

Complementary Roles of the Main and Accessory Olfactory Systems in Mammalian Mate Recognition

Michael J. Baum¹ and Kevin R. Kelliher²

¹Department of Biology, Boston University, Boston, Massachusetts 02215;
email: baum@bu.edu

²Department of Biological Sciences, University of Idaho, Moscow, Idaho 83844;
email: kelliher@uidaho.edu

Annu. Rev. Physiol. 2009. 71:141–60

First published online as a Review in Advance on
September 25, 2008

The *Annual Review of Physiology* is online at
physiol.annualreviews.org

This article's doi:
10.1146/annurev.physiol.010908.163137

Copyright © 2009 by Annual Reviews.
All rights reserved

0066-4278/09/0315-0141\$20.00

Key Words

pheromone, olfactory bulbs, medial amygdala, sex behavior, major histocompatibility complex

Abstract

We review studies conducted in mouse and ferret that have specified roles of both the main and the accessory olfactory nervous systems in the detection and processing of body odorants (e.g., urinary pheromones, extraorbital lacrimal gland secretions, major histocompatibility complex peptide ligands, and anal scent gland secretions) that play an essential role in sex discrimination and attraction between males and females leading to mate choice and successful reproduction. We also review literature that compares the forebrain processing of inputs from the two olfactory systems in the two sexes that underlies heterosexual partner preferences. Finally, we review experiments that raise the possibility that body odorants detected by the main olfactory system contribute to mate recognition in humans.

Main olfactory

system: composed of the MOE that contains OSNs that project to the MOB. MOB mitral cells project to olfactory cortex and amygdala and include a recently identified projection to the medial (vomeronasal) amygdala

Accessory olfactory

system: composed of the VNO neuroepithelium, which contains VSNs that project to the AOB. AOB mitral cells project to nuclei in the medial (vomeronasal) amygdala that in turn project to the BST, MPA, and VMH

Pheromone: a social chemosignal produced by one member of a species that regulates the endocrine function, behavior, or development of a conspecific (105). Signaler pheromones are chemosignals that specify reproductive status so as to contribute to mammalian mate recognition (106)

INTRODUCTION

The olfactory brain can be segregated into different classical and nonclassical components. Classical olfactory processing occurs through olfactory receptor neurons located in the main olfactory epithelium (MOE). In addition to the classical pathway, chemical cues that enter the nose can be detected by sensory neurons within the vomeronasal organ (VNO) epithelium, the septal organ, and the Grueneberg ganglion as well as by free nerve endings in the trigeminal system. Each of these larger systems comprises subsystems defined by differences in receptor mechanisms, signal transduction pathways, and physical location within the nasal cavity. To date, most research concerning mechanisms of mate recognition has focused on the detection of chemosensory cues by either the VNO neuroepithelium or, more recently, the MOE. Nothing is known about the role of the other above-mentioned olfactory systems in mate recognition. Therefore, this review concentrates on the roles of the main and the accessory olfactory systems in mammalian mate recognition. We focus on data for the mouse and ferret (species in which we have both conducted experiments) as well as for the human.

THE MAIN OLFACTORY SYSTEM

In all vertebrates the main olfactory system detects and processes the vast majority of chemical cues that enter the nasal cavity. This system is a chemical categorizer, first detecting a chemical and then cataloging it in the cortex for later recall. The role of the main olfactory system in the detection and processing of chemosensory cues that trigger innate, hard-wired physiological, or behavioral responses is still somewhat controversial but has recently received considerable attention (1–3).

Chemical cues are detected in the MOE after they bind to G protein-coupled receptors on the cilia of olfactory sensory neurons (OSNs). OSNs project their axons to the main olfactory bulb (MOB), where OSNs expressing a single

type of olfactory receptor target one or two individual glomeruli (4, 5). Within the MOB, mitral cells that extend an apical dendrite to a single glomerulus in turn extend axons via the lateral olfactory tract to central brain regions including the anterior olfactory nucleus (AO), anterior cortical amygdala (ACo), olfactory tubercle (Tu), piriform cortex (Pir), and entorhinal cortex (Ent) (**Figure 1**). Recent results (details below) also show that MOB mitral cells project to the anterior medial amygdala (Me), where signals may be integrated with inputs coming from the VNO/accessory olfactory system. Inputs from the vomeronasal amygdala, which includes the Me and the posterodorsal Me (MePD), have potential access to limbic targets including the bed nucleus of the stria terminalis (BST), the medial preoptic area (MPA), and the ventromedial hypothalamus (VMH) (6, 7). Thus, odors detected by the MOE may activate neural circuits involved in cortical as well as limbic processing. Until recently, it was thought that the MOE detects only airborne volatile odors. In fact, murine OSNs can also respond to small nonvolatile major histocompatibility complex (MHC) peptide ligands that lead to changes in behavior (8) (more details below).

THE ACCESSORY OLFACTORY SYSTEM

Odorants emitted from other conspecifics (including classically defined pheromones) that are dissolved in nasal mucous gain access to the VNO via an active pumping mechanism (9). These nonvolatile odorants, in turn, bind G protein-coupled receptors located on vomeronasal sensory neurons (VSNs). VSNs extend their axons to the accessory olfactory bulb (AOB) (**Figure 1**). AOB mitral cells send their axons to the vomeronasal amygdala, which in turn, sends projections to several hypothalamic nuclei either directly or via the BST (7, 10). Choi et al. (11) have suggested that interactions between subnuclei of the Me and their projections to the VMH are important for the differential regulation of

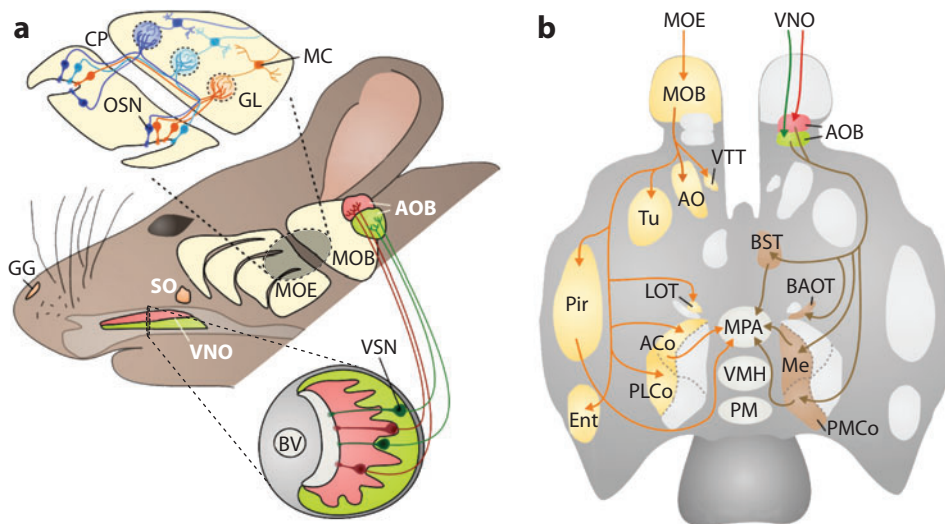


Figure 1

Diagrams showing the projection pathways of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). (a) Olfactory sensory neurons (OSN) in the MOE project through the cribriform plate (CP) to glomeruli (GL) in the main olfactory bulb (MOB). OSNs (purple, blue, and orange) expressing the same olfactory receptor genes converge on single GL in the MOB. MOB mitral cells (MC) extend an apical dendrite into abutting GL and extend axons to the forebrain. Vomeronasal sensory neurons (VSN) extend axons from the VNO to the accessory olfactory bulb (AOB). VSNs in the apical zone (red, left) of the VNO project to the rostral AOB; VSNs in the basal zone (green, right) of the VNO project to the caudal AOB. Additional abbreviations used: BV, blood vessel; GG, Gruenberg ganglion; SO, septal organ. (b) Forebrain projection pathways of the MOB (orange, left) and the AOB (brown, right). Abbreviations used: VTT, ventral tenia tecta; AO, anterior olfactory nucleus; Tu, olfactory tuber; Pir, piriform cortex; Ent, entorhinal cortex; LOT, nucleus of the lateral olfactory tract; ACo, anterior cortical amygdala; PLCo, posterolateral cortical amygdala; BST, bed nucleus of the stria terminalis; BAOT, bed nucleus of the accessory olfactory tract; Me, medial amygdala; PMCo, posteromedial cortical amygdala; MPA, medial preoptic area; VMH, ventromedial hypothalamus; PM, premammillary nucleus. Adapted from Reference 104 with permission.

reproductive versus defensive behaviors. It has been presumed that the VNO preferentially responds to heavy-molecular-weight, nonvolatile molecules. However, VSNs respond in vitro to both volatile and nonvolatile chemosensory stimuli (12, 13) (more details are given, below).

THE MAIN OLFACTORY SYSTEM DETECTS VOLATILE ODORS IMPORTANT FOR MATE RECOGNITION IN MICE

Most common odorants activate a subset of MOE neurons that express one of up to ~1000

olfactory receptor genes (14). This same family of odorants receptors is also expressed in spermatozoa and may guide sperm to eggs that release chemical cues that bind to these receptors (15). The same family of olfactory receptors, expressed in the MOE, plays an important role in sex discrimination, sexual attraction, and mate recognition in female as well as in male mice.

An early study (16) showed that female mice were significantly more attracted to volatile odors emitted by urine of gonadally intact versus castrated males. This preference was displayed by females that had never had mating experience and was irrespective of stage of their

estrous cycle. In a subsequent study (17), virgin female mice, regardless of stage of the estrous cycle, again preferred to investigate volatile odors emitted from gonadally intact versus castrated males. In addition, two volatile compounds isolated from gonad-intact male mouse urine, thiazoline and dehydrobrevicomine, when mixed with castrate male urine, made this urine as attractive to females as was gonad-intact male urine. Females were not attracted to these compounds unless they were dissolved in castrate male urine. Female mice preferred to approach urinary stimuli from normal males as opposed to males from which the preputial glands had been surgically removed (18). Two constituents of preputial gland secretions, α and β farnesene, when added to water, were preferentially approached by virgin female mice over water alone (19). Although volatile odors can gain access to the VNO, and these particular urinary compounds activate VSNs (13), there is no clear evidence that the accessory olfactory system alone can mediate mate recognition in response to these volatile odors.

One study reported that a highly volatile constituent of male urine, (methylthio)methanethiol (MTMT), enhanced the attractiveness of castrate male urine to sexually experienced female mice (20). In addition, females preferred to approach MTMT dissolved in water versus water alone. This study also identified a relatively small number of mitral cells in the ventral and lateral portions of the MOB of female mice that were electrically activated by urinary volatiles from both sexes. Approximately one-third of these urine-responsive neurons were also activated by MTMT. These experiments extend other studies (20–22) showing that volatile urinary odors from males with different MHC haplotypes stimulated *c-fos* expression in the periglomerular cells of different clusters of glomeruli located in the ventral MOB of female mice. Other investigators (23) also used functional magnetic resonance imaging (fMRI) to show that male urinary volatiles activated the ventral MOB of female subjects.

DAMAGE TO THE MAIN OLFACTORY EPITHELIUM DISRUPTS MATE RECOGNITION IN MICE

Several studies in which the function of the MOE was disrupted point to a critical role of the main olfactory system in heterosexual mate recognition in female mice. Thus, females given zinc sulfate lesions of the MOE lost their preference to investigate soiled bedding from a gonadally intact versus a castrated male (24). More recently (25), this result was extended to show that females with zinc sulfate lesions of the MOE lost both (*a*) their preference to approach volatile odors emitted from anesthetized, gonadally intact males versus castrated males and (*b*) their preference to approach urinary volatiles emitted from intact males. Likewise, selective destruction of the MOE in transgenic female mice, in which the expression of a bacterial nitroreductase gene was linked to the olfactory marker protein, eliminated subjects' ability to locate urinary odors from males (26). In another study (27) virgin female mice were exposed for seven days to volatile odorants emitted from the bedding of a dominant, as opposed to a subordinate, male. The females previously exposed to odors from a dominant male later preferred to approach volatile odors emitted from a dominant versus a subordinate male. In addition, there was significantly more incorporation of newly born neurons into the granule cell layer of the MOB in females previously exposed to dominant-male odors than in naive females. These results show that the main olfactory system plays an essential role in identifying volatile odors from potential male mating partners and in motivating females to seek out these conspecifics.

Sexually naive adult male mice preferred to approach urinary volatiles from females (estrous cycle stage not monitored) as opposed to water, whereas males with mating experience strongly preferred to approach urinary volatiles from females as opposed to either water or male urinary volatiles (28). Thus, in male mice, as in females, the ability to detect and

prefer opposite-sex urinary volatiles appears to be hard wired, without any requirement for prior mating experience. Zinc sulfate destruction of MOE neurons caused sexually experienced male mice to stop displaying a preference to approach either volatile body odors or odors emitted from soiled bedding of an estrous versus an ovariectomized female (29). The VNO remained intact and able to convey input to the AOB in males with MOE lesions. Yet in the absence of a functional MOE, males showed no preference to seek out opposite-sex urinary volatiles. As stated above, volatile body odors can be detected by VNO sensory neurons (12, 13, 30); however, to date there is no clear evidence that odors detected in this manner motivate approach to opposite-sex conspecifics.

Volatile odorants that influence mate recognition in both sexes initially bind to olfactory receptor proteins expressed in MOE neurons that, in turn, activate a cAMP signaling pathway (31). In one study (32) male mice with a null mutation of the type 3 adenylyl cyclase ($AC3^{-/-}$) showed neither behavioral (approach) nor MOE electrophysiological responses to urinary volatiles or to a putative mouse pheromone, 2-heptanone. Likewise, these $AC3^{-/-}$ males failed to display aggressive behavior toward a male intruder or sexual behavior toward a receptive female. A targeted null mutation of the cyclic nucleotide-gated channel subunit A2 ($CNGA2$) also eliminated the preference of sexually naive male mice to investigate female versus male urine (33). Like $AC3^{-/-}$ males, $CNGA2$ mutant males failed to display aggressive behavior toward a male intruder or mating behavior with an estrous female. Whereas $CNGA2$ null mutant males failed to prefer female over male urinary odors, mice with a null mutation of the $CNGA2$ retained MOE electrical responsiveness to several putative pheromones, including 2-heptanone (34). Also, in $CNGA2$ null mutant mice, pheromonal odors activated (stimulated c-fos expression in) a small set of MOB necklace glomeruli abutting the AOB. These necklace

glomeruli are innervated by MOE neurons that express the cone subunit of the CNG channel ($CNGA3$) instead of $CNGA2$ and use a cGMP instead of a cAMP signaling system. In addition, another set of glomeruli located primarily in the ventral MOB remained responsive to putative pheromones in $CNGA2$ null mutant mice. Neurons innervating these glomeruli appear to use a phospholipase C (PLC) signaling pathway, which may involve the transient receptor potential channel M5 ($TRPM5$) (35). In male mice, as in females, exposure to urinary volatiles of male and female conspecifics activated (stimulated c-fos expression in periglomerular cells) overlapping but distinct clusters of glomeruli located in the ventral MOB (36). Finally, a new family of MOE odor receptor genes that encode trace amounts of volatile amines [trace amine-associated receptors (TARRs)] has been characterized in mice (37). HEK293 cells cotransfected with expression vectors encoding murine TAARs and a cAMP reporter gene (CRE-SEAP) were activated by several different volatile amines as well as by volatiles from adult male urine, but not by volatiles from castrate male or from female urine.

THE MAJOR HISTOCOMPATIBILITY COMPLEX INFLUENCES MURINE MATE CHOICE VIA THE MAIN OLFACTORY EPITHELIUM

Although sex discrimination and attraction to the opposite sex play critical roles in mate recognition, identification of individuals' immune status based on characteristics of body odorants also makes a contribution. Thus, as first shown by Yamazaki et al. (38), the adaptive immune system is directly involved in mate preference. These authors found that mice could discriminate between individuals with different MHC haplotypes and preferred to approach opposite-sex individuals with divergent MHC haplotypes. In the intervening 30 years, odor-based MHC mate preferences have been observed in many species, including

humans (39, 40). The potential benefits of a mate preference based on MHC status include (a) inbreeding avoidance, leading to increased reproductive fitness; (b) increased MHC heterozygosity, resulting in offspring that are more resistant to multiple infections; and (c) provision of a moving target against pathogen escape, thereby decreasing the possibility that a pathogen may permanently avoid MHC immune recognition (41). If one or all of these hypotheses hold, then the recognition of individual MHC genotype likely plays an important role in mate preference. Studies in mice using a Y-maze or an olfactometer suggested that volatile urinary odors mediate discrimination of and preference for individuals with different MHC genotypes (38). Hypotheses about how the adaptive immune system produces a unique odor type have ranged from the breakdown products of the MHC class I molecules being excreted in urine to different patterns of body microflora producing unique patterns and concentrations of volatile body odors (42). Recently, Boehm & Zufall (39) proposed that the same peptide ligands that are used by the MHC class I molecules to recognize self versus nonself can also be detected by sensory neurons in the MOE and VNO. Although these peptide ligands are presumably nonvolatile molecules, they can activate OSNs in the MOE, leading male mice to prefer female urinary odors on the basis of the presence of peptides dissimilar to their own MHC genotype (8).

A COMPLEMENTARY ROLE FOR THE ACCESSORY OLFACTORY SYSTEM IN MURINE MATE RECOGNITION

Early anatomical data demonstrating a projection pathway from the VNO to the AOB (10, 43) and on to the Me and hypothalamus (see **Figure 1**) (7, 44) raised the possibility that this accessory olfactory system is the primary mediator of pheromonal actions on aspects of reproduction such as sex discrimination, attraction, and mate recognition (45). Early studies

(46, 47) showed that the ability of sexually naive male mice to display ultrasonic vocalizations after nasal contact with urine or soiled bedding from females was eliminated after VNO inputs to the AOB were destroyed. Males that were exposed to female odors on several occasions prior to VNO removal showed somewhat smaller deficits in ultrasonic vocalizations, implying that MOE inputs could partially compensate for the absence of VNO inputs (47). This view was further supported (48) by the observation that the ability of sexually experienced male mice to display ultrasonic vocalizations in response to female odors was partially attenuated by disruption of either main or accessory olfactory signaling but was totally eliminated by disruption of both olfactory systems. These results established the role of VNO signaling in the display of male-typical social behaviors toward same-sex conspecifics. However, none of this early work specifically identified a role of the VNO in males' ability to discriminate odor cues on the basis of conspecifics' sex or endocrine status.

DISRUPTION OF VOMERONASAL ORGAN SIGNALING IMPAIRS MOTIVATIONAL ASPECTS OF MATE RECOGNITION BUT NOT SEX DISCRIMINATION

Behavioral experiments exploring accessory olfactory system functions languished during the 1990s. However, the discovery (49) that the type 2 transient receptor potential channel (TRP2C) plays an essential role in the generation of action potentials in VNO sensory neurons following the detection of pheromonal stimuli via VR1 and/or VR2 receptors motivated the production of mice with a null mutation of this cation channel. VNO neurons of TRP2C mutant mice showed greatly reduced electrical responses to urine (50). Moreover, TRP2C mutant males displayed no aggressive behavior toward male intruders. These results supported earlier findings that VNO removal eliminated aggressive behavior

otherwise displayed by VNO-intact males toward intruder males (51, 52). Instead of aggressive behavior, TRP2C males showed significantly more mounting behavior than did wild-type or heterozygous TRP2C mutant control males toward a male intruder. Finally, TRP2C mutant males indiscriminately displayed ultrasonic vocalizations toward female and male conspecifics, whereas control males vocalized only in the presence of a female. An independent study (53) obtained a similar set of results, including the observation that TRP2C male mice displayed normal mating behavior with an estrous female. However, these mutant males also attempted to mount male intruders instead of attacking them. More recently, it was reported (54) that female TRP2C mutant mice indiscriminately mounted and produced ultrasonic vocalizations toward either a male or a female intruder (presented on separate occasions). By contrast, wild-type and heterozygous TRP2C control females displayed low levels of these two types of behavior toward an intruder female. Wild-type females from which the VNO was surgically removed also showed mounting and ultrasonic vocalization toward a female intruder. All three studies (50, 53, 54) concluded that VNO signaling plays a critical role in sex discrimination and, by inference, in mate recognition.

Surprisingly, none of the three studies that characterized the social behaviors of TRP2C mutant male and female mice assessed subjects' ability to discriminate body odorants from males versus females or to show a preference to investigate urinary odors from animals of the two sexes in different endocrine conditions. In a related study (55), surgical removal of the VNO from sexually naive mice failed to disrupt their ability to discriminate volatile odors from males versus estrous females in habituation/dishabituation tests given in the home cage. VNO removal also failed to disrupt males' sexual behavior in tests with an estrous female. Furthermore, when presented simultaneously with an estrous female and a castrated

male (with urine from a gonadally intact male swabbed on the castrated male's back), VNO-lesioned males and VNO-intact males showed an equivalent preference to mount the estrous female, although both groups directed a minority (~35%) of their mounts toward the castrated male. The only significant behavioral effect observed was that VNO removal eliminated the preference of both sexually naive mice and sexually experienced male mice to make nasal contact with urine from an estrous female versus a gonadally intact male. In another study (56) sexually naive male mice with intact versus lesioned VNOs showed an equivalent preference to make nasal contact with estrous urine versus water in the two goal boxes of a Y-maze. However, when the choice was between investigating male versus estrous female urine, VNO-intact controls spent significantly more time investigating female urine, whereas VNO-lesioned males spent an equivalent time investigating these two stimuli. Likewise, sexually naive VNO-intact females preferred to approach and nasally contact soiled bedding or urine from a male versus an estrous female in Y-maze tests, whereas VNO-lesioned females did not show such a preference (57). Both VNO-intact females and VNO-lesioned females preferred to approach volatile body odors (anesthetized stimulus animals in a goal box) or volatile urinary odors from a male as opposed to an estrous female when direct nasal access to the stimuli was prevented in Y-maze tests. Presumably, VNO signals generated when males or females come into direct nasal contact with opposite-sex urinary odorants motivate mice to remain in close proximity to these reproductively relevant stimuli. Contrary to the claims of papers describing the phenotype of TRP2C null mutant mice (50, 53, 54), there is no evidence that the accessory olfactory system plays an obligatory role in animals' ability to discriminate the sex of conspecifics on the basis of their pheromone profile. Instead, evidence already reviewed strongly implicates the main olfactory system in this critical first step in mate recognition.

DETECTION AND PROCESSING OF PHEROMONES BY THE ACCESSORY OLFATORY SYSTEM IS SEXUALLY DIFFERENTIATED IN MICE

Several studies that have directly monitored the responses of murine VNO neurons to pheromones suggest that subjects' sex and endocrine status modulates VNO responsiveness. In one study (12) action potentials of VNO neurons were monitored *in vitro* with a flat array of 61 extracellular electrodes. Many VNO neurons from each sex responded selectively to different dilutions of male versus female urine, regardless of the individual identity of donors of each sex. In two other studies (58, 59) the expression of immediate early genes (IEGs) *c-fos* or *EGR-1* were used to monitor the activation of murine VNO neurons in response to pheromones emitted from soiled bedding. Both studies revealed that male odorants stimulated significantly higher IEG expression in the female than in the male VNO, and showed that this effect was upregulated by administering estradiol to gonadectomized male and female subjects. In addition, castrated male subjects showed maximal IEG induction to female bedding odorants, and this effect was further enhanced by testosterone. In another study (60) a male mouse-specific peptide (exocrine gland-secreting peptide 1) isolated from the extra-orbital lacrimal gland stimulated *c-fos* expression in sensory neurons located in the basal zone of the female's VNO. Pheromonal cues, detected by the murine VNO, activate a projection circuit that includes the AOB, the Me, and other targets in the hypothalamus. Exposure of male mice to soiled bedding from either male or female conspecifics stimulated IEG expression in the AOB (58, 59, 61). Likewise, nasal application of whole male urine or the low-molecular-weight (but not high-molecular-weight) component of male urine to female mice stimulated *c-fos* expression in the AOB (57). VNO removal attenuates AOB *c-fos* responses in both sexes (55, 57).

Male urine stimulates *c-fos* expression in AOB mitral cells that project to the Me, as

shown by retrograde labeling after cholera toxin B (CTB) injections into the Me (62). Interestingly, CTB-Fos-labeled AOB mitral cells were significantly greater in gonadectomized, estradiol-primed females than in males. In another study (63) electrophysiological recordings were made from putative AOB mitral cells in freely behaving mice. These cells were only activated when subjects made direct physical contact with the bodies of stimulus mice—especially with the ano-genital or head/face regions, which are sources of urinary pheromones and exocrine gland-secreting peptide 1. Individual AOB mitral cells were preferentially activated by odors from one sex or mouse strain, raising the possibility that these cells respond to the selective activation of a single type of VNO receptor.

The ability of male body odors to stimulate *c-fos* expression in the Me, MePD, BST, and MPA is sexually differentiated; only females are responsive (59). This sexually differentiated response appears to be organized by perinatal androgen exposure in the male. Tfm (androgen receptor mutant) male mice showed a female-typical profile of Fos expression in these sites (64). By contrast, neonatal treatment of female mice with the androgen dihydrotestosterone defeminized (eliminated) the later ability of male bedding odors to induce *c-fos* responses in the BST and MPA (65).

INTEGRATED FUNCTIONING OF THE MAIN AND ACCESSORY OLFATORY SYSTEMS IN MICE

Most investigators who study murine pheromone signaling accept the dogma that AOB mitral cells project to the vomeronasal medial amygdalar nuclei (Me and MePD), whereas a subset of MOB mitral cells projects to the olfactory amygdala [including the ACo and posterolateral cortical amygdala (PLCo)] (10, 43). Volatile odorants that have the potential to function as pheromones (e.g., MTMT and TAAR ligands) are preferentially detected by the MOE sensory neurons and are processed via the MOB. In contrast,

nonvolatile pheromones [e.g., major urinary proteins (MUPs)] are dissolved in nasal mucus and gain access to the VNO lumen, where they activate VNO sensory neurons leading to information processing in the AOB. Several recent studies suggest that volatile pheromones derived from opposite-sex conspecifics selectively activate the AOB following their detection by the MOE and processing in the MOB. An early indication that such signaling may occur came from the observation (23) that in female mice a blood-oxygen-level-dependent (BOLD) fMRI signal was induced in the MOB immediately after exposure to male urinary volatiles whereupon a peak increase in the BOLD signal occurred in the females' AOB several minutes later. This result raises the possibility that volatile urinary odors, initially detected by the MOE, lead to an activation of the AOB. A study (36) confirmed this hypothesis, showing that ovariectomized female mice (given no replacement hormone) showed an increase in AOB mitral and granule layer c-fos expression in response to urinary volatiles from male, but not female, conspecifics. Interestingly, this response was sexually dimorphic in that castrated male mice (again, given no hormones) showed an increase in AOB c-fos expression in response to urinary volatiles from an estrous female, but not a male conspecific. In both sexes these respective odor-induced AOB c-fos responses were blocked by prior zinc sulfate lesioning of the MOE. Additional experiments showed that VNO-AOB signaling remained viable in females previously given intranasal zinc sulfate (nasal application of male urine stimulated significant AOB c-fos responses in these lesioned females). Thus, the absence of an AOB response to urinary volatiles in zinc sulfate-lesioned mice was likely not an artifact of inadvertent damage to the VNO.

There is evidence (66) that centrifugal inputs to the murine AOB originate in the Me and in the bed nucleus of the accessory olfactory tract (BAOT). A recent study (K.M. Martel & M.J. Baum, unpublished results) replicated this anatomical result after injection of the retrograde tracer CTB into the AOB.

In female mice that received AOB injections of CTB, the percentage of retrogradely labeled Me neurons that coexpressed c-fos was significantly increased in animals exposed to urinary volatiles from a male mouse as opposed to a female mouse, a cat, or food odors. This outcome is illuminated by other recent findings (N. Kang, M.J. Baum & J.A. Cherry, unpublished results) showing that a subset of mitral cells, located in the medial and ventral-central MOB, projects axons directly to the Me and MePD, as opposed to the more conventional MOB mitral projection to the ACo and PLCo. Again, after CTB injections into the Me of estrous female mice, exposure to urinary volatiles from male, but not female, conspecifics stimulated CTB/c-fos double labeling of MOB mitral cells that project to the Me. This input is perhaps responsible for the activation of the AOB seen in response to opposite-sex urinary volatiles. The functional significance of AOB activation that originates with MOE signaling remains unclear. Whether MOB-mediated activation of the AOB is a retrograde signaling mechanism or a means whereby the MOB gates the responsiveness of the AOB to opposite-sex odors remains to be determined. However, a recent study (67) showed that sexually naive male mice investigated volatiles emitted from an estrous female as opposed to those from a male when the stimuli were presented in sequence (regardless of order of presentation). A striking reduction in investigation of estrous female, but not of male, urinary volatiles was seen in male mice given either complete or incomplete bilateral lesions of the AOB. As in the case of VNO lesions (55), AOB lesions failed to disrupt males' mating behavior in tests with an estrous female.

These results suggest that the AOB/accessory olfactory system enhances the salience of the volatile opposite-sex pheromones in sexually naive animals, thereby increasing their motivation to investigate these cues. Some additional support for this view is provided by the observation (36) that female subjects showed enhanced c-fos responses in the MPA and nucleus accumbens shell in

response to urinary volatiles from male, but not female, conspecifics. This latter region is a projection target of dopamine neurons in the ventral tegmental area that have been implicated in learning and reward functions.

SEXUALLY DIMORPHIC ODOR CUES CONTRIBUTE TO MATE RECOGNITION IN THE FERRET

An early study (68) of feral ferrets showed that males and females establish separate territories in which contact between adults of the two sexes occurs only during the breeding season. The absence of direct social interaction between male and female ferrets raises the possibility that olfactory cues play an important role in ferrets' ability to find a mate. In this respect we would expect to find that scent marks left behind by both male and female ferrets communicate information about conspecifics' sex and reproductive fitness. Clapperton and coworkers (69) used gas chromatography to demonstrate that the composition of anal scent gland secretions is sexually dimorphic in ferrets. Zhang et al. (70) later confirmed and extended these findings with the observation that the composition of volatile compounds contained in urine from male versus female ferrets also differed significantly. Another classic study (71) showed that ferrets display a repertoire of scent-marking behaviors, including urogenital wiping and flank rubbing. Observations of gonadally intact ferrets showed that during the spring breeding season males displayed significantly higher levels of both types of scent-marking behavior than did females. More recently Chang et al. (72) found that after adult gonadectomy and in the absence of any hormone replacement, male ferrets displayed significantly more urogenital wipes than did female ferrets. Furthermore, administration of either estradiol or testosterone augmented the display of this wiping behavior, again with males still displaying significantly higher levels of the behavior than did females. Taken together, these data suggest that some combination of anal scent gland and/or urinary odorants is available to

communicate gender as well as breeding (endocrine) status of ferrets.

Clapperton and coworkers (69) provided some of the earliest evidence that ferrets' anal scent gland odorants attract the opposite sex. Thus, in Y-maze tests gonadally intact male and female ferrets were more likely initially to approach anal scent gland odorants from the opposite sex. More recently, Kelliher & Baum (73) monitored the amount of time that subjects spent in proximity to volatile odors emitted from anesthetized estrous female versus breeding male ferrets that were placed in the two arms of an air-tight Y-maze. First, in the absence of previous mating experience, gonadectomized male and female ferrets given either estradiol benzoate (EB) or testosterone propionate (TP) treatments showed no preference for opposite-versus same-sex odors. However, when subjects were retested after receiving mating experience, ovariectomized females treated with either EB or TP spent significantly more time in the vicinity of the Y-maze goal box containing an anesthetized male versus the female stimulus, whereas castrated males given EB or TP preferred the goal box containing an anesthetized female versus the male stimulus. The outcome of this study was corroborated in another study (72) that assessed the time that sexually experienced ferrets spent investigating blocks of wood that had previously been soiled (scent marked) by an estrous female versus a breeding male ferret. Gonadectomized male and female ferrets given no sex hormones showed equal preference for briefly investigating both wood blocks soiled by a male and those soiled by a female. However, following EB or TP treatment, ovariectomized females preferred to investigate blocks soiled with male odors. By contrast, castrated males given TP preferred to investigate blocks soiled with female odors. At first blush, these results imply that ferrets prefer to approach body odors from opposite-sex conspecifics only after they have received mating experience. However, in the study by Kelliher & Baum (73), in significantly more Y-maze trials sexually naive subjects (ovariectomized females treated with EB; castrated males treated with

TP) preferred to approach volatile odors emitted by an anesthetized opposite-sex ferret as opposed to volatile odors emitted by same-sex animals. (Subjects were placed in a start box prior to each trial in the maze.) This same method was used with sexually experienced male and female ferrets to assess the contribution of anal scent gland odorants to subjects' preference to approach opposite-sex body odorants (74). Gonadectomized male (TP-treated) and female (EB-treated) subjects preferred to approach the Y-maze goal box that emitted volatile odorants from opposite-sex anal scent glands; however, these same subjects persisted in their preference to approach a goal box containing an opposite-sex conspecific even if that ferret had been surgically descented. Thus, there appears to be a hard-wired preference among ferrets to approach opposite-sex volatile body odors that likely include a sexually dimorphic suite of compounds emitted from both urine and anal scent glands and/or additional putative odor sources such as lacrimal or skin glands. This preference is only revealed when adult ferrets have sex hormones circulating, and the preference is enhanced in both sexes after they receive mating experience.

THE MAIN OLFACTORY SYSTEM CONTROLS MATE RECOGNITION IN THE FERRET

A series of studies asked whether the main or the accessory olfactory systems play a preferential role in any contribution that pheromones from conspecifics make to heterosexual mate recognition. To the extent that ferrets, like other mammalian species, prefer to approach purely volatile odorants from opposite-sex conspecifics, it appears that any olfactory contribution to mate recognition may depend initially on odor detection and processing via the main olfactory system. Indeed, nasal contact with soiled bedding from opposite-sex conspecifics caused a significant stimulation of c-fos expression in MOB granule cells but not in the cell layer of the AOB (75). In a subsequent study (76) exposure to soiled bedding

from estrous female ferrets again stimulated c-fos expression on the MOB granule cell layer of gonadectomized male and female ferrets, and the magnitude of this response significantly increased after TP administration. Again, there was no odor-induced stimulation of AOB Fos responses in either male or female subjects. When gonadectomized, TP-treated male and female ferrets were exposed to soiled male bedding, and there was, again, a significant induction of Fos expression in MOB granule cells, but not in the AOB. In a parallel set of results (77), male and female ferrets taken at postnatal day 15 were placed in physical (nasal) contact with their anesthetized mother following a 4-h period of separation. Again, Fos responses were reliably seen in the MOB granule cell layer, but not in the AOB. In a final study (78) breeding ferrets of both sexes were exposed to volatile anal scent gland odorants from male versus female conspecifics in breeding condition. The presence of Fos-IR in the periglomerular cells of MOB glomeruli was taken as an index of glomerular activation (22). Groups of estrous female ferrets showed overlapping, although statistically distinct, profiles of glomerular activation in the ventral MOB 90 min after the onset of exposure to male versus female anal scents. A very similar profile of MOB glomerular activation was seen in breeding male subjects following exposure to these same odorants. There was no evidence in either sex that anal scents induced Fos in any cells of the AOB. These data suggest that pheromonal cues activate the main olfactory system in ferrets; however, the absence of an odor-induced Fos response in the AOB cannot be taken as definitive evidence that the ferret's VNO-accessory olfactory system is not activated by pheromones.

Experiments were undertaken selectively to disable either main or accessory olfactory function in ferrets to determine whether either procedure would disrupt heterosexual partner preference or mating behavior itself. An initial study (79) took advantage of the fact that the ferret (a carnivore), unlike rodent species, is able to breathe via the mouth after total occlusion of the nasal sinuses. Infusing dental impression

material into the nares of gonadectomized (TP-treated) males and (EB-treated) females eliminated subjects' ability to use a peppermint odor to locate food in Y-maze tests and eliminated all MOB c-fos expression. Even prior to receiving mating experience, sham-occluded females preferred to approach volatile male body odors from an anesthetized male in the Y-maze goal box. Not surprisingly, nares-occluded (anosmic) females approached the volatile odors from male and female stimuli equally. Male ferrets are 30% larger than females. Yet when awake stimulus animals were placed behind a transparent barrier (with holes to allow the passage of odors and sounds) in the goal boxes, nares-occluded females still showed no preference to approach the male stimulus (in contrast to sham-occluded controls). When placed in a small compartment with a stud male ferret, the nares-occluded females, like the control females, readily displayed all aspects of sexually receptive sexual behavior. Later, when again allowed to choose in Y-maze tests between male and female odors alone, or between the suite of odor, visual, and auditory cues emitted from male versus female stimulus ferrets, nares-occluded females (in contrast to control females) displayed no preference for one stimulus over the other. This was even the case in a final series of Y-maze tests in which subjects were allowed to have a brief physical interaction with tethered male versus female stimulus animals on each trial. An identical profile of Y-maze results was obtained in male subjects following bilateral nares occlusion, except that control males in that experiment showed a significant preference to approach the odor stimuli emitted from the estrous female stimulus animals. Although these results alone did not definitively rule out a possible role for the accessory olfactory system in heterosexual mate recognition, they did establish in both sexes the essential role of the main olfactory system in this process.

More definitive evidence that the accessory olfactory system plays no role in the ability of female ferrets to identify and seek out volatile body odors from male conspecifics came from a study (80) in which surgical removal of the

VNO from ovariectomized, EB-treated female ferrets failed to disrupt such a preference in Y-maze testing. By contrast, females from which the VNO had been removed spent significantly less time than VNO-intact control females investigating spots of male urine when they were first presented in a sequence of odor stimuli on a piece of filter paper adhered to the front of the subject's home cage. These same two groups of females investigated (made nasal contact with) same-sex urine stimuli for equal times. Both groups investigated either male or female anal scent gland odorants for equal times when they were first presented. It thus appears that VNO inputs generated by odorants emitted from male (opposite-sex) urine, but not anal scents, prolonged subjects' motivation to remain in close proximity to this stimulus. Therefore, whereas VNO-AOB inputs are not required for sex discrimination and heterosexual mate recognition, they may facilitate the motivation to remain in nasal contact with opposite-sex urinary stimuli. As in the case of disrupting main olfactory processing of odors, VNO destruction had no significant effect on the display of receptive sexual behaviors by female ferrets.

FOREBRAIN PROCESSING OF PHEROMONES IS SEXUALLY DIFFERENTIATED IN THE FERRET

Several studies suggest that body odors, detected and initially processed via the main olfactory system, are processed differently in male and female ferrets by projection pathways to the amygdala and on to the hypothalamus. Thus, exposure to soiled bedding from opposite-sex stimulus ferrets stimulated c-fos expression in the MOB granule cell layer (but not in the AOB) and in the medial amygdalar nucleus of both male and female subjects; however, only female subjects showed increased c-fos expression in the MPA and VMH (75). In a subsequent study (76) gonadectomized male and female ferrets (regardless of whether they received TP or oil vehicle) showed significant increases in c-fos

expression in the MOB granule cell layer (but not in the AOB), Me, and BST after exposure to soiled female bedding. However, only female subjects showed a significant c-fos response in the MPA. In addition, exposure of gonadectomized, TP-treated male and female subjects to soiled male bedding again activated Fos in the MOB granule cell layer, Me, and BST of both sexes. In addition, however, odorants in soiled male bedding stimulated c-fos expression selectively in the MPA and VMH of female, but not male, subjects. Taken together, these results suggest that body odorants that are initially detected by the MOE and processed via the MOB, Me, and BST in both sexes are differentially processed in the hypothalamus as a function of subjects' sex and odor source (male versus female).

Additional studies suggest that the differential processing of body odorants in different subregions of the hypothalamus may determine the different preferences of male and female ferrets to approach volatile body odors emitted from potential mating partners (male versus female). Early studies (81–83) suggested that the ability of adult male ferrets to display male-typical neck grip, mount, and pelvic thrusting behaviors (that lead to penile intromission) depends on the perinatal actions of testosterone and/or estradiol in the brain. As explained above, gonadectomized, adult ferrets given sex hormones show a sex difference in their preference to approach male versus female stimulus animals (males prefer to approach females; females prefer to approach males). Perinatal treatment of female ferrets with testosterone masculinized their later partner preference profile just as it augmented females' capacity to show male-typical mating responses later in life (84). It is noteworthy that exposing female ferrets to soiled male bedding stimulated c-fos expression in a forebrain circuit leading from the MOB to the Me and terminating in the VMH (75, 76). The functional implication of this observation was revealed by a study (85) in which bilateral electrolytic lesions of the female ferret's VMH eliminated the females' motivation to approach volatile odors emitted from

an anesthetized male ferret housed behind an opaque barrier in the goal box of a Y-maze. Females with VMH lesions also showed a reduction in their motivation to approach and interact briefly with a tethered male in Y-maze tests. By contrast, other females with bilateral lesions of the MPA/AH (anterior hypothalamus) resembled sham-operated control females in displaying a strong preference to approach odors emitted from either an anesthetized male or a tethered male in Y-maze tests. These results suggest that odor inputs processed by the neural circuit from the Me to the VMH encode maleness in female ferrets.

The male ferret, like the male rat (86), possesses a sexually dimorphic structure in the medial MPA/AH. Ferrets have a cluster of large neurons that differentiates solely in males during the last quarter of the 41-day gestation in response to the action of estradiol formed via neural aromatization of testosterone (87, 88). Localized electrolytic lesions of the adult male ferret's sexually dimorphic nucleus failed to disrupt the display of male-typical mating behavior. By contrast, however, destruction of this nucleus in males caused them to display a female-typical profile of estradiol-induced approach responses to a tethered male kept in the goal box of an L-shaped maze (89). Likewise, placement of either excitotoxic (90) or electrolytic (91) lesions in the male ferrets' sexually dimorphic MPA/AH caused them to prefer to approach and interact sexually with a tethered same-sex (male) versus a female conspecific in T-maze tests. Finally, electrolytic lesions of the sexually dimorphic MPA/AH caused male ferrets to switch their preference from female to male body odors in Y-maze tests (92). Male ferrets with bilateral lesion damage to the male nucleus of the MPA/AH showed a female-typical Fos response in the MPA (a site rostral to the lesion) after exposure for 90 min to soiled male bedding.

Taken together, these results suggest that the female-typical default situation is for male odors to activate neurons in the VMH. These odor inputs originate primarily from the main olfactory system, although accessory olfactory

inputs may also contribute to the females' motivation to remain in proximity to urinary odor deposits from a male. As a result of hypothalamic sexual differentiation, the male ferret develops a circuit that tonically blocks inputs of social odorant cues to this brain region. For reasons that are not understood, the male thus develops a preference to approach female-derived body odors. After destruction of the male's sexually dimorphic nucleus of the MPA/AH, he partially reverts to the female-typical profile of Fos responsiveness to male odors while showing a female-typical preference to seek out male, as opposed to female, body odors.

MATE RECOGNITION IN HUMANS: EVIDENCE FOR A POSSIBLE ROLE OF THE MAIN OLFACTORY SYSTEM

Several studies have addressed the actions of the volatile odorous steroid 4,16-androstadien-3-one (androstadienone), which is excreted in sweat from the axillary region of men and, to lesser extent, women (93). The MOE odorant receptor OR7D4 mediates responses to androstadienone, and single-nucleotide polymorphisms in the OR7D4 gene reduce humans' ability to detect this odorant (94), resulting in considerable variability in its detection threshold (95). Application of androstadienone to the upper lip exerted a subtle positive effect on mood in female subjects (96), enhanced the masculinity rating of men's faces in male subjects (97), and enhanced cortisol secretion in women (98). However, to date there is no report that androstadienone has any effect on at-

titudes of women toward or preference for particular men or on genital arousal in women. The current consensus (99, 100) is that the VNO is not functional in adult humans, perhaps because there is no AOB to which VSNs can project. Thus, any pheromone signaling that occurs in humans presumably depends on pheromone detection by receptor neurons in the MOE and further processing via the MOB, the Me, and the hypothalamus. Savic and colleagues (101) used positron emission tomography (PET) scans to assess the distribution of forebrain sites activated by androstadienone as a function of genetic sex. Androstadienone augmented activity in the hypothalamus of self-identified heterosexual women, but not in the hypothalamus of heterosexual men. The PET method used in this study was not sufficiently sensitive to provide unambiguous resolution of the subnuclei of the hypothalamus that were activated in women by androstadienone. Even so, there is an apparent correspondence between this outcome in humans and previously described results from ferret (76) and mouse (59) in which putative male pheromones more strongly stimulated Fos expression in hypothalamic nuclei of females than in those of males. It has yet to be determined whether the male mouse gender signal, MTMT, is able to duplicate the effects of male mouse urine on the female-specific activation of forebrain c-fos expression. However, there is an obvious analogy between the behavioral (attractant) actions of MTMT in mice and a potential attractant role of androstadienone in humans. Finally, there are reports (102, 103) that women more positively rated body odors from men with dissimilar MHC type.

SUMMARY POINTS

1. The main olfactory system detects volatile pheromones important for sex discrimination and mate recognition in mice.
2. Major histocompatibility complex peptide ligands influence murine mate recognition after their detection by the main olfactory system.

3. The accessory olfactory system contributes to motivational aspects of mate recognition, including attraction to opposite-sex pheromones.
4. Detection and processing of pheromones by the accessory olfactory system are sexually differentiated in mice.
5. Sexually dimorphic processing of pheromones in the hypothalamus controls heterosexual partner preference in ferrets.
6. More research is needed to determine whether either the odorous androgen androstadienone or MHC peptide ligands contribute to mate recognition in humans after their detection and processing via the main olfactory system.

FUTURE ISSUES

1. Do OSNs that express murine TAAR receptors also express TRPM5 cation channels?
2. Do OSNs dedicated to detecting pheromones project to MOB glomeruli that provide direct access via abutting mitral cells to the medial (vomeronasal) amygdala?
3. Is the salience of opposite-sex pheromones that are initially detected by the MOE enhanced by further processing via the AOB prior to these inputs being passed back to the forebrain?
4. Which forebrain circuits bias males to seek out female pheromones and vice versa?
5. Do MHC peptides exist in humans, and are such peptides detected by the OSNs?
6. Do MHC molecules or androstadienone function as human pheromones that contribute to sexual attraction or mate recognition?

DISCLOSURE STATEMENT

The authors are not aware of any bias that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENT

We acknowledge support from NIH grants HD044897 and HD21094 (M.J.B.) and CD006603 (K.R.K.).

LITERATURE CITED

1. Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T, et al. 2007. Innate versus learned odour processing in the mouse olfactory bulb. *Nature* 450:503–8
2. Restrepo D, Arellano J, Oliva AM, Schaefer ML, Lin W. 2004. Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. *Horm. Behav.* 46:247–56
3. Shepherd GM. 2006. Behaviour: smells, brains and hormones. *Nature* 439:149–51
4. Ressler KJ, Sullivan SL, Buck LB. 1994. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79:1245–55

5. Vassar R, Chao SK, Sitcheran R, Nunez JM, Vossahl LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79:981-91
6. Dong HW, Petrovich GD, Swanson LW. 2001. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res. Brain Res. Rev.* 38:192-246
7. Kevetter GA, Winans SS. 1981. Connections of the corticomедial amygdala in the golden hamster. I. Efferents of the "vomeronasal amygdala." *J. Comp. Neurol.* 197:81-98
8. Spehr M, Kelliher KR, Li XH, Boehm T, Leinders-Zufall T, Zufall F. 2006. Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. *J. Neurosci.* 26:1961-70
9. Meredith M, Marques DM, O'Connell RO, Stern FL. 1980. Vomeronasal pump: significance for male hamster sexual behavior. *Science* 207:1224-26
10. Davis BJ, Macrides F, Youngs WM, Schneider SP, Rosene DL. 1978. Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the hamster. *Brain Res. Bull.* 3:59-72
11. Choi GB, Dong HW, Murphy AJ, Valenzuela DM, Yancopoulos GD, et al. 2005. Lhx6 delineates a pathway mediating innate reproductive behaviors from the amygdala to the hypothalamus. *Neuron* 46:647-60
12. Holy TE, Dulac C, Meister M. 2000. Responses of vomeronasal neurons to natural stimuli. *Science* 289:1569-72
13. Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, et al. 2000. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* 405:792-96
14. Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65:175-87
15. Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, et al. 2003. Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* 299:2054-58
16. Scott JW, Pfaff DW. 1970. Behavioral and electrophysiological responses of female mice to male urine odors. *Physiol. Behav.* 5:407-11
17. Jemiolo B, Alberts J, Sochinski-Wiggins S, Harvey S, Novotny M. 1985. Behavioural and endocrine responses of female mice to synthetic analogues of volatile compounds in male urine. *Anim. Behav.* 33:1114-18
18. Bronson FH, Caroom D. 1971. Preputial gland of the male mouse; attractant function. *J. Reprod. Fertil.* 25:279-82
19. Jemiolo B, Xie TM, Novotny M. 1991. Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals. *Physiol. Behav.* 50:1119-22
20. Lin DY, Zhang SZ, Block E, Katz LC. 2005. Encoding social signals in the mouse main olfactory bulb. *Nature* 434:470-77
21. Schaefer ML, Yamazaki K, Osada K, Restrepo D, Beauchamp GK. 2002. Olfactory fingerprints for major histocompatibility complex-determined body odors II: relationship among odor maps, genetics, odor composition, and behavior. *J. Neurosci.* 22:9513-21
22. Schaefer ML, Young DA, Restrepo D. 2001. Olfactory fingerprints for major histocompatibility complex-determined body odors. *J. Neurosci.* 21:2481-87
23. Xu F, Schaefer M, Kida I, Schafer J, Liu N, et al. 2005. Simultaneous activation of mouse main and accessory olfactory bulbs by odors or pheromones. *J. Comp. Neurol.* 489:491-500
24. Lloyd-Thomas A, Keverne EB. 1982. Role of the brain and accessory olfactory system in the block to pregnancy in mice. *Neuroscience* 7:907-13
25. Keller M, Douhard Q, Baum MJ, Bakker J. 2006. Destruction of the main olfactory epithelium reduces female sexual behavior and olfactory investigation in female mice. *Chem. Senses* 31:315-23
26. Ma D, Allen ND, Van Bergen YC, Jones CM, Baum MJ, et al. 2002. Selective ablation of olfactory receptor neurons without functional impairment of vomeronasal receptor neurons in OMP-ntr transgenic mice. *Eur. J. Neurosci.* 16:2317-23
27. Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, et al. 2007. Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. *Nat. Neurosci.* 10:1003-11
28. Nyby J, Day E, Bean N, Dahinden Z, Kerchner M. 1985. Male mouse attraction to airborne urinary odors of conspecifics and to food odors: effects of food deprivation. *J. Comp. Psychol.* 99:479-90

29. Keller M, Douhard Q, Baum MJ, Bakker J. 2006. Sexual experience does not compensate for the disruptive effects of zinc sulfate-lesioning of the main olfactory epithelium on sexual behavior in male mice. *Chem. Senses* 31:753-62
30. Sam M, Vora S, Malnic B, Ma W, Novotny MV, Buck LB. 2001. Neuropharmacology. Odorants may arouse instinctive behaviours. *Nature* 412:142
31. Firestein S. 2001. How the olfactory system makes sense of scents. *Nature* 413:211-18
32. Wang Z, Balet Sindreu C, Li V, Nudelman A, Chan GC, Storm DR. 2006. Pheromone detection in male mice depends on signaling through the type 3 adenylyl cyclase in the main olfactory epithelium. *J. Neurosci.* 26:7375-79
33. Mandiyan VS, Coats JK, Shah NM. 2005. Deficits in sexual and aggressive behaviors in *Cnga2* mutant mice. *Nat. Neurosci.* 8:1660-62
34. Lin W, Arellano J, Slotnick B, Restrepo D. 2004. Odors detected by mice deficient in cyclic nucleotide-gated channel subunit A2 stimulate the main olfactory system. *J. Neurosci.* 24:3703-10
35. Lin W, Margolskee R, Donnert G, Hell SW, Restrepo D. 2007. Olfactory neurons expressing transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals. *Proc. Natl. Acad. Sci. USA* 104:2471-76
36. Martel KL, Baum MJ. 2007. Sexually dimorphic activation of the accessory, but not the main, olfactory bulb in mice by urinary volatiles. *Eur. J. Neurosci.* 26:463-75
37. Liberles SD, Buck LB. 2006. A second class of chemosensory receptors in the olfactory epithelium. *Nature* 442:645-50
38. Yamazaki K, Boyse EA, Mike V, Thaler HT, Mathieson BJ, et al. 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. *J. Exp. Med.* 144:1324-35
39. Boehm T, Zufall F. 2006. MHC peptides and the sensory evaluation of genotype. *Trends Neurosci.* 29:100-7
40. Eggert F, Muller-Ruchholtz W, Ferstl R. 1998. Olfactory cues associated with the major histocompatibility complex. *Genetica* 104:191-97
41. Slev PR, Nelson AC, Potts WK. 2006. Sensory neurons with MHC-like peptide binding properties: disease consequences. *Curr. Opin. Immunol.* 18:608-16
42. Penn D, Potts W. 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proc. Biol. Sci.* 265:1299-306
43. Scalia F, Winans SS. 1975. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *J. Comp. Neurol.* 161:31-55
44. Kevetter GA, Winans SS. 1981. Connections of the corticomедial amygdala in the golden hamster. II. Efferents of the "olfactory amygdala." *J. Comp. Neurol.* 197:99-111
45. Wysocki CJ. 1979. Neurobehavioral evidence for the involvement of the vomeronasal system in mammalian reproduction. *Neurosci. Biobehav. Rev.* 3:301-41
46. Bean NJ. 1982. Olfactory and vomeronasal mediation of ultrasonic vocalizations in male mice. *Physiol. Behav.* 28:31-37
47. Wysocki CJ, Nyby J, Whitney G, Beauchamp GK, Katz Y. 1982. The vomeronasal organ: primary role in mouse chemosensory gender recognition. *Physiol. Behav.* 29:315-27
48. Sipos ML, Wysocki CJ, Nyby JG, Wysocki L, Nemura TA. 1995. An ephemeral pheromone of female house mice: perception via the main and accessory olfactory systems. *Physiol. Behav.* 58:529-34
49. Liman ER, Corey DP, Dulac C. 1999. TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. *Proc. Natl. Acad. Sci. USA* 96:5791-96
50. Stowers L, Holy TE, Meister M, Dulac C, Koentges G. 2002. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. *Science* 295:1493-500
51. Bean NJ. 1982. Modulation of agonistic behavior by the dual olfactory system in male mice. *Physiol. Behav.* 29:433-37
52. Clancy AN, Coquelin A, Macrides F, Gorski RA, Noble EP. 1984. Sexual behavior and aggression in male mice: involvement of the vomeronasal system. *J. Neurosci.* 4:2222-29
53. Leybold BG, Yu CR, Leinders-Zufall T, Kim MM, Zufall F, Axel R. 2002. Altered sexual and social behaviors in *trp2* mutant mice. *Proc. Natl. Acad. Sci. USA* 99:6376-81

54. Kimchi T, Xu J, Dulac C. 2007. A functional circuit underlying male sexual behaviour in the female mouse brain. *Nature* 448:1009–14
55. Pankevich DE, Baum MJ, Cherry JA. 2004. Olfactory sex discrimination persists, whereas the preference for urinary odorants from estrous females disappears in male mice after vomeronasal organ removal. *J. Neurosci.* 24:9451–57
56. Pankevich DE, Cherry JA, Baum MJ. 2006. Effect of vomeronasal organ removal from male mice on their preference for and neural Fos responses to female urinary odors. *Behav. Neurosci.* 120:925–36
57. Keller M, Pierman S, Douhard Q, Baum MJ, Bakker J. 2006. The vomeronasal organ is required for the expression of lordosis behaviour, but not sex discrimination in female mice. *Eur. J. Neurosci.* 23:521–30
58. Halem HA, Baum MJ, Cherry JA. 2001. Sex difference and steroid modulation of pheromone-induced immediate early genes in the two zones of the mouse accessory olfactory system. *J. Neurosci.* 21:2474–80
59. Halem HA, Cherry JA, Baum MJ. 1999. Vomeronasal neuroepithelium and forebrain Fos responses to male pheromones in male and female mice. *J. Neurobiol.* 39:249–63
60. Kimoto H, Haga S, Sato K, Touhara K. 2005. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. *Nature* 437:898–901
61. Dudley CA, Moss RL. 1999. Activation of an anatomically distinct subpopulation of accessory olfactory bulb neurons by chemosensory stimulation. *Neuroscience* 91:1549–56
62. Kang N, Janes A, Baum MJ, Cherry JA. 2006. Sex difference in Fos induced by male urine in medial amygdala-projecting accessory olfactory bulb mitral cells of mice. *Neurosci. Lett.* 398:59–62
63. Luo M, Fee MS, Katz LC. 2003. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. *Science* 299:1196–201
64. Bodo C, Rissman EF. 2007. Androgen receptor is essential for sexual differentiation of responses to olfactory cues in mice. *Eur. J. Neurosci.* 25:2182–90
65. Bodo C, Rissman E. 2008. The androgen receptor is selectively involved in organization of sexually dimorphic social behaviors in mice. *Endocrinology* 149:4142–50
66. Barber PC. 1982. Adjacent laminar terminations of two centrifugal afferent pathways to the accessory olfactory bulb in the mouse. *Brain Res.* 245:215–21
67. Jakupovic J, Kang N, Baum MJ. 2008. Effect of bilateral accessory olfactory bulb lesions on volatile urinary odor discrimination and investigation as well as mating behavior in male mice. *Physiol. Behav.* 93:467–73
68. Moors S, Lavers R. 1981. Movements and home range of ferrets at Pukepuke Lagoon, New Zealand. *N. Z. J. Zool.* 8:413–23
69. Clapperton B, Minot E, Crump D. 1988. An olfactory recognition system in the ferret. *Anim. Behav.* 36:541–53
70. Zhang JX, Soini HA, Bruce KE, Wiesler D, Woodley SK, et al. 2005. Putative chemosignals of the ferret (*Mustela furo*) associated with individual and gender recognition. *Chem. Senses* 30:727–37
71. Clapperton B. 1989. Scent-marking behaviour of the ferret. *Anim. Behav.* 38:436–46
72. Chang YM, Kelliher KR, Baum MJ. 2000. Steroidal modulation of scent investigation and marking behaviors in male and female ferrets. *J. Comp. Psychol.* 114:401–7
73. Kelliher K, Baum M. 2002. Effect of sex steroids and coital experience on ferrets' preference for the smell, sight and sound of conspecifics. *Physiol. Behav.* 76:1–7
74. Cloe AL, Woodley SK, Waters P, Zhou H, Baum MJ. 2004. Contribution of anal scent gland and urinary odorants to mate recognition in the ferret. *Physiol. Behav.* 82:871–75
75. Wersinger SR, Baum MJ. 1997. Sexually dimorphic processing of somatosensory and chemosensory inputs to forebrain luteinizing hormone-releasing hormone neurons in mated ferrets. *Endocrinology* 138:1121–29
76. Kelliher KR, Chang YM, Wersinger SR, Baum MJ. 1998. Sex difference and testosterone modulation of pheromone-induced NeuronalFos in the ferret's main olfactory bulb and hypothalamus. *Biol. Reprod.* 59:1454–63
77. Chang YM, Kelliher KR, Baum MJ. 2001. Maternal odours induce Fos in the main but not the accessory olfactory bulbs of neonatal male and female ferrets. *J. Neuroendocrinol.* 13:551–60

78. Woodley SK, Baum MJ. 2004. Differential activation of glomeruli in the ferret's main olfactory bulb by anal scent gland odours from males and females: an early step in mate identification. *Eur. J. Neurosci.* 20:1025-32
79. Kelliher KR, Baum MJ. 2001. Nares occlusion eliminates heterosexual partner selection without disrupting coitus in ferrets of both sexes. *J. Neurosci.* 21:5832-40
80. Woodley SK, Cloe AL, Waters P, Baum MJ. 2004. Effects of vomeronasal organ removal on olfactory sex discrimination and odor preferences of female ferrets. *Chem. Senses* 29:659-69
81. Baum MJ. 1976. Effects of testosterone propionate administered perinatally on sexual behavior of female ferrets. *J. Comp. Physiol. Psychol.* 90:399-410
82. Baum MJ, Gallagher CA, Martin JT, Damassa DA. 1982. Effects of testosterone, dihydrotestosterone, or estradiol administered neonatally on sexual behavior of female ferrets. *Endocrinology* 111:773-80
83. Tobet SA, Baum MJ. 1987. Role for prenatal estrogen in the development of masculine sexual behavior in the male ferret. *Horm. Behav.* 21:419-29
84. Baum MJ, Erskine MS, Kornberg E, Weaver CE. 1990. Prenatal and neonatal testosterone exposure interact to affect differentiation of sexual behavior and partner preference in female ferrets. *Behav. Neurosci.* 104:183-98
85. Robarts DW, Baum MJ. 2007. Ventromedial hypothalamic nucleus lesions disrupt olfactory mate recognition and receptivity in female ferrets. *Horm. Behav.* 51:104-13
86. Gorski RA, Gordon JH, Shryne JE, Southam AM. 1978. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res.* 148:333-46
87. Cherry JA, Basham ME, Weaver CE, Krohmer RW, Baum MJ. 1990. Ontogeny of the sexually dimorphic male nucleus in the preoptic/anterior hypothalamus of ferrets and its manipulation by gonadal steroids. *J. Neurobiol.* 21:844-57
88. Tobet SA, Zahniser DJ, Baum MJ. 1986. Differentiation in male ferrets of a sexually dimorphic nucleus of the preoptic/anterior hypothalamic area requires prenatal estrogen. *Neuroendocrinology* 44:299-308
89. Cherry JA, Baum MJ. 1990. Effects of lesions of a sexually dimorphic nucleus in the preoptic/anterior hypothalamic area on the expression of androgen- and estrogen-dependent sexual behaviors in male ferrets. *Brain Res.* 522:191-203
90. Paredes RG, Baum MJ. 1995. Altered sexual partner preference in male ferrets given excitotoxic lesions of the preoptic area/anterior hypothalamus. *J. Neurosci.* 15:6619-30
91. Kindon HA, Baum MJ, Paredes RJ. 1996. Medial preoptic/anterior hypothalamic lesions induce a female-typical profile of sexual partner preference in male ferrets. *Horm. Behav.* 30:514-27
92. Alekseyenko OV, Waters P, Zhou H, Baum MJ. 2007. Bilateral damage to the sexually dimorphic medial preoptic area/anterior hypothalamus of male ferrets causes a female-typical preference for and a hypothalamic Fos response to male body odors. *Physiol. Behav.* 90:438-49
93. Nixon A, Mallet AI, Gower DB. 1988. Simultaneous quantification of five odorous steroids (16-androstenes) in the axillary hair of men. *J. Steroid Biochem.* 29:505-10
94. Keller A, Zhuang H, Chi Q, Vossell LB, Matsunami H. 2007. Genetic variation in a human odorant receptor alters odour perception. *Nature* 449:468-72
95. Lundstrom JN, Hummel T, Olsson MJ. 2003. Individual differences in sensitivity to the odor of 4,16-androstadien-3-one. *Chem. Senses* 28:643-50
96. Jacob S, Garcia S, Hayreh D, McClintock MK. 2002. Psychological effects of musky compounds: comparison of androstadienone with androstenol and muscone. *Horm. Behav.* 42:274-83
97. Kovacs G, Gulyas B, Savic I, Perrett DI, Cornwell RE, et al. 2004. Smelling human sex hormone-like compounds affects face gender judgment of men. *Neuroreport* 15:1275-77
98. Wyart C, Webster WW, Chen JH, Wilson SR, McClary A, et al. 2007. Smelling a single component of male sweat alters levels of cortisol in women. *J. Neurosci.* 27:1261-65
99. Brennan PA, Zufall F. 2006. Pheromonal communication in vertebrates. *Nature* 444:308-15
100. Meredith M. 2001. Human vomeronasal organ function: a critical review of best and worst cases. *Chem. Senses* 26:433-45
101. Savic I, Berglund H, Gulyas B, Roland P. 2001. Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. *Neuron* 31:661-68

102. Jacob S, McClintock MK, Zelano B, Ober C. 2002. Paternally inherited HLA alleles are associated with women's choice of male odor. *Nat. Genet.* 30:175–79
103. Wedekind C, Furi S. 1997. Body odour preferences in men and women: Do they aim for specific MHC combinations or simply heterozygosity? *Proc. Biol. Sci.* 264:1471–79
104. Spehr M, Spehr J, Ukhanov K, Kelliher KR, Leinders-Zufall T, Zufall F. 2006. Parallel processing of social signals by the mammalian main and accessory olfactory systems. *Cell Mol. Life Sci.* 63:1476–84
105. Karlson P, Luscher M. 1959. 'Pheromones': a new term for a class of biologically active substances. *Nature* 183:55–56
106. McClintock MK. 2002. Pheromones, odors, and vasanas: The neuroendocrinology of social chemosignals in humans and animals. In *Hormones, Brain and Behavior*, ed. DW Pfaff, AP Arnold, AM Etgen, SE Fahrbach, RT Rubin, pp. 797–870. San Diego: Elsevier



Contents

CARDIOVASCULAR PHYSIOLOGY, *Jeffrey Robbins, Section Editor*

Sex-Based Cardiac Physiology <i>Elizabeth D. Luczak and Leslie A. Leinwand</i>	1
---	---

CELL PHYSIOLOGY, *David E. Clapham, Associate and Section Editor*

Convergent Evolution of Alternative Splices at Domain Boundaries of the BK Channel <i>Anthony A. Fodor and Richard W. Aldrich</i>	19
Mechanisms of Muscle Degeneration, Regeneration, and Repair in the Muscular Dystrophies <i>Gregory Q. Wallace and Elizabeth M. McNally</i>	37
Plant Ion Channels: Gene Families, Physiology, and Functional Genomics Analyses <i>John M. Ward, Pascal Mäser, and Julian I. Schroeder</i>	59
Polycystins and Primary Cilia: Primers for Cell Cycle Progression <i>Jing Zhou</i>	83
Subsystem Organization of the Mammalian Sense of Smell <i>Steven D. Munger, Trese Leinders-Zufall, and Frank Zufall</i>	115

ECOLOGICAL, EVOLUTIONARY, AND COMPARATIVE PHYSIOLOGY, *Martin E. Feder, Section Editor*

Complementary Roles of the Main and Accessory Olfactory Systems in Mammalian Mate Recognition <i>Michael J. Baum and Kevin R. Kelliber</i>	141
Pheromone Communication in Amphibians and Reptiles <i>Lynne D. Houck</i>	161

ENDOCRINOLOGY, *Holly A. Ingraham, Section Editor*

Transcriptional Control of Mitochondrial Biogenesis and Function <i>M. Benjamin Hock and Anastasia Kralli</i>	177
--	-----

GASTROINTESTINAL PHYSIOLOGY, *James M. Anderson, Section Editor*

The Functions and Roles of the Extracellular Ca²⁺-Sensing Receptor
along the Gastrointestinal Tract
John P. Geibel and Steven C. Hebert 205

Neuroendocrine Control of the Gut During Stress:
Corticotropin-Releasing Factor Signaling Pathways in the Spotlight
Andreas Stengel and Yvette Taché 219

Stem Cells, Self-Renewal, and Differentiation in the
Intestinal Epithelium
Laurens G. van der Flier and Hans Clevers 241

NEUROPHYSIOLOGY, *Roger Nicoll, Section Editor*

Dendritic Spine Dynamics
D. Harshad Bhatt, Shengxiang Zhang, and Wen-Biao Gan 261

Endocannabinoid Signaling and Long-Term Synaptic Plasticity
Boris D. Heifets and Pablo E. Castillo 283

Sensing Odorants and Pheromones with Chemosensory Receptors
Kazushige Touhara and Leslie B. Vosshall 307

Signaling at Purinergic P2X Receptors
Annamarie Surprenant and R. Alan North 333

RENAL AND ELECTROLYTE PHYSIOLOGY, *Gerhard H. Giebisch, Section Editor*

Activation of the Epithelial Sodium Channel (ENaC)
by Serine Proteases
Bernard C. Rossier and M. Jackson Stutts 361

Recent Advances in Understanding Integrative Control of Potassium
Homeostasis
Jang H. Youn and Alicia A. McDonough 381

RESPIRATORY PHYSIOLOGY, *Richard C. Boucher, Jr., Section Editor*

The Contribution of Epithelial Sodium Channels to Alveolar Function
in Health and Disease
Douglas C. Eaton, My N. Helms, Michael Koval, Hui Fang Bao, and Lucky Jain 403

The Role of CLCA Proteins in Inflammatory Airway Disease
Anand C. Patel, Tom J. Brett, and Michael J. Holtzman 425

Role of HDAC2 in the Pathophysiology of COPD
Peter J. Barnes 451

SPECIAL TOPIC, ASTHMA, *Jeffrey M. Drazen, Special Topic Editor*

Aspirin-Sensitive Respiratory Disease
Sophie P. Farooque and Tak H. Lee 465

Immunobiology of Asthma
Qutayba Hamid and Meri Tulic 489

Noncontractile Functions of Airway Smooth Muscle Cells in Asthma
Omar Tliba and Reynold A. Panettieri, Jr. 509

Indexes

Cumulative Index of Contributing Authors, Volumes 67–71 537

Cumulative Index of Chapter Titles, Volumes 67–71 540

Errata

An online log of corrections to *Annual Review of Physiology* articles may be found at
<http://physiol.annualreviews.org/errata.shtml>