; maternal bonding; olfactory bulb; pregnancy block; social recognition; vomeronasal

1. INTRODUCTION

The mammalian lifestyle with its high degree of maternal care has led to the evolution of complex social systems in which the ability to distinguish and recognize individuals is vital for reproductive success. It is particularly important in the formation of parent–offspring bonds, enabling maternal resources to be directed to related individuals and denied to others. Individual recognition also forms the basis of territorial behaviour, identifying the individual or group, defending resources such as

mates, food or nest sites and allowing the detection of intruders and the rejection of unfamiliar animals from a social group. It further extends to the choice of mate, in which the ability to assess the degree of relatedness of a potential mate is thought to reduce inbreeding and maximize the fitness of offspring, especially in competitive natural environments (Meagher *et al.* 2000). Information from a range of senses can be used for discrimination among conspecifics, including visual recognition of physical features, such as faces, or vocal cues, such as those in whale song or in human speech. However, for most mammals, olfaction is their dominant sense and their behaviour is heavily influenced by the social chemosignals secreted by individual conspecifics (Wyatt 2003).

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One contribution of 14 to a theme issue ‘The neurobiology of social recognition, attraction and bonding’.

2. MAMMALIAN SOCIAL CHEMOSIGNALS

Mammals release an enormous variety of molecules into the environment, either as specific chemosignals or as products of metabolic processes. These range from small volatile molecules to large proteins, and are released by a variety of routes including urine, faeces or the secretions of skin, reproductive tract or specialized scent glands. They provide a wealth of information about the producer, such as their sex, age, health and reproductive state which contribute to the odour profile of the animal. Some of these, such as the 5 α -androst-16-en-3-one and 5 α -androst-16-en-3-ol produced by boars in their saliva, can act as classical pheromones in releasing a specific behavioural response (Brennan & Keverne 2004). Being volatile, these airborne steroids are sensed at a distance by the main olfactory system of sows, in which they elicit approach behaviour and lordosis, to allow mating (Dorries *et al.* 1997). Other chemosignals, such as those signalling individuality, are components of highly complex mixtures. The main olfactory system is able to integrate the pattern of volatile molecules produced by an animal into an overall odour signature for that individual. However, many of the constituent molecules will be subjected to environmental influences, such as changes in diet, or microbial flora, and may prove unreliable as cues for recognizing the individual on subsequent occasions. There is thus a strong argument that reliable cues for individual discrimination and recognition should be based on differences in individual genotype.

3. GENETIC DETERMINANTS OF CHEMOSENSORY INDIVIDUALITY

Although many genotypic differences have the potential to affect chemosensory identity, most interest has focused on the genes of the major histocompatibility complex (MHC). This large and highly polymorphic family of genes is involved in the ability of the immune system to distinguish self from non-self at the cellular level as a defence against invading pathogens. In addition to this immunological role, MHC genotype can determine individual identity at the behavioural level (Boyse *et al.* 1987). For instance, there is strong evidence that MHC genotype influences the urine odour of mice. In a series of experiments, Yamazaki & Beauchamp's group trained mice to discriminate between urine from different individuals in a Y maze to obtain a water reward. They showed that mice could discriminate between the urine odours of congenic mice that differed genetically only at the H2 locus of their MHC (Yamaguchi *et al.* 1981; Yamazaki *et al.* 1990). Although this odour conditioning procedure required extensive training, untrained mice have also been shown to be capable of similarly fine discriminations using a habituation/dishabituation test (Penn & Potts 1998c). Humans too can discriminate and recognize the odours from different individuals. Fathers, grandmothers and aunts have been shown to be able to identify the odour of a related infant compared with an unrelated one, independent of prior experience with the infant (Porter *et al.* 1986).

Such an ability to identify genetic relatedness through social odours influences mate choice in mice. Studies by Yamazaki *et al.* (1976, 1988) have demonstrated

MHC-dependent mate choice in congenic strains of mice differing only in their MHC genotype. When given the choice between two individuals, under laboratory conditions, mice generally choose to mate with the MHC-dissimilar individuals (Jordan & Bruford 1998). This pattern of disassortative mating has also been observed in colonies of mice living in semi-natural enclosures, which produced fewer MHC homozygous offspring than expected from random matings (Potts *et al.* 1991). There is even limited evidence that MHC genotype may play a similar role in human mate choice. Genetic analysis of human leukocyte antigen (HLA) haplotype (the human MHC) in reproductively isolated Hutterite communities has found fewer HLA matches than expected, suggesting that they avoid spouses with similar HLA haplotype to their own (Ober *et al.* 1997). Other studies have found that on average, human subjects rate the odours of other individuals as more pleasant if they have a few matches of HLA alleles, rather than either none or a high degree of similarity (Wedekind & Furi 1997; Jacob *et al.* 2002). Furthermore, these preferences were based on the matches with only paternally inherited HLA type, suggesting that the preference was dependent on own genotype, rather than having been learnt during exposure to related individuals (Jacob *et al.* 2002). These findings are consistent with the idea of disassortative mating preferences, but human mate choice is difficult to study and much more work needs to be done in this area to establish whether HLA type plays a significant role in complex human societies.

MHC genotype also influences parent-offspring interactions. For instance, although female mice nest communally with other females and nurture each other's pups indiscriminately, they are more likely to nest with individuals of MHC-similar genotype (Manning *et al.* 1992). This co-operative behaviour minimizes the delivery of maternal resources to genetically unrelated individuals. Maternal recognition of offspring is not as important as in altricial mammals, such as rodents, where their young are confined to a nest, as it is in mammals that have more mobile, precocial young, such as sheep. Nevertheless, when presented with scattered pups, mice preferentially retrieve those of the same MHC type as themselves (Yamazaki *et al.* 2000). Furthermore, pups learn from an early age to recognize the odours to which they are exposed in the nest. When tested in a Y maze, mouse pups were found to prefer nest odours of maternal and sibling MHC type rather than an unfamiliar MHC type (Yamazaki *et al.* 2000).

4. CHEMOSENSORY CUES OF MHC IDENTITY

Gas chromatographic analysis of the urinary volatiles from MHC-congenic mice have been shown to differ in the relative proportions of volatile carboxylic acids, which are necessary and sufficient to convey MHC-chemosensory identity (Singer *et al.* 1997; Schaefer *et al.* 2002). However, despite extensive research and a range of different hypotheses, the mechanism linking the MHC's role in conveying individuality at the immunological and behavioural levels has remained unclear (Penn & Potts 1998a). The H2 region of mouse chromosome 17 codes for MHC proteins of classical

class I type that are expressed on the cell membrane of nearly all the cells in the body. Their immunological role is to bind peptides produced by the proteosomal degradation of endogenous and foreign proteins and present them to the cell surface, providing the immune system with a dynamic view of intracellular protein composition (Singh 2001). The immune system is able to recognize peptides of endogenous origin as self, whereas peptides derived from foreign proteins activate cytotoxic T lymphocytes and cell destruction. The MHC class I proteins belong to a highly polymorphic gene family with structurally diverse peptide binding grooves. Therefore, a particular MHC class I protein will bind a specific subset of the pool of peptides produced by proteosomal degradation. These are estimated to be up to 100 alleles at the *H-2K* and *H-2D* loci, with less polymorphism at the *H-2L* locus, producing greater than 10^9 unique phenotypes in outbred mice (Singh 2001). A similar amount of MHC diversity is believed to occur in other mammalian species, implying that each individual within a population essentially has a different MHC genotype, and consequently a unique combination of peptide ligands bound by its MHC class I proteins.

The ability of mice to discriminate urine odours of MHC-congenic strains is related to the degree of amino acid divergence in the peptide-binding groove of their MHC class I proteins (Carroll *et al.* 2002). Several theories have been proposed as to how these differences could be related to differences in the profile of urinary volatiles (Penn & Potts 1998a). According to one hypothesis, differences in profiles of urinary volatiles could arise from endogenous and/or microbial breakdown of the individual-specific pool of MHC-bound peptides, although, as mice are able to distinguish urine odours of germ free mice, it appears that microbial processes are not essential. Another suggestion, known as the carrier hypothesis, proposes that when MHC class I proteins are cleaved from the cell surface, a conformational change causes the peptide to dissociate from the binding cleft. This would enable the MHC class I protein to bind plasma volatiles and transport them into the urine. Differences in the structure of the binding grooves would thus lead to differences in the profile of urinary volatiles. Small, 27 kDa fragments of MHC class I proteins have been found in mouse urine, albeit at low concentrations (Singh *et al.* 1987), although there is no evidence that they bind volatiles.

More recent evidence has shown that the MHC peptide ligands themselves can function as individuality chemosignals, forming a direct link between individuality at the immunological and behavioural levels. These peptides are nine amino acids long in mice, a size determined by proteosomal processing. The major factor determining their binding is large hydrophobic side chains of particular amino acids, known as anchor residues, which occupy characteristic pockets in the MHC-binding groove. The position and shape of two, or rarely three, anchor pockets vary among different MHC class I proteins and determine the specificity of peptide binding. For example, the MHC class I D^b molecule encoded by the *H-2^b* haplotype found in C57BL/6 mice, preferentially binds to peptides having asparagine (N) at position 5, such as AAPDNRETF, whereas the K^d

Figure 1. MHC class I peptide ligands act as vomeronasal chemosignals of MHC identity owing to the binding characteristics of their anchor residues. Both endogenous and foreign proteins are degraded into nine amino acid peptides by the proteosomal degradation pathway. MHC class I proteins are loaded with the subset of peptides possessing anchor residues that specifically bind to their peptide binding groove. When these peptides are released in body secretions they can act as ligands at the V2R class of vomeronasal receptor, in which the peptide binding specificity is determined by the MHC-dependent positions of their anchor residues.

molecule encoded by the *H-2^d* haplotype found in BALB/c mice, preferentially binds to peptides with tyrosine (Y) at position 2, such as SYFPEITHI. Therefore, any receptor system with similar binding characteristics to the MHC class I molecules will be activated specifically by peptides associated with a certain MHC type (figure 1).

Receptors with these binding characteristics have been found in mouse vomeronasal sensory neurons (VSNs) of the V2R class (Leinders-Zufall *et al.* 2004). Synthetic peptides of BALB/c-type (SYFPEITHI) or C57BL/6-type (AAPDNRETF) were found to elicit selective responses from largely separate sub-populations of VSNs. Furthermore, the specificity of the responses was not affected if the amino acid residues between the anchor residues were varied. In contrast, replacement of the characteristic anchor residues with alanines or scrambling the sequence of amino acids to change the locations of the anchor residues both abolished the receptor response. These findings confirm that MHC peptides can signal individual information via the vomeronasal system. This chemosensory system is

found in most mammals, with the notable exception of Old World primates, including humans. The vomeronasal organ (VNO) is a blind-ended tubular structure situated in the nasal septum and connected to the nasal cavity via a narrow duct (Døving & Trotier 1998). The VSNs are located in the sensory epithelium along the medial side of the lumen with a large blood sinus running laterally. Changes in the blood flow to this sinus result in pressure changes in the lumen, which pumps mucus and chemosignals into the organ (Meredith & O'Connell 1979; Meredith 1994).

The vomeronasal system has been shown to convey individuality information in the pregnancy block effect (also known as the Bruce effect). This occurs when recently mated female mice are exposed to urinary chemosignals from an unfamiliar male, which elicits a high incidence of pregnancy failure (Bruce 1959). Although the mating male also produces pregnancy-blocking chemosignals in its urine, they do not block its mate's pregnancy. This is because the female learns to recognize the individual identity of her mate's urinary chemosignals, during a sensitive period at the time of mating, which prevents them from aborting her offspring (Keverne & de la Riva 1982). Selective lesions of the VNO abolish the pregnancy block effect (Bellringer *et al.* 1980; Rajendren & Dominic 1984), whereas lesions of the main olfactory epithelium are without effect on pregnancy block or selective recognition of the mating male (Lloyd-Thomas & Keverne 1982; Ma *et al.* 2002).

The biological effectiveness of MHC peptide ligands in conveying strain identity has been demonstrated in the context of the pregnancy block effect (Leinders-Zufall *et al.* 2004). Addition of C57BL/6 peptides was sufficient to alter the strain identity and confer pregnancy-blocking effectiveness to BALB/c urine, following mating with a BALB/c male, whereas the addition of BALB/c-specific peptides was ineffective. The converse was true for females that had mated with C57BL/6 males. Hence, the addition of BALB/c type peptides to C57BL/6 urine changed its strain identity and caused it to be treated as unfamiliar, resulting in pregnancy failure (Leinders-Zufall *et al.* 2004). These results are consistent with earlier findings (Yamazaki *et al.* 1983) that congenic mice differing from the mating male at only the H2 locus of the MHC were effective in blocking pregnancy.

Therefore, these peptides form robust and specific chemosignals of individuality, which may be of importance in other vertebrate species and behavioural contexts. The importance of MHC peptide ligands as potential signals of individuality has been enhanced by the recent finding that they elicit responses in olfactory sensory neurons (OSNs) of the main olfactory epithelium of mice (Spehr *et al.* 2006). The responses of OSNs to MHC peptide ligands differ from those of VSNs in their dependence on the presence of anchor residues and their lower sensitivity of around 10^{-10} M, suggesting that different receptor mechanisms are involved. These peptides therefore form robust and specific chemosignals of individuality, which may be of importance in other vertebrate species. However, nine amino acid peptides are unlikely to be very volatile and probably could not account for the discriminability of urine odours at a distance. It therefore appears that there are both peptide and volatile MHC genotype

signals that are sensed by separate chemosensory systems, and may be used in different behavioural contexts.

5. MAJOR URINARY PROTEINS AND COUNTERMARKING BEHAVIOUR IN MICE

The MHC is not the only story when it comes to a genetic basis for chemosensory identity. Members of a lipocalin family of ligand binding proteins are found in body secretions, saliva and urine, and are thought to play a chemosensory signalling role in a variety of species and behavioural contexts. These lipocalins possess a β -barrel structure enclosing a ligand-binding calyx, and they often bind and transport small volatile chemosignals (Flower 1996). This is certainly the case for lipocalins found in rodent urine such as the major urinary proteins (MUPs). The 18–20 kDa MUPs bind small volatile urinary chemosignals including (*R,R*)-3,4-dehydro-exo-brevicomin (DB), (*S*)-2-sec-butyl-4,5-dihydrothiazole (BT), *E,E*- α -farnesene, *E*- β -farnesene and 6-hydroxy-6-methyl-3-heptanone (Robertson *et al.* 1993; Novotny 2003). These testosterone-dependent chemosignals advertize the presence of a reproductively active male. Thus, they elicit aggressive behaviour in male mice (Novotny *et al.* 1985) and play a role in regulating the reproductive state of females, including the acceleration of puberty (Novotny *et al.* 1999) and the induction and synchronization of oestrus cycles (Jemiolo *et al.* 1986).

Male mouse urine contains extremely high concentrations of MUPs of up to 70 mg ml^{-1} , constituting up to 99% of its protein content (Humphries *et al.* 1999). Therefore, their production represents a significant energetic cost and reflects their important chemosensory role in mouse territorial behaviour. Many animals display territorial behaviour to defend a certain area and its attendant resources from competitors (Wyatt 2003). Individual mammals or groups typically identify their territory by depositing urine, faeces or marks from specialized scent glands throughout their territory, but especially at the boundaries or along major access routes. Such odour signalling has the advantage that the chemical cues are long lasting and are generally less costly than other mechanisms of advertizing their presence. The maintenance of fresh odour marks throughout the territory not only signals the fitness of the producer, but also their identity, allowing an intruder to recognize the territory owner and vice versa. For instance, dominant male mice deposit urine marks throughout their territory and will countermark any marks left by intruder males and can match a urine mark with the individual odour of the male that produced it (Hurst 1993; Hurst *et al.* 2005).

The MUPs in male mouse urine act as a reservoir for volatile chemosignals that attract investigation from females and other males, prolonging their release from dried urine marks (Hurst *et al.* 1998). However, the countermarking behaviour of males is driven by the non-volatile MUPs rather than the volatiles released from urine marks. Moreover, the MUPs form a highly polymorphic family of genes of which a wild mouse produces between 4 and 15 MUP variants in its urine (Beynon & Hurst 2003). This MUP profile differs among inbred mouse strains in the laboratory and

among individual mice in the wild, forming an identity code with diversity comparable with that of the MHC (Robertson *et al.* 1997). Indeed, Hurst's group has demonstrated that MUPs convey the individual ownership of urine marks (Hurst *et al.* 2001). They showed that changing the MUP profile of urine marks, by the addition of artificially produced MUPs, increased the countermarking rate of territory owners. Moreover, male mouse countermarking behaviour depended on MUP profile rather than MHC genotype.

The mechanism by which MUPs signal individuality remains to be resolved. They could potentially influence the profile of volatile urinary chemosignals. However, the fact that male countermarking behaviour is driven by non-volatile components of urine leads to the speculation that they may interact directly with vomeronasal receptors to provide a reliable signal of urine mark identity (Flower 1996). Mouse urine is clearly a complex chemosensory cocktail with MHC-dependent volatile and non-volatile, and MUP-dependent cues, which are each capable of signalling individual identity in different behavioural contexts.

6. CODING OF CHEMOSENSORY INDIVIDUALITY

The complex mixture of molecules that constitutes the airborne odour signature of an individual appears to contain few, if any, unique compounds. Rather, it is the relative proportions of common volatiles in the odour profile that convey the information about individual identity (Singer *et al.* 1997; Schaefer *et al.* 2002). Such complex odour discrimination tasks can be readily accomplished by the main olfactory system, which is ideally suited for recognizing the profiles of airborne volatiles that constitute an individual odour signature. Each OSN expresses a single receptor protein, which typically responds to a relatively broad range of odourants with related molecular structures. Consequently, each OSN will respond to a range of related odourants, and a single odourant will stimulate OSNs expressing different receptor types. A complex profile of odourants that constitute the odour of an individual will therefore be represented as a pattern of activity across the receptor repertoire. OSNs project their axons to the glomeruli of the main olfactory bulb (MOB) where they provide input to the primary dendrites of mitral and tufted cell projection neurons. Each glomerulus receives input from a single receptor type and therefore acts as a fundamental unit of odour representation. Therefore, individual odour profiles are represented as specific patterns of glomerular activity across the MOB (figure 2; Schaefer *et al.* 2001, 2002). Indeed, exposure to urine from congenic mice differing in MHC at only the H-2^K gene results in patterns of glomerular activity in the MOB that can be distinguished using principal components analysis (Schaefer *et al.* 2002).

The different modes of stimulus access to the main olfactory epithelium and the VNO have the consequence that they are specialized for detecting different types of stimuli. The main olfactory epithelium senses highly volatile stimuli that are carried in the nasal airstream. In contrast, the VNO appears to be specialized to detect relatively non-volatile stimuli, such as peptides and

proteins present in skin secretions or scent marks which are only taken into the VNO following direct contact (Wysocki *et al.* 1985; Luo *et al.* 2003). The degree of this specialization has recently been called into question, as VSNs respond highly specifically and sensitively to urinary volatiles *in vitro* (Leinders-Zufall *et al.* 2000). Furthermore, calcium imaging of VSNs found that they responded to 18 out of a panel of the 82 general odourants tested. This suggests that there is at least the potential for overlap in the chemosignals that are detected by the main olfactory and vomeronasal systems (Sam *et al.* 2001). If this actually occurs will depend on whether such volatile stimuli gain access to the VNO under normal conditions. Evidence from type-3 adenylyl cyclase knockout mice that lack functional OSNs, but retain functional VSNs, has suggested that this could be the case (Trinh & Storm 2003). These mice show a behavioural response to volatile urinary pheromones, such as 2-heptanone and dimethylpyrazine when presented on a cotton swab without any direct contact. However, the extent to which main olfactory system function is abolished in these mice has been questioned and the behavioural discrimination of these volatile odours could be mediated by atypical OSNs using alternative transduction mechanisms (Lin *et al.* 2004). Therefore, the ability of volatile stimuli to gain access to the VNO in natural situations remains in doubt.

The two classes of vomeronasal receptors that have been identified are seven-transmembrane-domain G-protein coupled receptors, but they share little homology with each other or with main olfactory receptors, which suggests that they may respond to different types of ligand (Herrada & Dulac 1997; Matsunami & Buck 1997; Ryba & Tirindelli 1997). The VSNs expressing receptors from each class are segregated in the vomeronasal epithelium. VSNs that express V1rs are located in the apical zone, whereas V2r-expressing VSNs are found in the deeper basal zone. At least 137 functional receptors of the V1r class have been identified in the mouse genome (Rodriguez *et al.* 2002) and it has been estimated that there are around 60 functional receptors in the V2r class (Yang *et al.* 2005). Unlike the OSNs that typically respond to a range of related odourant molecules, VSNs respond highly selectively to specific chemosignals (Leinders-Zufall *et al.* 2000, 2004). *In vitro* recordings have revealed that VSNs of the V1r class respond to small volatile molecules, such as 2-heptanone (Boschat *et al.* 2002) which is found in male mouse urine and extends the length of female oestrus cycles (Novotny 2003). In addition to being highly selective, V1r VSNs are highly sensitive, with typical thresholds of 10^{-10} – 10^{-11} M, and unlike OSNs their selectivity does not broaden as stimulus concentration is increased (Leinders-Zufall *et al.* 2000).

The V2r class of VSNs is even more sensitive and V2rs respond specifically to MHC peptide ligands at concentrations down to 10^{-13} M (Leinders-Zufall *et al.* 2004). The structure of V2rs differs from that of the V1Rs in the presence of a large extracellular N-terminal domain (Herrada & Dulac 1997). This accounts for most of the receptor diversity and is likely to form the ligand-binding domain that interacts with MHC peptide ligands. Interestingly, the V2rs are co-expressed with non-classical MHC Ib proteins, in VSNs in the basal zone of

Figure 2. Distinct patterns of glomerular activity in the main olfactory bulb are found in response to urine odours from mice of difference MHC type. (a) The schematic shows the positional relationship between regions of the main olfactory bulb and the two-dimensional contour maps. Average *c-fos* glomerular activation patterns in the main olfactory bulbs of H-2^d female mice in response to; (b) clean air, (c) H-2^b male urine odour and (d) H-2^k male urine odour. Colour bar to the right of b shows the density of active glomeruli for b–d (number of positive glomeruli per bin). (e) Colour contour map of the difference between H-2^b and H-2^k odour representations assessed by Mann–Whitney *U* test. Colour bar to the right of e shows the *p* values. The black border indicates the critical value for the differences to be regarded as significant. Modified with permission from Schaefer *et al.* (2002). Copyright 2002 by the Society for Neuroscience.

the VNO, suggesting they may have a role in vomeronasal function. There are nine members of the non-classical MHC Ib family, M1, M9, M10.1 to M10.6 and M11 (Loconto *et al.* 2003; Ishii *et al.* 2003) with sequence variability concentrated in the region around the peptide binding pocket. Whether they bind MHC peptide ligands is unclear, as several of the amino acid residues that are normally involved in peptide binding are missing (Loconto *et al.* 2003). However, certain combinations of MHC Ib proteins are expressed with particular V2rs

Figure 3. Accessory olfactory bulb mitral cells respond selectively to the strain identity of anaesthetized stimulus animals. Significant excitatory responses are indicated in red and significant inhibitory responses are indicated in green. Non-significant responses are shown in black and hatched boxes indicate stimulus animals that were not tested. Colour scale at the right represents response indices ranging from -2 to 3. Numbers to the left are identifiers of the individual neurons, some of which were excited by specific strain–sex combinations (7.11–2.8), others by animals of both sexes of a single strain (10.9 and 2.5y). Neuron 9.2x was excited by the majority of stimulus animals and did not show strain specificity. Reprinted with permission from Luo *et al.* (2003). Copyright 2003 AAAS.

(Ishii *et al.* 2003), and it is tempting to speculate that they might convey innate differences in responsiveness to different MHC types.

As expected from their differences in epithelial location and receptor structure, the different classes of VSN expressing either V1rs or V2rs appear to handle different types of vomeronasal information. The V1r class handles chemosensory information of a specific pheromonal nature, conveying signals such as sex, whereas the V2r class conveys information about MHC identity. These two receptor classes are not only segregated at the level of the vomeronasal epithelium, but also project to separate sub-regions of the mouse accessory olfactory bulb (AOB; Halpern *et al.* 1998). The V1r class of VSNs projects to the anterior sub-region, while the V2r class projects to the posterior sub-region. *In vitro* recordings from the guinea-pig AOB have demonstrated that stimulation of the afferents to one sub-region elicits activity that propagates through that sub-region, but that does not propagate across the anatomical boundary to the other sub-region (Sugai *et al.* 1997). This suggests that information about MHC identity might be processed separately from other vomeronasal stimuli in the rodent AOB.

Unlike the mitral cells in the MOB which project a single primary dendrite to collect information from a single glomerulus (Mombaerts *et al.* 1996), mitral/tufted (M/T) projection neurons in the anterior sub-region of the AOB send a branched primary dendritic tree that collects information from typically 15–30 glomeruli (Rodriguez *et al.* 1999; Belluscio *et al.* 1999). This pattern of connectivity appears to mediate the integration of different types of pheromonal information at the level of the AOB, at least for the V1r class of VSNs (Wagner *et al.* 2006).

This view is consistent with electrophysiological recordings from the mouse AOB reported by Katz's group (Luo *et al.* 2003). Although they found M/T neurons that responded specifically to strain identity regardless of sex of an anaesthetized mouse, many M/T neurons were found to be excited or inhibited by specific combinations of sex and inbred strain identity (figure 3). For instance, a single M/T neuron in the AOB of a CBA male was strongly excited during investigation of a BALB/c male, but did not respond to either a CBA male or a BALB/c female. The same neuron was strongly inhibited during investigation of a CBA female and weakly inhibited in response to a C57/B6 female. It is difficult to see how MHC peptide ligands could convey information about both sex and strain identity of the producer. Therefore, it appears that information about sexual and individual identity is integrated in the AOB, although whether this involves convergent input from V1r and V2r receptor types remains unclear.

7. NEURAL MECHANISMS UNDERLYING INDIVIDUAL RECOGNITION

Much of what is known of the neural basis of olfactory learning in mammals has come from studying a small range of species, especially rodents and sheep, in which learning occurs in particular contexts often vital for reproductive success. These not only involve cues for individuality but occur in the context of other arousing sensory signals. Somatosensory stimulation plays an important role in many contexts of olfactory learning. However, attractant chemosignals may also play an important, if subtle, role in olfactory recognition by promoting attention towards the individuality chemosignals that have to be learned (Lévy *et al.* 2004). For instance, male mouse urine contains (methylthio) methanethiol, a chemical that is partly responsible for its attractiveness to other mice (Lin *et al.* 2005). This attraction will promote initial investigation of urine, which will facilitate learning of the individuality cues.

Attractant chemosignals are particularly important in the formation of mother offspring bonds, and chemosignals in amniotic fluid appear to play an important role in lamb odour learning in sheep. Amniotic fluid is normally unattractive to sheep but it becomes highly attractive immediately after parturition, a change that is dependent on the associated vaginocervical stimulation (Poindron & Lévy 1990). Ewes that receive peridural anaesthetic during parturition are not attracted to amniotic fluid, but interestingly the attraction can be induced by intracerebroventricular infusion of oxytocin (OT; Lévy *et al.* 1990b). This attraction to amniotic fluid promotes licking of the newborn lamb and facilitates maternal behaviour and the development of selective lamb recognition. Many species of mammal use attractant pheromones to encourage neonates to the nipples for suckling. Perhaps, the most extreme example of this is in rabbits that nurse their young for only a 3–4-min period once a day. A single molecule, identified as 2-methylbut-2-enal, is produced in milk and elicits the stereotyped search behaviour that results in the rapid location of

the nipples (Schaal *et al.* 2003). This arousing behavioural context induces learning of the maternal odours by the rabbit pups, which reinforces the nipple search response (Kindermann *et al.* 1994). The breast odour of human mothers also elicits attraction of newborn babies, which will crawl towards the odour, despite the relative immaturity of their motor system development (Varendi & Porter 2001).

8. NEONATAL ODOUR LEARNING IN RODENTS

The preference of mouse pups to approach maternal odours is influenced by neonatal learning, as it can be reversed by cross-fostering (Yamazaki *et al.* 2000). However, this learning not only forms the basis for their ability to find their way back to their nest as their mobility increases, but it also has longer term effects on their behaviour as adults. MHC-associated mate preferences in adulthood can be reversed by cross-fostering neonatal mice to a mother of different MHC type (Yamazaki *et al.* 1988; Penn & Potts 1998b). Thus, adult mice appear to bias their choice of mate towards dissimilarity from the parental and sibling odours to which they were exposed in the nest environment. The finding that the approach of neonates to maternal odours could not be completely reversed suggests that there may be a component of introspection involved, in which behaviour is influenced by 'knowledge of self'. Direct evidence for such phenotype matching in rodents is lacking. It has been claimed that hamsters' investigation of scent marks from different individuals is based on preferences acquired partly through introspection (Mateo & Johnston 2000). However, the hamsters in this study were not cross-fostered until several hours following birth. Therefore, there was a chance for learning of maternal odour to occur perinatally or even *in utero*. Although the main olfactory system is not fully developed at birth, there is good evidence from many species, including humans, that fetuses can learn *in utero* about the odours of food eaten by their mothers during gestation. Thus, it is perfectly plausible that mammals could also learn odours associated with maternal MHC type *in utero*.

The neural mechanisms underlying neonatal olfactory learning have been investigated using a conditioning procedure to artificial odours that mimics the natural learning of maternal and sibling nest odours (Wilson & Sullivan 1994). The licking and grooming of rat pups by their mother acts as a strong unconditioned stimulus for learning of maternal and sibling odours. This can be conveniently studied in a laboratory environment by placing rat pups in a beaker with peppermint scented wood shavings (conditioned stimulus) and stroking them for a few minutes with a paintbrush (unconditioned stimulus). When tested with a choice of the peppermint-scented pine shavings versus unscented pine shavings, the neonates spent more time over the conditioned peppermint odour compared with naive pups. This has features of classical conditioning, as learning does not occur in the backward pairing condition in which the stroking occurs before odour exposure. This form of learning only occurs during a developmentally determined

sensitive period before postnatal day 10. After this time, stroking loses its ability to act as an unconditioned stimulus (Woo & Leon 1987).

The unconditioned stimulus of tactile stimulation increases noradrenaline (NA) release in the MOB of neonatal rats by centrifugal fibres from the locus ceruleus (Rangel & Leon 1995). This noradrenergic transmission is necessary for learning to occur, as lesions of the noradrenergic fibres to the MOB prevent odour conditioning (Sullivan *et al.* 1989). Similarly, odour conditioning is prevented by local infusions of the β -noradrenergic antagonist propranolol into the MOB during the odour conditioning procedure (Sullivan *et al.* 1992). The association of increased NA release in the MOB and odour input results in substantial morphological and functional changes at the glomerular level, which are restricted to consistently located odour 'hotspots' (Coopersmith & Leon 1984). Following odour conditioning, the activity of mitral cells in these hotspots is more likely to be suppressed than excited in response to the conditioned odour. In contrast, mitral cells recorded in the hotspots of control animals were more likely to be excited rather than suppressed in response to the same odour (Wilson *et al.* 1987). This suggests that the inhibitory input to mitral cells from local inhibitory interneurons is increased following learning in response to the conditioned odour.

Stroking results in a higher and more prolonged release of NA in the MOB prior to postnatal day 10, when tactile stimulation is effective as an unconditioned stimulus. Furthermore, whereas local infusions of acetylcholine into the locus ceruleus stimulate the release of NA in the MOB before postnatal day 10, they lose their effectiveness after this sensitive period (Moriceau & Sullivan 2004). The change in neonatal odour conditioning around postnatal day 10 coincides with an increase in the ratio of $\alpha 2$ - $\alpha 1$ noradrenergic receptors in the locus ceruleus. This would act to limit the duration of locus ceruleus neuronal activity in response to tactile stimulation and consequently reduce the amount of NA released in MOB by stroking (Moriceau & Sullivan 2004). This developmental change is associated with the functional maturation of other, more sophisticated, learning systems. In adults, pairing an odour with an aversive consequence such as a mild shock leads to the subsequent avoidance of the odour. However, pairing odour and shock in neonatal rats younger than postnatal day 10 leads to a lasting odour preference. This makes sense biologically as rat pups depend on the relationship with their mother for their early survival. Even if their mother treats them roughly, they cannot afford to form an aversion to her or her odour as they depend on her maternal care and the safe environment of the nest. The developmental changes to the neonatal brain around day 10 include the onset of amygdala function and the appearance of innate fear responses and fear conditioning, so that aversive stimuli such as footshock now support aversive conditioning (Sullivan *et al.* 2000). These are required when their visual, auditory and motor systems have developed enough to move away from the security of the nest for short periods.

9. LAMB-ODOUR RECOGNITION IN SHEEP

Unlike maternal care in rodents, where their pups are born at an early stage of development and are confined to a nest, sheep give birth to precocial young that are able to stand and move within a few hours of birth. Being seasonal breeders, a large number of lambs are born over the same period. Although they show some evidence for being able to recognize their own mother within the first 12 h after birth using a combination of odour, vocal and visual cues, they need 2–4 weeks to become completely proficient at doing so and will often try to suckle from other ewes (Kendrick 1994). Therefore, the mother has to have a highly efficient system for recognizing her own lamb and allow it to suckle, while rejecting the suckling attempts of 'strange' lambs (Poindron & Lévy 1990). This discrimination of own from strange lambs is vital for restricting the ewe's maternal resources to her own offspring and is mediated primarily by olfactory cues from their wool and skin. If the olfactory bulbs or the olfactory epithelium is lesioned, selectivity is lost and a ewe will accept any lamb. In contrast, cutting the vomeronasal nerve does not affect maternal selectivity, implying that the olfactory cues involved in lamb recognition are mediated by the main olfactory system (Lévy *et al.* 1995b).

The ewe learns about the odour of her newborn lamb during a sensitive period of 2–4 h following parturition. After this period, the ewe becomes increasingly selective and will accept suckling attempts from only her own lamb. This olfactory learning and the maternal acceptance behaviour are dependent on the hormonal environment of late gestation, but are triggered by the mechanical stimulation of the vagina and cervix that occurs during parturition (Keverne *et al.* 1983). Thus, a new sensitive period for lamb acceptance can be induced by vaginocervical stimulation 2–3 days following parturition (Kendrick & Keverne 1991). The development of maternal behaviour is facilitated by the odour of the amniotic fluid, which the ewe licks off the lamb immediately after birth. Washing lambs to remove the amniotic fluid reduces maternal licking behaviour and disrupts the acceptance of lambs by inexperienced ewes (Lévy & Poindron 1987). Conversely, applying amniotic fluid to a 1-day-old lamb increases its chance of acceptance by a parturient ewe.

Vaginocervical stimulation occurring during parturition increases the release of NA from locus ceruleus afferents in the MOB, which plays an essential role in odour learning (Lévy *et al.* 1993). Both specific noradrenergic lesions of the MOB using 6-hydroxydopamine (Pissonier *et al.* 1985) and the infusion of the β -noradrenergic antagonist propranolol into the MOB, during the sensitive period, prevent the selective recognition of the ewe's own lamb (Lévy *et al.* 1990a). Oxytocin release in the MOB also increases at birth and is likely to facilitate the learning process by enhancing the release of NA in the MOB (Kendrick 2000). There is also a role for the nitric oxide signalling pathway in the development of the selective response to own lamb odour. Neuronal nitric oxide synthase (nNOS) is present in the granule cells of the sheep MOB and can act as a retrograde messenger to enhance

glutamate release from the mitral cells via soluble guanylyl cyclase (SGC). Inhibition of this pathway by MOB infusions of inhibitors of nNOS or SGC prevents the selective recognition of own lamb odours (Kendrick *et al.* 1997). The effects of NOS, but not SGC inhibition, can then be reversed by localized infusions of nitric oxide donors (Kendrick *et al.* 1997).

Lamb odour learning is associated with dramatic changes in the electrophysiology and neurochemistry of the MOB. Kendrick *et al.* (1992) found that before parturition, the majority of mitral cells in a defined area of the MOB responded most strongly to food odours and none responded preferentially to lamb odours. However, when mitral cell responses were recorded from the same region of the MOB following parturition, the vast majority (60%) of them now showed a preferential response to lamb odours with some showing a preferential response to own lamb odour. Furthermore, *in vivo* microdialysis monitoring of neurotransmitter levels in the MOB showed that before parturition lamb odours have little effect on the release of any bulbar neurotransmitters. However, following parturition and the establishment of maternal selectivity, own lamb odour, but not the odour of a strange lamb, induced a significant increase in the release of both the excitatory amino acid glutamate and the inhibitory transmitter γ -aminobutyric acid (GABA) (Kendrick *et al.* 1992). Moreover, the ratio of glutamate to GABA was significantly lower in response to own lamb odour compared with strange lamb odour, suggesting that inhibitory neurotransmission in the MOB is increased in response to own lamb odour.

The first maternal experience has lasting consequences, as the development of selective recognition of the ewe's own lamb occurs more rapidly for subsequent births (Keverne *et al.* 1993; Kendrick 1994). This is reflected in the release of intrinsic MOB neurotransmitters (dopamine, GABA and glutamate), as well as modulatory neurotransmitters (acetylcholine and NA), and peptides (OT and vasopressin), which depends on previous maternal experience (Lévy *et al.* 1993, 1995a). In particular, NA levels only increase around 2 h following the first birth, suggesting that vaginocervical stimulation is not as efficient at stimulating NA release as it is in experienced ewes (Lévy *et al.* 1995a). The process of bonding with their offspring after the first birth therefore induces lasting changes in the MOB circuitry. This may well involve up regulation of OT receptors (Lévy *et al.* 1993) thereby sensitizing the system to vaginocervical stimulation and shortening the time taken to develop a selective response at subsequent births. Certainly, mRNA expression for the OT receptor is significantly enhanced in a number of brain regions as a function of maternal experience (Broad *et al.* 1999).

10. THE ROLE OF THE MOB IN SOCIAL RECOGNITION

Neonatal odour conditioning in rats and lamb odour learning in sheep occur in behavioural contexts that are vital for reproductive success. In both cases learning is contingent on somatosensory stimulation, which evokes increased NA release in the MOB, and is associated with extensive neural changes at the level of

Figure 4. Schematic of the major projections of the main olfactory system and the vomeronasal system in the rat. Selected second order connections are shown to highlight the interconnectivity of the two chemosensory pathways at the level of the amygdala and their convergence on outputs to the hypothalamus and BNST (Pitkänen 2000). Abbreviations: ACo, anterior cortical nucleus; AOB, accessory olfactory bulb; AON, anterior olfactory nucleus; BAOT, bed nucleus of the accessory olfactory tract; BNST, bed nucleus of the stria terminalis; ENT, entorhinal cortex; Me, medial nucleus; MOB, main olfactory bulb; MOE, main olfactory epithelium; NLOT, nucleus of the lateral olfactory tract; OT, olfactory tubercle; PIR, piriform cortex; PMCo, posterior medial cortical nucleus; PLCo, posterior lateral cortical nucleus; VNO, vomeronasal organ.

the MOB. A common feature of these changes is the increased inhibitory control of MOB mitral cells in response to the learned olfactory stimulus (Brennan & Keverne 2000). These forms of olfactory learning are somewhat specialized in that they occur during a defined sensitive period. However, similar neurochemical changes have been reported to occur in the MOB following a simple appetitive odour conditioning procedure in adult mice (Brennan *et al.* 1998). Changes in the inhibitory control of mitral cells in the MOB therefore appear to be a general feature of odour learning, but an understanding of how these changes are involved in odour recognition requires a consideration of the network properties of the MOB.

In addition to their single primary dendrite, mitral cells in the vertebrate MOB possess an extensive secondary dendritic tree. This extends tangentially over a large area of the MOB making reciprocal dendrodendritic synapses at spines on granule cell inhibitory interneurons (Mori *et al.* 1983). This neural architecture is highly suited for mediating lateral inhibitory interactions with the surrounding mitral cells, as has been demonstrated in the rabbit MOB (Yokoi *et al.* 1995). Lateral inhibition is further

facilitated by the presence of mGluR₂ receptors on the granule cell side of reciprocal synapses which locally inhibit GABA release at spines receiving mitral cell input (Bischofberger & Schild 1996). These reduce self-inhibition while permitting lateral inhibition onto neighbouring mitral cells that are less active. Electroencephalogram (EEG) recordings from the surface of the rabbit MOB have provided evidence for learning-dependent changes in the pattern of activity in the MOB (Freeman & Schneider 1982). The global pattern of EEG activity across the MOB was observed to change during learning. However, once an odour had become specifically associated with a behavioural response, the pattern was invariant for succeeding odour presentations. Moreover, when the reward contingencies of the odour changed, the pattern of EEG activity elicited by the odour also changed, generating a new odour response pattern specific for the learned odour.

There is also evidence for a role of the granule cells in synchronizing the activity of mitral cells activated by different odourant features, which could facilitate their binding as a single odour representation at the level of the piriform cortex (Kashiwadani *et al.* 1999; Wilson & Stevenson 2003). Changes in the efficacy of inhibitory control of mitral neurons by granule inhibitory interneurons could therefore change the spatial pattern, temporal pattern or synchrony of the mitral cells that respond to an odour. This is hypothesized to play an important role in odour learning by pulling apart the bulbar representations of similar odours, enabling them to be discriminated more reliably. For example, prior to parturition sheep do not have to make fine discriminations between lamb odours, as they have the same meaning to the animal and are largely ignored. However, following parturition, the failure to discriminate between the odours of her own and alien lambs would be highly undesirable. The change in the MOB representation of own lamb odour following parturition, would differentiate it from representations of the highly similar odours of alien lambs, and increase the reliability with which it is linked to a different behavioural response by more central brain areas.

11. VOMERONASAL MECHANISMS OF MATE RECOGNITION

The ability of a female mouse to recognize the pheromones of the male with which she has mated is one of the simplest examples of social recognition (Brennan *et al.* 1990; Brennan & Keverne 1997). Exposure of a recently mated female mouse to the urinary chemosignals of an unfamiliar male causes a high rate of pregnancy failure, but this does not occur when she is exposed to the pheromones of the mating male, as she is able to recognize her mate's chemosignals (Bruce 1961). Learning her mate's identity occurs during a sensitive period lasting a few hours immediately after mating and is contingent on the vaginocervical stimulation of coitus (Rosser & Keverne 1985). The pregnancy block effect depends on a testosterone-dependent chemosignal in male mouse urine, as pregnancy-blocking effectiveness is lost following castration (Bruce 1965) and restored by testosterone injections (Hoppe 1975). Such hormonal

manipulations would not be expected to affect the production of MHC peptide ligands, suggesting that pregnancy-blocking effectiveness and the individual identity are likely to be conveyed by separate chemosignals.

Both the pregnancy block effect and ability of females to recognize the male with which they mated are mediated solely by the vomeronasal system (Lloyd-Thomas & Keverne 1982; Ma *et al.* 2002). However, there is also evidence for a luteotrophic effect of male exposure, which lasts up to 7 days following mating and is mediated by the main olfactory system (Archunan & Dominic 1990; Acharya & Dominic 1997). This cannot explain the mate recognition in the pregnancy block effect which lasts for at least 30 days after mating (Kaba *et al.* 1988), far longer than the recognition underlying the luteotrophic effect. Nevertheless, it suggests that the main olfactory and vomeronasal systems may have synergistic roles in mediating mate recognition in mice.

The vomeronasal pathway forms a relatively direct route by which chemosensory stimuli mediate effects on endocrine state and reflexive behaviour. The M/T neurons of the AOB receive input from the VSNs and project on to the cortico-medial regions of the amygdala, then via the medial hypothalamus to the tuberoinfundibular dopaminergic (TIDA) neurons in the arcuate nucleus of the hypothalamus (Li *et al.* 1989, 1990, 1992). Activation of this pathway by pregnancy-blocking chemosignals results in dopamine release from the TIDA neurons, which inhibits prolactin production by the anterior pituitary (Reynolds & Keverne 1979; Ryan & Schwartz 1980; Marchelwska-Koj 1983). As prolactin is luteotrophic in mice, the fall in serum prolactin withdraws luteotrophic support, leading to the failure of progesterone production by the corpora lutea. This change in hormonal state prevents implantation of the developing embryos and causes the female to return to oestrus (Parkes & Bruce 1961; Dominic 1966). Thus, male chemosignals are only effective at blocking pregnancy when the exposure is coincident with the twice-daily peaks of prolactin during the vulnerable pre-implantation period (Rosser *et al.* 1989). Similarly, electrical stimulation of the AOB is effective in blocking pregnancy, but again only when it is timed to coincide with the prolactin surges (Li *et al.* 1994).

The locus for the neural changes underlying mate recognition has been established by infusing the local anaesthetic lignocaine into each site along the vomeronasal pathway, to temporarily disrupt neural activity during the sensitive period for memory formation (Kaba *et al.* 1989). Infusions of lignocaine into the corticomedial amygdala were without effect on memory formation, whereas recognition of the mating male was prevented when lignocaine was infused into the AOB. Along with the necessary control procedures, these experiments imply that the neural changes underlying mate recognition occur in the AOB, at the first level of processing of the chemosensory information.

The synaptic circuitry of the AOB is comparatively simple (Mori 1987). M/T cells receive vomeronasal nerve input from multiple glomeruli and project to the cortico-medial amygdala. They form reciprocal dendrodendritic synapses with inhibitory interneurons at two levels in the AOB. Periglomerular interneurons

provide feedback inhibition onto M/T neurons at the glomerular level, while granule cell interneurons provide feedback inhibition on their primary dendritic tree. Granule cell dendritic spines are depolarized by excitatory glutamatergic input from M/T neurons, providing lateral inhibition between M/T neurons and feedback inhibition onto the same M/T neurons via GABA release at their reciprocal synapses. Mate recognition can therefore be explained by an increased efficacy of the reciprocal synapses of the sub-population of M/T neurons responding to the mating male (Brennan *et al.* 1990; Kaba & Nakanishi 1995). This would result in a long-lasting increase in feedback inhibition onto the sub-population of M/T neurons receiving the mating male's chemosignal. The outcome would be to selectively disrupt the mating male's pheromonal signal at the level of the AOB, preventing it from reaching the hypothalamus and blocking pregnancy. Chemosignals from an unfamiliar male would activate a different sub-population of M/T neurons that were not subjected to the enhanced feedback inhibition and would convey the pregnancy-blocking signal unimpeded.

This long-standing hypothesis is consistent with recent findings that M/T neurons in the AOB are excited highly selectively in response to males of individual inbred strains (figure 3). Furthermore, M/T neurons are subjected to powerful and highly selective inhibition (Luo *et al.* 2003). This may be a consequence of the morphology of AOB M/T neurons, which differ from MOB mitral cells both in their highly branched primary dendritic tree and in lacking an extensive lateral dendritic tree. Inhibitory feedback is provided by granule cell inhibitory neurons at reciprocal synapses on the primary dendritic tree of the M/T neurons, where it is ideally located to inhibit the transmission of information from individual glomeruli (Mori *et al.* 1987).

The external plexiform and granule cell layers of the AOB receive a dense noradrenergic innervation from the locus ceruleus, which is activated by the vaginocervical stimulation at mating. This increases NA release in the AOB for at least 4 h following mating, signalling that mating has occurred (Rosser & Keverne 1985; Brennan *et al.* 1995). 6-Hydroxydopamine lesions of NA fibres or local infusions of the α -noradrenergic antagonist phentolamine into the AOB have demonstrated a vital role for NA in this learning. Both of these treatments prevent memory formation and subsequent recognition of the mating male (Rosser & Keverne 1985; Kaba & Keverne 1988). Although NA plays a vital role in olfactory learning in both the MOB and AOB, the pharmacology of its effects differs. The primary role of NA in the AOB is likely to be an α_2 mediated suppression of the GABA-mediated granule cell inhibition of M/T neurons, similar to that observed in cultured MOB neurons (Trombley & Shepherd 1992). This would be consistent with the effect of vaginocervical stimulation in causing disinhibition of spontaneous M/T neuron activity in the AOB of anaesthetized mice (Otsuka *et al.* 2001).

The NA-induced disinhibition of M/T neurons is hypothesized to result in a long-lasting enhancement of

the glutamatergic synapses onto granule cells. This can be mimicked by local infusions of the GABA_A-receptor antagonist bicuculline into the AOB, which disinhibit M/T neurons by blocking the feedback inhibition from granule cells. This widespread pharmacological disinhibition of M/T neurons leads to the formation of a non-specific memory without mating, so that all males are recognized as familiar (Kaba *et al.* 1989). In the natural memory formation process, the disinhibition of M/T cells is likely to be more selective owing to the action of mGluR₂ metabotropic glutamate receptors, which are expressed at high levels by granule cells. These mGluR₂ receptors presynaptically inhibit the release of GABA from granule cells receiving M/T input and therefore selectively disinhibit the M/T neurons responding to the mating male chemosignals (Hayashi *et al.* 1993). Hence, local infusions of the mGluR₂ agonist (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV) into the AOB, during exposure to male chemosignals, promote the formation of a selective memory for that male without mating having occurred (Kaba *et al.* 1994).

The importance of the granule cell side of the reciprocal synapses as the locus for memory induction is supported by experiments involving infusions of non-selective ionotropic glutamate antagonists into the AOB during the sensitive period after mating. These prevent memory formation, despite causing the disinhibition of M/T neurons, as they prevent ionotropic glutamatergic input to granule cells (Brennan & Keverne 1989; Brennan 1994). Memory formation can also be prevented by local infusions the calmodulin inhibitor calmidazolium into the AOB (Nakazawa *et al.* 1995), suggesting that in common with other examples of synaptic plasticity, the level of Ca²⁺ within the spine is likely to be the important trigger for memory induction. Nitric oxide is also likely to play a role in memory formation, as granule cells of the AOB have one of the highest levels of NOS of any region of the mouse brain (Bredt *et al.* 1991). Inhibition of NOS activity does not disrupt memory formation as it does in the sheep MOB (Brennan & Kishimoto 1993; Okere *et al.* 1995). However, infusions of the nitric oxide donor sodium nitroprusside into the AOB during exposure to male chemosignals result in the formation of a selective memory without mating (Okere *et al.* 1996). Therefore, although nitric oxide is not essential, it does appear to facilitate memory formation in the AOB.

The hypothesis that mate recognition involves an increase in the inhibitory feedback onto M/T neurons at reciprocal synapses is supported by investigations of the neurochemical changes in the AOB following learning (Brennan *et al.* 1995; Brennan & Binns 2005). Following mating, exposure chemosignals from the mating male resulted in a significant decrease in the ratio of excitatory to inhibitory neurotransmitters, compared with females that had received the same chemosensory exposure without mating. Consistent with this, the length of postsynaptic densities of the glutamatergic synapses on granule cells has been found to increase following learning (Matsuoka *et al.* 1997). Interestingly, this change in the glutamatergic side of the reciprocal synapse is only apparent during the first

5 days after mating, after which the length of the GABAergic postsynaptic densities onto M/T neurons increased (Matsuoka *et al.* 2004). These changes lasted until day 20 following mating but had declined by day 50, mirroring the time course of the memory for the mating male. Increases in efficacy of both the excitatory and inhibitory sides of the reciprocal synapses have the same overall effect of increasing the inhibitory feedback to M/T neurons.

As in the MOB, this tight inhibitory feedback in the AOB results in oscillatory properties of the network, which would be expected to have the effect of synchronizing M/T activity and their output to central brain areas. Oscillations in the gamma frequency range have been observed in recordings from slices of guinea pig AOB *in vitro*, which were mediated by GABAergic inhibition from local interneurons (Sugai *et al.* 1999). Changes in the inhibitory gain of the reciprocal synapses in the AOB associated with learning might therefore be speculated to involve changes in the synchrony or frequency of such network activity, which could impair the activation of neurons downstream in the cortico-medial amygdala. This might provide a subtle way of disrupting the transmission of the pregnancy-blocking signal without completely inhibiting M/T neuron activity at the level of the AOB (Taylor & Keverne 1991). Recent evidence has shown that oscillations of the local field potential recorded from the AOB do change following learning (Binns & Brennan 2005). The differential responses to chemosignals from the mating compared with an unfamiliar male, are consistent with increased granule cell inhibition of the mate's signal. Furthermore, individual neurons in the medial amygdala were found to respond more strongly to urine from an unfamiliar male than the mating male (Binns & Brennan 2005). This is consistent with the reduced c-Fos expression found in the medial amygdala in response to the mating male chemosignals compared with those from an unfamiliar male (Halem *et al.* 2001). These findings provide further support for the hypothesis that the pregnancy-blocking signal from the mating male is selectively suppressed at the level of the AOB and therefore less effective in activating neurons at the level of the corticomedial amygdala that elicit pregnancy block.

12. CENTRAL AREAS INVOLVED IN OLFACTORY RECOGNITION OF INDIVIDUALS

The importance of the corticomedial amygdala and the peptide OT in handling social information in adult animals is apparent from tests of social recognition in rodents. Many variants of this test are used in a variety of rodent species. They generally involve an adult male, which will readily investigate a juvenile or ovariectomized female that is introduced to its cage for a period of *ca* 5 min. The stimulus animal is then removed and reintroduced after a delay of *ca* 30 min. If the male is able to recognize the reintroduced animal as familiar, it shows a decreased amount of investigation compared with the first encounter with the animal or to the high level of investigation to a novel animal. In rats, this is a relatively short-term memory

that decays over a period of around an hour, so that by *ca* 120 min, following the first exposure, the reintroduced animal is investigated as intensively as a novel animal. However, in mice kept in a social environment, the memory can be maintained for a week or more (Kogan *et al.* 2000).

OT is crucial for the processing of these social odours, as OT knockout mice fail to recognize the reintroduced animal as familiar (Ferguson *et al.* 2000). However, this ability to recognize the familiar animal is rescued by infusions of OT into the medial amygdala (Ferguson *et al.* 2001). Furthermore, infusions of an OT antagonist into the medial amygdala but not the MOB of wild-type mice prevent recognition. Although these findings point to the important role played by the OT in the corticomedial amygdala in social recognition, the duration of the memory in rats can be extended to 120 min by infusing OT into the MOB (Dluzen *et al.* 2000). This effect of oxytocin is likely to be mediated by enhancing the release of NA in the MOB, which acts to prolong the duration of the memory via α -noradrenergic receptors, perhaps involving a disinhibition of mitral cell activity. Therefore, although OT release in the MOB appears to enhance social recognition, the crucial site for its action is the medial amygdala.

The importance of the corticomedial amygdala has also been shown in the context of selective maternal bonding in sheep. Medial and corticomedial amygdala infusions of lignocaine were found to disrupt lamb recognition in ewes, in that they were still maternal but no longer selective (Keller *et al.* 2004). These findings reflect the fact that the cortical and medial nuclei of the amygdala form a hub in the networks governing mammalian social behaviour. Although c-Fos expression has been found to increase in the anterior medial amygdala of hamsters in response to both conspecific and heterospecific chemosensory stimuli, the posterior medial amygdala was found to respond only to socially relevant conspecific stimuli (Meredith & Westberry 2004).

There are considerable species differences in the afferent projections to the medial amygdala, but in most mammals they receive a predominantly chemosensory input. In mice, the separate streams of vomeronasal information from anterior and posterior sub-regions of the AOB converge in completely overlapping projections to the corticomedial amygdala, the bed nucleus of the accessory olfactory tract and the bed nucleus of the stria terminalis (von Campenhausen & Mori 2000). The corticomedial amygdala is interconnected with the main olfactory pathway and is therefore a major site for the integration of V1r, V2r and main olfactory information (figure 4). Moreover, the high levels of expression of steroid receptors in this area also make it sensitive to hormonal state. Consequently, whereas VNO lesions disrupt mating behaviour in sexually naive hamsters, such lesions are ineffective following sexual experience as mating behaviour can be sustained by the main olfactory system. Thus, odours conveyed by the main olfactory system may become associated with vomeronasal stimuli, at the level of the medial amygdala, and may

subsequently become sufficient to drive the same behavioural response (Meredith 1998).

The main olfactory system also sends projections to the hippocampus via the entorhinal cortex and to the frontal cortex. These regions, together with the piriform cortex, show enhanced expression of genes associated with neural plasticity, such as brain-derived nerve growth factor (BDNF) and its receptor *trk-B* following the development of lamb recognition in sheep (Broad *et al.* 2002). A number of studies in rodents have reported that formation of long-term social and non-social recognition memories requires an intact hippocampus or pathways projecting to it (Maaswinkel *et al.* 1996; Kogan *et al.* 2000). However, individual recognition by male hamsters in the context of the Coolidge effect (i.e. ability to distinguish a novel from a familiar female) has been found to be disrupted by lesions of the perirhinal and entorhinal cortices but not the hippocampus (Petrulis & Eichenbaum 2003). Neurons in the entorhinal cortex of hamsters have been reported to be responsive to individual social odours (Petrulis *et al.* 2005). Furthermore, temporary inactivation of the entorhinal cortex using the anaesthetic tetracaine prevents selective recognition of lambs by maternal ewes but without disrupting maternal behaviour. However, after consolidation of the memory, similar inactivation of the entorhinal cortex does not prevent recognition (Sánchez-Andrade *et al.* 2005). There is enhanced activation (*c-Fos* protein expression) in the frontal cortex of maternal ewes in response to their own lambs after memory consolidation (Keller *et al.* 2004). However, this may have more to do with strengthening of perception-action patterns, since temporary inactivation of the medial prefrontal cortex has been found to disrupt a maternal ewe's ability to show selective recognition, in terms of her motor response (aggression towards strange lambs), but not to prevent memory formation *per se* (Broad *et al.* 2002).

While the role of central projections of the main olfactory system in social recognition memory still requires further clarification, it is clear at this stage that multiple systems downstream of the olfactory bulbs and piriform cortex are initially involved, including the corticomedial amygdala, entorhinal, perirhinal and frontal cortex, and possibly the hippocampus as well. Post-consolidation maintenance of the recognition memory seems to become restricted to the olfactory bulb and piriform cortex, and the links between the frontal cortex and systems controlling motor and motivational responses. In contrast, in the vomeronasal system only the AOB seems to be of importance in all phases of the social recognition process. However, it seems probable that with recognition systems involving both vomeronasal and main olfactory systems, AOB projections to the corticomedial amygdala may also be important.

13. CONCLUSIONS

Mammals produce complex mixtures of social chemosignals that are still poorly understood. Single molecules can act as pheromones exerting specific control over physiology and behaviour, while genetically determined cocktails of molecules enable the

recognition of individuals. There is no single means of signalling this individuality information, but rather a variety of complementary systems, based on highly polymorphic genetic loci that are adapted for use in different behavioural contexts, such as mate choice or territorial marking. The vomeronasal system has long been thought of as specialized to sense pheromonal signals using labelled line systems to engage relatively simple stereotyped responses. However, this view has been rethought in light of the evidence that the main olfactory system also conveys highly specific pheromonal information via labelled lines. Conversely, the vomeronasal system is able to convey information about MHC individuality as effectively as the main olfactory system.

So can the roles of the main olfactory and vomeronasal systems be neatly summarized? Maybe not presently, but there do at least seem to be common themes related to the means of stimulus access. The main olfactory system is specialized to sense volatile molecules present in the nasal airstream and therefore at a distance. In contrast, the vomeronasal system is specialized to pump in relatively non-volatile stimuli following direct contact with the stimulus source. Of course, individual chemosignals may be able to stimulate both systems, such as the mouse urinary volatiles that when dissociated from MUPs can be sensed as volatile signals by the main olfactory system, but that can be taken up into the VNO when tightly bound by MUPs. The two systems have complementary roles in mediating the effects of social chemosignals. Volatile attractant chemosignals, sensed via the main olfactory system, drive more direct investigation that enables non-volatile cues to be taken up and analysed by the vomeronasal system.

The recognition of individuals depends on learning their chemosensory profile, which involves changes at all levels of neural processing. The involvement of noradrenaline release in the olfactory bulb in inducing sensitive periods is a common feature of olfactory learning across different species and different behavioural contexts. Changes in the inhibitory control of the mitral cell projection neurons following learning may be a common feature to differentiate odour representations that need to be reliably discriminated. However, olfactory learning must also involve changes in odour processing at more central levels, including the pivotal role of the corticomedial amygdala in integrating social information from different sensory systems and hormonal states. Finally, although the sense of smell is often relegated to a minor role in human social communication, evidence is starting to accumulate that odours have not lost the ability to influence human behaviour. The extent to which social odours play a role in modern human society is an open but intriguing question for future research.

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