Olfactory Regulation of the Sexual Behavior and Reproductive Physiology of the Laboratory Mouse: Effects and Neural Mechanisms

Kevin R. Kelliher and Scott R. Wersinger

Abstract

In many species, chemical compounds emitted by conspecifics exert profound effects on reproductive physiology and sexual behavior. This is particularly true in the mouse, where such cues advance and delay puberty, suppress and facilitate estrous cycles, and cause the early termination of pregnancy. They also facilitate sexual behavior and inform mate selection. The mouse has a rich and complex repertoire of social behaviors. The technologies of molecular genetics are well developed in the mouse. Gene expression can be experimentally manipulated in the mouse relatively easily and in a time- and tissue-specific manner. Thus, the mouse is an excellent model in which to investigate the genetic, neural, and hormonal bases by which chemical compounds released by other mice affect physiology and behavior. These chemical cues are detected and processed by the olfactory system and other specialized but less well characterized sensory organs. The sensory information reaches brain regions that regulate hormone levels as well as those that are involved in behavior and alters the function of these brain regions. The effects of these chemical compounds have important implications for the laboratory animal facility as well as for researchers. We begin with an overview of the basic structure and function of the olfactory system and of the connections among brain regions that receive olfactory stimuli. We discuss the effects of chemosensory cues on the behavior and physiology of the organism along with what is known about the neural and hormonal mechanisms underlying these effects. We also describe some of the implications for the laboratory animal facility.

Key Words: behavior; Bruce effect; chemosensory cues; main olfactory; *Mus musculus*; pheromones; reproductive physiology; vomeronasal

Introduction

Sexual behavior and reproductive physiology are regulated by a number of factors. In many species, both wild and domestic, chemical cues released by conspecifics exert a profound influence on these processes. For example, the female silkmoth (*Bombyx mori*) releases a lipid molecule called bombykol that attracts male silkmoths (Matsumoto et al. 2007; Regnier and Law 1968). Female roughskin newts (*Taricha granulosa*) release a chemical that facilitates copulatory behavior in male newts (Thompson and Moore 2000). Male sheep, cattle, and goats detect chemicals present in the anogenital region of females that allow them to assess the females' estrous state (Rekwot et al. 2001). Domestic boar (*Sus scrofa*) saliva contains a chemical, androstenone, that not only attracts sows but also facilitates the display of a characteristic mating posture (Dorries et al. 1997; Signoret 1970). A comprehensive description of all species in which chemical cues affect reproduction is well beyond the scope of this article; these examples should illustrate the point that chemical cues released by members of the same species have significant effects on reproductive behavior and physiology in very diverse species.

We focus on the mouse (*Mus musculus*) for several reasons. First, mice are macrosmotic animals, meaning they have a highly developed sense of olfaction. Second, the reproduction and behavior of the mouse are profoundly influenced by chemicals released by conspecifics. In addition, these effects are robust and reliably elicited, making them easier to study. Thus, the mouse is an exceptional model organism to study the chemosensory regulation of reproductive physiology and behavior. A third reason is that the molecular techniques that allow manipulation of the genome are still optimal in the mouse, enabling manipulations that are far more difficult, if not impossible, in other species. Finally, nearly all laboratory animal facilities house mice, and the effects we review in this paper have important implications for animal husbandry.

When considering the effects of chemical cues, it is useful to take an evolutionary approach because, as with all phenotypes, these effects are the result of evolutionary processes. This approach considers how and why these effects evolved. Many researchers and facility staff may prefer a strictly pragmatic perspective. How do these phenomena affect husbandry? How can they be circumvented (or

Kevin R. Kelliher, PhD, is an assistant professor in the Department of Biological Sciences at the University of Idaho, Moscow, and Scott R. Wersinger, PhD, is an assistant professor in the Department of Psychology at the University at Buffalo, the State University of New York.

Address correspondence and reprint requests to Dr. Scott R. Wersinger, Department of Psychology, B-72 Park Hall, University at Buffalo, the State University of New York, Buffalo, NY 14260 or email sw39@buffalo.edu.

exploited) to maximize reproductive output? How can they be used to control pest populations? Indeed, there has been great interest in using sex pheromones to control gypsy moth populations, and some hunters claim that pheromones can be used to attract their quarry.

No matter which of these questions is of interest, the answer relies on an understanding of the mechanism by which the chemical cues exert their effects. After reading this review, we hope the reader will have a basic understanding of the sensory systems that detect chemical compounds in the environment (which we interchangeably call chemosensory cues) and the part of the brain involved in the processing of these cues. We review the major effects of chemosensory cues on the behavior and reproduction of the mouse to convey the extent of their impact on the mouse, in particular, but by generalization, all species. As the reader becomes familiar with all the effects of chemosensory cues, we are certain that it will be clear that these cues must be considered by all researchers and other personnel involved in the use and husbandry of animals in the laboratory animal facility.

Overview of the Olfactory System

Some chemical compounds in the environment, working independently or in concert with other cues (not necessarily chemical in nature), exert profound effects on physiology and behavior. Many of these behaviors or physiological responses to chemical cues have been either correctly or incorrectly associated with pheromonal responses. The term pheromone was first introduced in the literature 50 years ago by Karlson and Luscher (1959), who defined pheromones as "substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction." Thus, some, but not all, chemical compounds in the environment are correctly labeled pheromones (nonpheromonal chemical cues do not elicit a specific reaction in a conspecific). The definition of a pheromone in relation to mammalian chemical cues has changed and often taken new meaning. Kelliher has suggested that the term be used as a concept, not a discrete entity, and defines the concept as "a chemical cue that is secreted by an individual *with the sole or primary function of communicating* with a member of the same species" (Kelliher 2007; emphasis added). Based on this definition, many of the effects we review here are mediated by pheromones.

To understand the mechanism by which chemosensory cues, including pheromones, affect the receiver, the reader must understand the basic organization and function of the olfactory system. The olfactory system, the sensory system that detects chemicals in the environment, comprises multiple subdivisions that presumably serve different functions (although these functions have not been fully elucidated). For example, the septal organ and the Gruenberg ganglion are two anatomically distinct organs with sensory receptor cells that send axons to the olfactory bulb (Breer et al. 2006). Most of what is known about chemical cues and reproductive physiology has come from studies of the two largest subdivisions, the main olfactory system and the vomeronasal system (until recently called the accessory olfactory system). Since the vast majority of mammals, reptiles, and many amphibians studied to date possess both of these systems, it is reasonable to conclude that the common ancestors of these classes of species possessed functional main and vomeronasal systems. (Species such as the Old World primates, including humans, and aquatic mammals lost the functional vomeronasal system during evolution.)

Figure 1 is a general illustration of the brain regions that receive sensory information from the main and vomeronasal systems. Although the information converges in the medial amygdala, many aspects of the anatomy and function of the two systems are distinct and we therefore discuss them separately.

The Main Olfactory System

Detection

The main olfactory epithelium detects the vast majority of chemosensory cues and discriminates between thousands of different odorants. These enter the nasal cavity and come in contact with the ciliated dendrites of highly specialized bipolar olfactory receptor neurons (ORNs), which, along with sustentacular supporting cells and protogenic basal cells, make up the pseudostratified sensory epithelium in the nose. While the amount of sensory epithelium compared to respiratory epithelium may differ depending on the species, the sensory epithelium is generally located in the dorsal caudal nasal cavity. Classical ORNs use a canonical cyclic adenosine monophosphate (cAMP) signaling pathway and are the most common olfactory receptor type in the main olfactory epithelium. ORNs project their axons to the olfactory bulbs where they arborize in only two individual glomeruli in the main olfactory bulb (MOB) (Ressler et al. 1994; Vassar et al. 1994), maintaining a functional topography between the epithelium and the MOB (Mori et al. 1999). Figure 2 depicts the general structure of the main olfactory system from the olfactory epithelium to the MOB. Transmission of any information to the central nervous system requires a fully intact circuit among these components.

Perception

Perception is generally defined as awareness of a sensory stimulus. Perception of odor and the identification of stimuli begin in the olfactory bulb. As illustrated in Figure 2, mitral cells in the olfactory bulb arborize in a single glomerulus and therefore receive information from only a single type of OR. The main olfactory bulb is more than just a relay between receptor neurons and the primary olfactory cortex. The laminar structure of the MOB is thought to be important

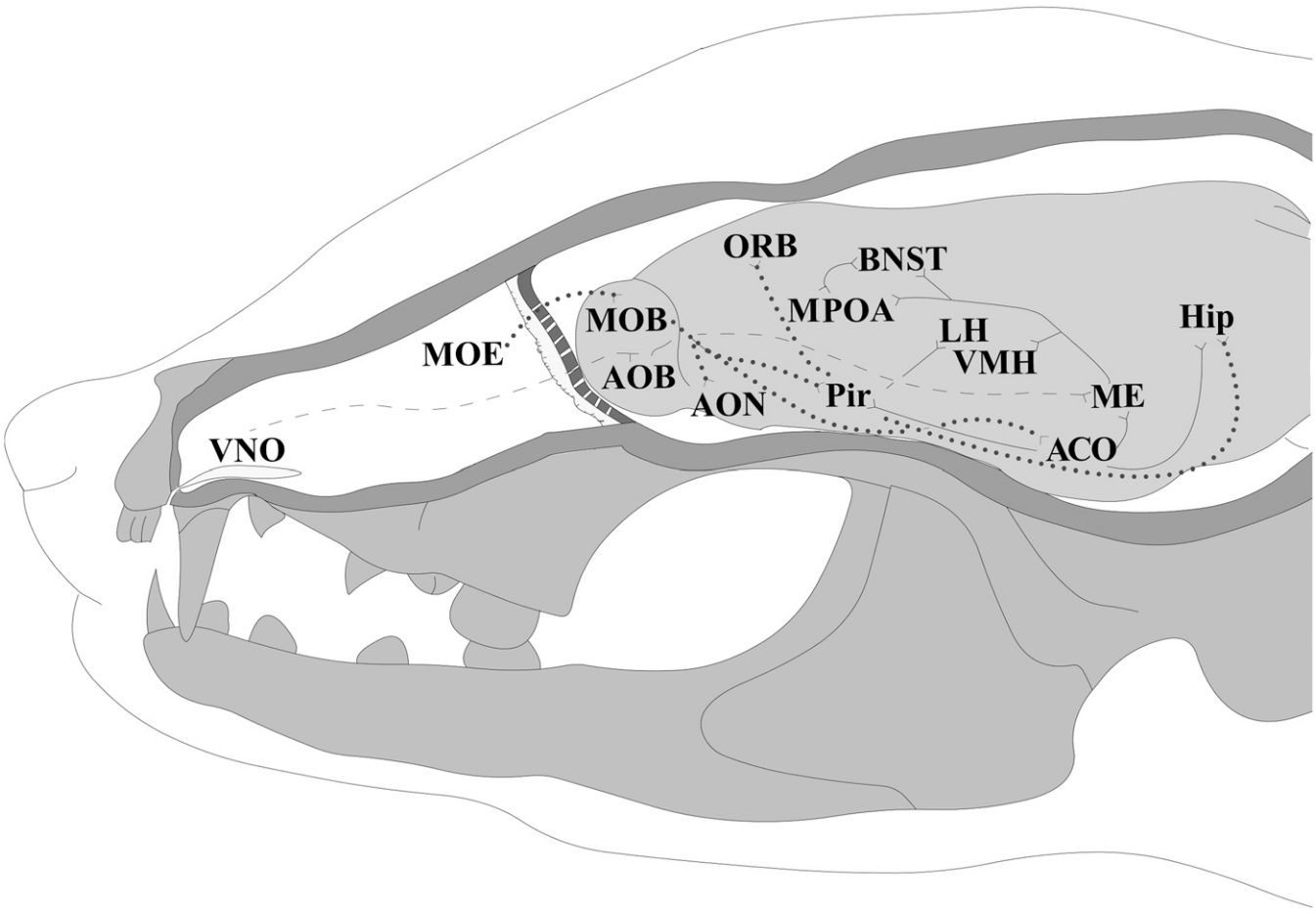

Figure 1 The mouse chemosensory response circuit. The vomeronasal system (dashed line) begins with the sensory epithelium in the vomeronasal organ (VNO). The VNO projects to the accessory olfactory bulb (AOB), which in turn projects directly to the medial amygdala (ME). The medial amygdala has broad projections to regions including the bed nucleus of the stria terminalis (BNST), the medial preoptic area (MPOA), and the ventromedial nucleus of the hypothalamus (VMH). The main olfactory system (dotted line) begins with the main olfactory epithelium (MOE), which projects to the main olfactory bulb (MOB). The MOB sends projections to the accessory olfactory nucleus (AON), the piriform cortex (Pir), and the corticomedial nucleus of the amygdala (ACO). The ACO projects to the Pir, the ME, and the hippocampus (Hip). Thus, sensory stimuli carried by the main and vomeronasal systems converge in the ME. The Pir projects to the lateral hypothalamus (LH) and the orbitofrontal cortex (ORB). Solid lines represent pathways after possible convergence of the main olfactory and vomeronasal systems.

for the initial processing of olfactory signals and involved in the formation of memories (Leise 1990; Shepherd 1992). In fact, the lateral inhibition between mitral cells, which enhances the specificity of responses between two closely related compounds, performs a similar function to lateral inhibition in the visual system (Haberly 2001; Yokoi et al. 1995).

The mitral cells project via the lateral olfactory tract to multiple higher olfactory structures—the anterior olfactory nucleus, anterior cortical amygdala, olfactory tubercle, tenia tectum, piriform cortex, and entorhinal cortex (Shipley and Ennis 1996). These regions in turn project to other cortical, subcortical, and limbic regions and provide feedback to the olfactory bulb itself (Haberly 2001; Shipley and Ennis 1996). Individual mitral cells project to multiple regions in the piriform cortex in addition to many other higher olfactory areas (Ojima et al. 1984; Scott 1981).

Although the piriform cortex had long been thought of as the primary olfactory cortex, the main olfactory bulb may in fact take on more of this role and the piriform cortex may instead function more as an associative cortex. Cells in the piriform cortex fire in response not only to odors but also to reward components present during an olfactory discrimination task. These firing rates increase in response to an increased incentive and will even precede the odor response, predicting the onset of the task (Schoenbaum and Eichenbaum 1995). Using c-Fos as a marker for neuronal activation Kippin and Pfaus (Kippin et al. 2003) found that the piriform cortex responded to a nonsocial odor (almond extract) detected by the main olfactory system only after it had been previously paired with copulation. These findings suggest that one role of the piriform cortex is to respond to odors that have been learned or associated with reward and that it is thus a likely site for odor perception (Haberly 2001).

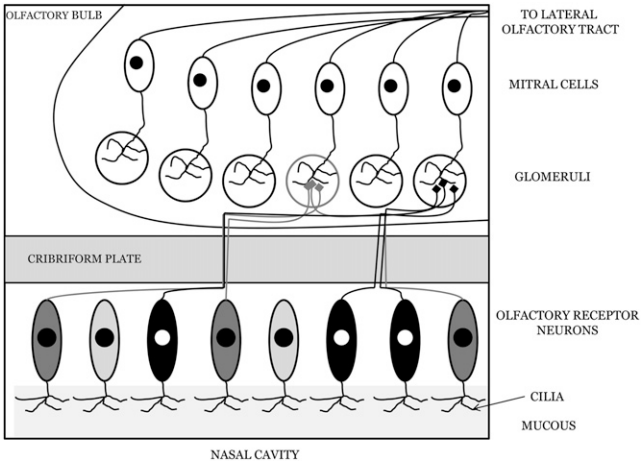

Figure 2 A schematic representation of the anatomy and projections of the main olfactory system. Cilia from olfactory receptor neurons (ORNs) extend into the nasal cavity where they interact with chemical compounds. Each ORN contains one type of olfactory receptor. Although olfactory receptor neurons with the same olfactory receptor are distributed throughout the sensory epithelium, their axons pass through the cribriform plate of the skull and project to the same glomerulus in the main olfactory bulb (thus the black ORNs in the drawing all project to the same glomerulus). In the glomerulus, the terminals of the ORNs synapse onto dendrites of the mitral cells, and the mitral cell axons converge to form the lateral olfactory tract, which projects to the brain regions summarized in Figure 1.

Assignment of Meaning

An animal that detects a stimulus must identify familiar stimuli and recall a meaning associated with them. For novel stimuli, the animal will assign meaning based on the circumstances of detection and memory formation. Areas of the amygdala, hypothalamus, and cortex are all important for assigning and recalling meaning. The basolateral amygdala (BLA) and the orbital frontal cortex both receive input from the piriform cortex and are important for associative learning (Everitt et al. 1999; Fanselow and LeDoux 1999; Schoenbaum et al. 2002). Cells in both of these regions encode neural responses to odors presented during operant training tasks (Schoenbaum et al. 1999). The ventral striatum, which encompasses the nucleus accumbens, receives projections from both the basolateral amygdala and the orbital frontal cortex. It has also been implicated as a major integration site for associative learning and reward. The BLA and the ventral striatum work together to encode and respond to motivationally significant cues (both rewarding and aversive), which ultimately guide the behavior of the animal (Cardinal 2002; Schoenbaum et al. 2003; Setlow et al. 2002). Activation of this pathway is evident in male rats trained to associate a neutral odor (almond extract) with copulation: they show significant increases in c-Fos expression in the piriform cortex, basolateral amygdala, nucleus accumbens (NAcc), and lateral hypothalamus after exposure to almond odor, compared to rats exposed to the same odor but without training (Kippin et al. 2003). In summary, it is the interconnections between the piriform cortex, basolateral amygdala, orbitofrontal cortex, and the nucleus accumbens that appear to encode odor perception and the motivational significance or meaning to odors.

The Vomeronasal System

The vomeronasal (or accessory olfactory) system is a distinct subsystem present in most vertebrate animals with the exception of avian species, aquatic mammals, and Old World primates, including humans (Wysocki 1979). Chemosensory cues are first detected by vomeronasal receptor neurons (VRNs) located in a bilaterally paired set of organs at the base of the nasal septum. These cues bind to vomeronasal receptors located on microvilli that project into the vomeronasal lumen. In mammals such as rodents and marsupials, in which the system is particularly well defined, the vomeronasal organ is separated into two subsystems, an apical layer and a basal layer, distinguished by differences in the signal transduction cascades. Apical neurons have a vomeronasal receptor from the V1R family of receptors, which are coupled to a specific second messenger system (the Gαi protein) and require the use of a transient receptor potential channel with a TRPC2 subunit (Dulac and Torello 2003; Herrada and Dulac 1997; Tirindelli et al. 1998). In contrast, basal neurons have a vomeronasal receptor from the V2R family of receptors that is G protein–coupled and have a TRPC2 subunit but can also process chemosensory cues in a TRPC2-independent manner (Kelliher et al. 2006). While the functional significance of these two subsystems is still unclear, some chemical cues are clearly detected only by the apical VRNs and some by the basal VRNs (Leinders-Zufall et al. 2000, 2004).

As shown in Figure 2, vomeronasal receptor neurons send their axons directly to the glomeruli of the accessory olfactory bulb (AOB[1]), which is located caudally on the dorsomedial portion of the olfactory bulb and which maintains the zonal segregation of the VRNs. Axons from apically located VRNs arborize in glomeruli situated in the rostral AOB whereas axons from basally located VRNs arborize in the caudal AOB (Halpern et al. 1998). Axons from the VRNs enter the AOB glomeruli and make synaptic contact with mitral cell dendrites.

Mitral cells project their axons to forebrain structures—the anterior olfactory nucleus, bed nucleus of the accessory olfactory tract (BAOT), medial amygdala (ME), posteromedial cortical amygdala, and bed nucleus of the stria terminalis (BNST)—via the lateral olfactory tract (Scalia and Winans 1975). There appear to be some species differences as to whether the zonal segregation observed in the vomeronasal

[1]Abbreviations used in this article: AOB, accessory olfactory bulb; Fos-ir, Fos-like immunoreactivity; HPG, hypothalamic-pituitary-gonadal axis; LH, luteinizing hormone; MUPs, major urinary proteins; VNO, vomeronasal organ

epithelium and AOB is maintained in the posteromedial cortical amygdala. In the opossum, axons from posterior mitral cells (those receiving input from the basal layer of the vomeronasal epithelium) terminate more deeply than mitral cells from the anterior zone of the AOB (Martinez-Marcos and Halpern 1999), whereas no such segregation has been observed in the medial amygdala of the mouse (Salazar and Brennan 2001).

Connection with Limbic and Hypothalamic Regions

Further projections to the medial preoptic area (MPOA), ventrolateral portion of the ventromedial hypothalamic nucleus (VLH), and premammillary nucleus originate from both the medial amygdala and the BNST (Canteras et al. 1992a,b, 1995; Scalia and Winans 1975; Winans and Scalia 1970). The vomeronasal pathway is distinct and separate from the main olfactory pathway, although there is a likely convergence at the medial amygdala. Mitral cells from the main olfactory bulb project to the endopiriform nucleus and the corticomedial amygdala, both of which then project to the medial and posteromedial cortical amygdala (Coolen and Wood 1998; Krettek and Price 1978a,b). Licht and Meredith (1987) demonstrated these projections in hamsters when they recorded activity in the ME after electrical stimulation of either the vomeronasal epithelium (VNE) or the main olfactory epithelium (MOE).

Researchers have generally believed that the convergence of the main and vomeronasal pathways is "one-way": olfactory information can flow to the vomeronasal pathway but vomeronasal information does not appear to gain access to cortical olfactory areas (Kevetter and Winans 1981a,b). Intra-amygdala connections, however, are highly complex. Whereas the vast majority of the connections flow from the cortical amygdala and the basolateral amygdala to the ME, there are also reciprocal connections between these brain regions (Coolen and Wood 1998). It is therefore possible that the vomeronasal system communicates with cortical olfactory areas, but this has yet to be demonstrated.

Effects of Olfaction on Physiology and Social Behavior

Chemosensory cues—a wide variety of compounds produced by conspecifics—are produced and released (actively or passively) by a "sender" animal and detected by the receiving animal, where they act on neural systems to affect behavior and physiology.

It is important to consider the chemical nature of the compounds because it affects their spread and detection. Compounds that stimulate the olfactory system take many forms with a broad range of characteristics: some are large proteins while others are small lipophilic molecules, some are airborne and highly volatile while others are not. The volatility likely plays a role in whether a chemical compound is detected at a distance or via direct physical contact. The traditional view was that volatile cues stimulate the main olfactory system and nonvolatile cues stimulate the vomeronasal system. Although reasonable based on the evidence available at the time, it is now clear that both the main and vomeronasal systems are capable of detecting both volatile and nonvolatile cues (Dulac and Torello 2003; Leinders-Zufall et al. 2000; Spehr et al. 2006b).

Some chemical cues alter hormone levels, which in turn affect behavior. Others may influence social behavior by conveying information (such as stage of the estrous cycle and therefore likelihood of behavioral receptivity) about individual animals. The processing and effect of these compounds may also vary with the experience of the receiver.

Although many of the chemical cues discussed in this paper fit the definition of pheromones, it is very important to note that chemical cues that are not pheromones affect behavior. For example, red fox urine suppresses locomotor activity of male (but not female) voles in the open field (Perrot-Sinal et al. 1996).

Pregnancy Block in the Mouse

Chemosensory cues can have very dramatic effects on pregnancy in the mouse—in fact, they are capable of terminating pregnancy (Bruce 1959; this effect has not been consistently reported in any other species). The termination of pregnancy by chemosensory cues, called the Bruce effect or pregnancy block, occurs when a recently impregnated mouse aborts her litter in response to chemosensory cues from an unfamiliar male.

In Bruce's initial experiment (Bruce 1959), female albino mice were allowed to copulate with a male albino stud. After insemination was confirmed (by the observation of a vaginal sperm plug), the males were removed from the females' cages. The females were then housed for 24 hours with either an unfamiliar albino male, an unfamiliar wild-type male, a castrated albino male, another albino female, or the original stud male. All the females housed with an albino female or the original stud male remained pregnant. Of females housed with an unfamiliar albino male, 28% lost their litter; of those housed with a castrated unfamiliar albino male, 26% lost their litter; and 71% of those housed with an unfamiliar wild-type male lost their litter. Furthermore, Bruce demonstrated that physical contact was unnecessary as the rate of pregnancy block was similar whether the female was housed with the male or alone in his cage, suggesting that the Bruce effect results from exposure to a substance released by the male rather than to the male per se.

Hormonal and Neural Bases of Pregnancy Block

Chemosensory cues from a strange male appear to activate neurons in the medial amygdala, which in turn affect activity

of the tuberoinfundibular dopamine neurons (Brennan 2004; Brennan and Peele 2003). When activated, these neurons release dopamine, which acts in the anterior pituitary gland to inhibit the release of prolactin from lactotrophs. In the mouse, prolactin is critical for the maintenance of the corpus luteum; without it, the corpus luteum regresses, progesterone levels decrease, and implantation fails. Treatment with prolactin or progesterone immediately (i.e., no more than 48 hours) after mating prevents pregnancy block (Dominic 1966b,c; Rajendren and Dominic 1987, 1988b).

The Bruce effect relies on a complex series of processes that we divide into two classes: pericopulatory and postcopulatory. The first process is the formation of an olfactory memory. At the time of mating, the female forms a memory of her mate's olfactory profile. This requires that the female (1) recognize that she has mated and (2) generate a signal that induces the formation of an olfactory memory of her mate (i.e., it triggers the synaptic events in olfactory areas that underlie the memory). The signal that might trigger these synaptic events has not been unequivocally identified; we speculate that the neuropeptide oxytocin, which is released by vaginal cervical stimulation during copulation, triggers the mouse's formation of the memory of the mate (Wersinger et al. 2008). Others suggest that it is a mating-induced release of norepinephrine in the accessory olfactory bulb (Brennan et al. 1990, 1995). These are not mutually exclusive hypotheses.

The memory of the mate forms within the first few hours of mating (3-7h) and can remain intact after 30 days (Keverne and de la Riva 1982). The formation of the memory requires a functional accessory olfactory bulb—females that received an infusion of lidocaine (a local anesthetic) in the AOB at the time of mating exhibited pregnancy block in response to their mate, suggesting a disruption of memory formation (Kaba et al. 1989). The synthesis of new protein is involved in later stages of memory formation. Pregnancy block was prevented in females that received a protein-synthesis inhibitor in the AOB toward the end of memory formation, 3 to 6 hours after copulation (Kaba et al. 1989), but not when treated immediately after mating (Kaba and Keverne 1988).

At the synaptic level, Brennan and colleagues (1998) have suggested that exposure to cues from a male at the time of mating potentiates glutamate synapses on granule cells. This potentiation enhances feedback inhibition of these granule cells on mitral cells that are activated by chemosensory cues from the male, and the effect of this increased inhibition is that stimuli activating the mitral cells are not transmitted to other regions of the brain. Cues from an unfamiliar male activate a different set of mitral cells that are free of inhibition, and without this inhibition the neural activation passes on to other brain regions, including, ultimately, the tuberoinfundibular dopamine (TIDA) neurons. As mentioned above, activation of the TIDA neurons increases dopamine levels, dopamine inhibits the release of prolactin, and without prolactin, implantation fails.

Irrespective of the mechanism, the empirical data are consistent with the presence of a filter in the accessory olfactory bulb that prevents olfactory stimuli from the familiar male from passing to the brain regions that regulate the TIDA neurons. Halem and colleagues (2001) demonstrated that the induction of Fos-like immunoreactivity in limbic and hypothalamic brain regions in female mice exposed to urine from stimulus males with which they had recently mated was attenuated compared to females exposed to urine from unfamiliar stimulus males. In the AOB, urine induced Fos-like immunoreactivity (Fos-ir[1]) in all groups. These results strongly indicate that signals from the familiar male are filtered at the level of the AOB.[2]

After copulation, a different set of processes occurs. The female is sensitive to the sex and endocrine status of conspecifics present in the environment. Gonad-intact reproductive males, but not castrated males, are capable of inducing pregnancy block (Rajendren and Dominic 1988a). Castrated males and females, which do not interfere with implantation, are capable when treated with androgen of disrupting pregnancy (Hoppe 1975; Rajendren and Dominic 1988a). Urine from adult or juvenile females housed eight per cage, or a pool of urine collected from eight individually housed females, disrupts pregnancy, although is it unclear if the mechanism underlying this effect is similar to that of the Bruce effect (Drickamer 1999).

The female compares olfactory cues from reproductive males in the environment with the olfactory memory of her mate. If the cues do not match that memory, the information passes through the chemosensory responsive circuit, activates the TIDA neurons, and triggers pregnancy block. Although the mate produces the molecules that induce pregnancy block, the stimuli do not activate the TIDA neurons of the female with which he has mated, possibly because the stimulus fails to exit the accessory olfactory bulb.

Characteristics and Identity of Chemosensory Cues Implicated in Pregnancy Block

As mentioned above, mice treated with androgen elicit pregnancy block (Bruce 1959; Drickamer 1999; Rajendren and Dominic 1988a). Although the vast majority of evidence suggests that the cue is present in urine (Dominic 1965, 1966a; Marchlewska-Koj 1977, 1981), at least one report suggests that urine alone is insufficient to reliably induce the block (de Catanzaro et al. 1995). The preputial gland is unnecessary for pregnancy block, since males without this gland (or a vesicular coagulating gland) induce pregnancy block (de Catanzaro et al. 1996; Zacharias et al. 2000).

Which cues signal individual identity and to which cues does the female form an olfactory memory? The cues must be present in the urine. Major histocompatability complex (MHC) peptides are viable candidates. Female mice recently mated with C57BL/6 males exhibit pregnancy block when exposed to the urine of BALB/c males but not when exposed to that of C57BL/6 males (Kelliher et al. 2006). And pregnancy

[2]Lesions of the hippocampus disrupt olfactory learning in the context of maze tests (Alvarez et al. 2002) but have no effect on the induction of the pregnancy block (Selway and Keverne 1990), suggesting that this structure plays no role in the type of olfactory memory that underlies pregnancy block.

block occurs in females mated with C57BL/6 males following exposure to urine from these males treated with MHC peptides from BALB/c males. Removal of the VNO prevents pregnancy block in response to urine from a strange male (Bellringer et al. 1980). If MHC peptides play a role in pregnancy block, then the VNO must be sensitive to them; it is, and it must be intact for MHC peptides to have an effect on pregnancy (Kelliher et al. 2006; Leinders-Zufall et al. 2004).

Like MHC peptide ligands, major urinary proteins (MUPs[1]) are involved in individual and mate recognition (Thom et al. 2008), but experimental evidence does not support their role in the pregnancy block. The fraction of urine containing high molecular weight molecules, which includes the MUPs, fails to induce pregnancy block whereas the fraction containing low molecular weight molecules, which includes volatile compounds, does (Peele et al. 2003). Thus the AOB is activated by the low, but not high, molecular weight fraction in urine. Although MUPs may play a role in other phenotypes regulated by chemosensory cues and are likely sources of chemosensory cues of individual recognition and mate preference (Thom et al. 2008), they are unlikely to play a major role in pregnancy block.

Implications for the Animal Facility

Mice are bred in laboratory animal facilities for many reasons, including the maintenance of transgenic lines, and pregnancy block may be a major factor in poor production of breeding colonies. In fact, it is believed that Bruce began investigating the social environment of the colony because reproductive output was low. In small facilities, breeding females may be routinely exposed to odor cues from unfamiliar males. If reproductive output of a line is low, many investigators conclude the line is subfertile because of the genetic manipulation. We suggest considering and, if necessary, addressing the olfactory environment of a breeding colony with low output, as relatively minor changes may dramatically increase the output of the colony. For example, the presence of the mate appears to protect the female from pregnancy block, so leaving the male with the female after copulation may help. Olfactory isolation of mated females from the remainder of the colony by the use of microisolator tops or single-sex rooms may further ameliorate the condition.

Effects on Puberty

Social cues from conspecifics affect the timing of puberty in the mouse by advancing or delaying it.[3] Both effects appear to be mediated by the olfactory system. Since the cues appear to be different, we discuss them separately.

[3]Puberty is the process by which organisms become reproductively competent. Because it is a process, it cannot be quantified by a single measure. In humans, Tanner stages are used to gauge where in the process a child is. In female rodents the onset of puberty is often defined as the day of vaginal opening, a distinctive, easily observed phenotype. To confirm puberty, vaginal lavage can be used to confirm the presence of estrous cycles.

Puberty Acceleration

Vandenbergh (1973) was the first to report the accelerating effect of male chemosensory cues on puberty in female mice. Juvenile females exposed to an adult, reproductively capable male enter puberty an average of 2 to 3 days earlier than juvenile females housed in the absence of males. Even exposure to male urine alone accelerates puberty. Later, Drickamer (1984) reported that urine from pregnant and lactating females accelerated sexual maturation in juvenile females whereas urine from multiparous, nonlactating singly housed females did not. The phenomenon has been well studied in laboratory mice and also occurs in feral populations of mice (Massey and Vandenbergh 1981), suggesting that it is not a simple artifact of laboratory housing.

Hormonal and Neural Bases

Puberty is characterized by increased activity of the hypothalamic-pituitary-gonadal axis (HPG[1]; Figure 3). Hypothalamic gonadotropin-releasing hormone (GnRH) neurons increase their activity, resulting in an increase in the pituitary

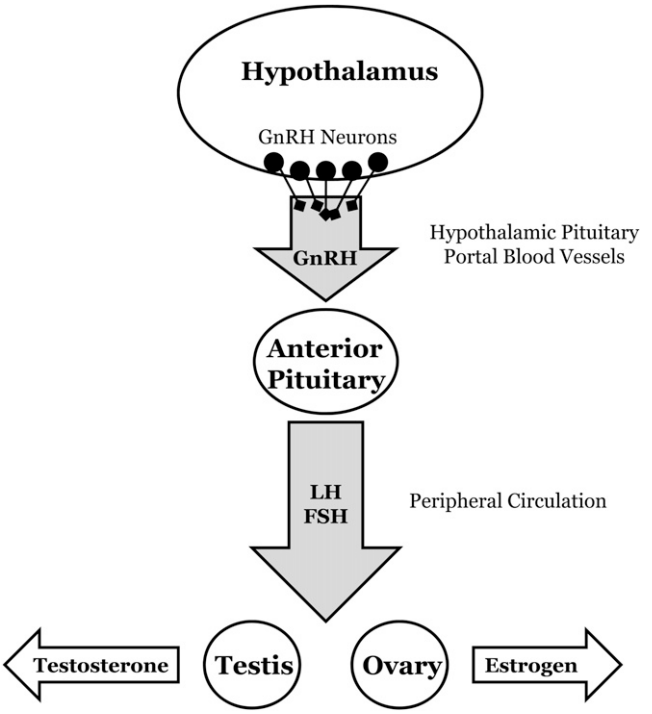

Figure 3 The hypothalamic-pituitary-gonadal axis. Neurons containing gonadotropin-releasing hormone (GnRH) are broadly distributed in the hypothalamus. When excited, these neurons release GnRH into the hypothalamic-pituitary portal blood vessels, which connect the hypothalamus with the anterior pituitary. In the anterior pituitary, GnRH increases the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the peripheral circulatory system by the gonadotrophs. These two hormones, together called the gonadotropins, act on the gonad to stimulate the synthesis of sex steroids (estrogen and testosterone) in the peripheral blood.

synthesis and release of luteinizing hormone (LH[1]) and follicle-stimulating hormone (FSH). These hormones then act at the gonad (the testis or ovary) to increase sex steroid synthesis.

Bronson and Maruniak (1976) demonstrated that exposure of prepubertal female mice to adult males or their urine increases LH output within 30 minutes of exposure. These stimuli had no immediate effect on FSH but over time decreased its levels. The effects after exposure to a stimulus male were greater than after exposure to urine, but the reason is not clear; it is possible that other cues from the male (e.g., tactile cues or chemosensory cues from other parts of the body) play a role. It is also possible that urine cues are more efficiently detected by the female when she is paired with him; for example, she may engage in anogenital investigation, which may provide a strong, concentrated source of odor cues. This suggests that male pheromones are capable of altering activity of the HPG, which provides a putative mechanism for the acceleration of puberty.

An intact vomeronasal organ (VNO[1]) is necessary for urine from males to accelerate puberty (Kaneko et al. 1980; Lomas and Keverne 1982; Wysocki and Lepri 1991), suggesting that the vomeronasal system mediates the effect. Drickamer (1986b) provided further support for this finding by demonstrating that treatment with zinc sulfate, which destroys the main olfactory epithelium without destroying the VNO, has no effect on male-induced puberty advance in females.

Characteristics and Identity of the Cues

Urine of adult male mice (Drickamer and Assmann 1981) or of estrous, pregnant, or lactating females (Drickamer 1983a, 1986a) accelerates puberty of juvenile females whereas urine from castrated males (Pandey and Pandey 1988), juvenile males (Drickamer 1992), and other (i.e., nonestrous, nonpregnant, nonlactating) gonad-intact females does not. Urine from androgen-treated juvenile (Drickamer 1992) or castrated males (Pandey and Pandey 1988) accelerates puberty, strongly suggesting that the accelerating cues are androgen-dependent. The dominance status of the male also affects the ability of his urine to accelerate puberty: urine from dominant males is more effective than that of subordinate males (Drickamer 1983b). Production of the cues that advance puberty does not require an intact VNO in either male or female stimulus animals (Lomas and Keverne 1982), and rendering the producer of the signal anosmic by disrupting the main olfactory system similarly has no effect on the ability of the signal to accelerate puberty (Drickamer 1986b). (As we discuss below, this is not the case with cues that suppress estrous cyclicity in the female.)

The cue itself has not been definitively characterized. Vandenbergh (Vandenbergh et al. 1975) first suggested that the puberty-accelerating compound present in the urine of males was a protein. Although one group reported that two amines (isobutylamine and isoamylamine) advanced vaginal opening in the mouse (Nishimura et al. 1989), a later study reported that compounds do not increase uterine weight in prepubertal females (Price and Vandenbergh 1992). There is a tight correlation between uterine weight and circulating levels of estrogen, so an exposure-induced increase in uterine weight has been used as a marker for the puberty-accelerating effects of various compounds. Thus, compounds that induce puberty via activation of the hypothalamic-pituitary-gonadal axis elevate estrogen levels and, consequently, uterine weight (Figure 3). A series of papers have reported a number of compounds that accelerate puberty in uterine weight assays (Jemiolo et al. 1989; Novotny et al. 1999a,b). Novotny's groups report small compounds that bind to the MUPs present in urine. It is interesting that a natural MUP isolated from male urine increased uterine weight whereas a recombinant MUP did not. This, along with other evidence, suggests that the cue that induced puberty advance is not the MUP per se but rather a complex of compounds and molecules that includes the MUP and its ligands.

Puberty Delay

The onset of puberty in group-housed female mice is significantly longer than in singly housed females or females housed with a male (Drickamer 1977; Massey and Vandenbergh 1980). The signals that delay puberty are present in the urine of group-housed females, as exposure to urine alone delays puberty as does exposure to the animals themselves (Drickamer 1977). The synthesis or release of the signal is density-dependent in laboratory mice. The urine of females housed in groups of four delays puberty after 2 weeks of group housing, while the urine of females housed in groups of six delays puberty after 1 week of group housing (Coppola and Vandenbergh 1985). The signal persists for 10 days after a female is moved from group to single housing. Unlike some pheromonal effects that have been described only in laboratory animals, feral mice populations also exhibit this delay in puberty (Massey and Vandenbergh 1980, 1981).

Several synthetic compounds that are analogues of urinary compounds have also been shown to delay puberty (Jemiolo et al. 1989; Jemiolo and Novotny 1994). A particularly interesting characteristic of this signal is that group-housed female mice do not produce it when they are rendered anosmic by treatment with zinc sulfate (Drickamer 1986b). Thus not only does the olfactory system mediate the response to this signal, it plays a key role in its production.

Implications for the Animal Facility

The effect of olfactory cues on the timing of puberty has important implications for the animal facility. Few researchers and facility staff understand that housing conditions can directly affect the timing of puberty. For studies of adult or very young animals, these effects may not affect the phenotype of interest, but for developmental or other studies involving

peripubertal subjects, differences in the timing of puberty may be a confounding variable if not carefully controlled.

Effects on the Estrous Cycle

Chemosensory cues from conspecifics of both sexes have effects on the estrous cycle of female mice. In 1955 researchers reported that group-housed females entered a state of spontaneous pseudopregnancy (Van Der Lee and Boot 1955), a state associated with sterile mating (or extensive vaginal cervical stimulation). Essentially, the physical stimulus results in the release of a luteotrophic factor and the female experiences hormonal changes identical to those of early pregnancy. A hallmark of pseudopregnancy is that the uterus displays a decidual cell reaction, which is associated with the ability of the uterus to support implantation of the zygote. The decidual cell reaction is assessed by experimentally inducing trauma (e.g., by injecting sesame oil into the lumen of the uterine horn) and measuring the proliferation of decidual cells in the uterine lining near the site of injection (Finn and Keen 1963).

Although the pseudopregnant female does not exhibit cyclicity, pseudopregnancy and anestrus are not the same; the mechanism that induces each state and the hormonal profile of each are very different. In anestrous animals, the HPG is inhibited and hormone levels are low. During pseudopregnancy, the corpus luteum persists and hormone levels are high. Despite the title of the original paper ("Spontaneous Pseudopregnancy in Mice"), the Lee-Boot effect is currently defined as a suppression of estrous cyclicity, as opposed to spontaneous pseudopregnancy, among group-housed females. The mechanism is a lengthening of estrous cycle length by prolonging the diestrous stage of the cycle. The cycle length of pairs of females does not differ significantly from singly housed females (Champlin 1971), but when the group size reaches 3 to 6, there is a significant lengthening of the estrous cycle (Champlin 1971). Whitten (1959) observed that in very large groups of females individuals become anestrous, and concluded that this was because of decreased ovarian weight and the absence of corpora lutea as well as the absence of the vaginal cytology associated with estrus. One study (Ryan and Schwartz 1977) reported that estrous cyclicity disappeared in group-housed female white Swiss mice, although, rather than experiencing a suppression of estrus, these animals appeared to become pseudopregnant. Thus the effect of group housing, a suppression of estrus, is similar in Swiss mice and other strains of mouse. However, the neural and hormonal mechanism appears to be different for this and other strains of *Mus musculus*.

Mechanisms of Action and Characteristics of the Cues

The effect of group housing on cyclicity is at least partially mediated by the vomeronasal organ, as one study has shown that disruption of the VNO reduces the number of females experiencing delayed estrus (Reynolds and Keverne 1979). This same study suggests that other olfactory sensory organs also play a role since disruption of the VNO does not completely eliminate the response to urine collected from group-housed females (Reynolds and Keverne 1979). As mentioned, the brain regions that constitute the chemosensory response circuit (Figure 1) are functionally connected to the HPG (Figure 3). Thus, it is easy to imagine that a chemosensory cue is detected by the olfactory system and that this stimulus is passed on to alter the function of the gonadotropin-releasing hormone neurons at the top of the HPG.

The factor that causes this suppression appears to be chemical in nature and its production mediated by the olfactory system: urine from females in which the VNO has been lesioned fails to alter the estrous cycle of recipient females. But the effect of the inhibitory pheromone is not dependent on the presence of the ovary since ovariectomized group-housed females suppress cyclicity in gonad-intact females. The adrenal gland, however, appears critical for the generation of the cue (Ma et al. 1998), as urine from group-housed, adrenalectomized females fails to suppress cycles, whereas urine from similar females treated with corticosterone inhibited cycles, suggesting the involvement of this hormone in the production of the suppressive factor.

Although cues from females inhibit cyclicity, cues from males appear to stimulate the HPG and the estrous cycle. This has been called the Whitten effect, after the researcher who first reported it (Whitten 1958, 1959; Whitten et al. 1968). Mice typically exhibit 4- to 5-day estrous cycles and mating behavior occurs on one day of this cycle. In a large colony of randomly cycling mice, approximately 20-25% of the females should be behaviorally receptive on any given day. Whitten, however, observed that approximately 50% of the females in his colony mated on the same night, a nonrandom pattern that suggests a synchronization of estrus in the colony. Females exposed to odor cues from a male for 2 days before testing were more likely to mate the first night, suggesting that male chemosensory cues influenced the timing of behavioral estrus and ovulation. Indeed, suppression of estrus combined with the Whitten effect has been used in laboratory animal facilities to help time pregnancy (Scharmann and Wolff 1980). This approach can also be effective in other paradigms when it is advantageous to have subjects in the same stage of the estrous cycle, although it can be difficult to generate sufficient numbers of subjects in a particular stage of the cycle. To produce 10 estrous female mice for treatment on a given day, one would need 40 subjects since, on average, only 25% are in estrus at any given time. The Whitten effect can, however, be exploited to reduce the variability in cycle stage; for example, Dalal and colleagues (2001) used it to generate female subjects in a consistent stage of the estrous cycle for a study delineating the hormonal dependence of gonorrhea vulnerability.

Implications for the Animal Facility

Chemosensory effects on the estrous cycle have many implications for the animal facility. As just mentioned, they may

be exploited to time pregnancies, an important ability for commercial as well as transgenic facilities. They have also been used to reduce phenotypic variability in female subjects by synchronizing or suppressing estrous cycles. However, if unaccounted for, these effects can introduce unintended biases. For example, failure to house animals in similarly sized groups or with the same gender can cause increased variability. And if, as sometimes happens during the breeding of transgenic mice and controls, subjects of the genotypes are consistently housed in different conditions, these effects could introduce a systematic bias. If a genetic manipulation alters the function of the olfactory system, the housing condition may produce further bias if one genotype is capable of responding to pheromonal cues while another is not. Animal facilities and investigators need to specify housing conditions that are optimal for the experimental protocol, given that group-housing females or the presence of males in a room can greatly affect the reproductive (and therefore behavioral) state of their subjects.

Effects on Male Hormone Levels

Although the vast majority of pheromonal effects on reproductive physiology have been reported in the female, pheromones also affect hormone levels in the male.

The exposure of male mice to female mice or to urine from female mice induces a luteinizing hormone (LH) surge (Maruniak and Bronson 1976) in both sexually naïve and sexually experienced males, although the release is larger in sexually experienced males. Coquelin and colleagues (1984) tested the role of the VNO in this phenomenon. In sexually experienced males, surgical removal of the VNO prevented the release of LH in response to female urine but not in response to an ovariectomized female. This finding demonstrates that the release of LH in response to a female involves more than one sensory system, and that the vomeronasal system in particular is necessary for part of this response. The main olfactory system or another sensory modality also contributes to the male response to a female stimulus animal.

Johnston and Bronson (1982) investigated the role of the ovary and the pituitary gland in the production of the pheromone that elicits an LH release from the male. The effect occurs independent of the ovarian state of the stimulus female (Maruniak and Bronson 1976). Indeed, the ovary is unnecessary (Johnston and Bronson 1982). The pituitary, however, is critical: females without a pituitary gland, or urine from such females, fail to induce the LH surge in males (Johnston and Bronson 1982). It is interesting that males are attracted to cues from intact females but not from ovariectomized or hypophysectomized females, suggesting that the ovary is essential for the attractiveness of the female but not for the cue that induces the LH surge (Johnston and Bronson 1982).

The neural mechanism of this response has not been well characterized in the mouse. The protein Fos, present in some neurons after presentation of a stimulus, is used in animal models as a marker of neuronal sensitivity to a stimulus (Bialy and Kaczmarek 1996; Dragunow and Faull 1989; Harris 1998; Hoffman et al. 1993) and is usually detected using immunocytochemical techniques. By comparing the pattern of Fos-ir in subjects exposed to a stimulus with that of unexposed subjects, it is possible to identify neurons sensitive to the stimulus. One important limitation of the technique is that not all neurons that alter their activity in response to a stimulus will express Fos-ir, so it is difficult to interpret the meaning of a lack of Fos-ir. However, interpretation of positive results is easy: the neuron expressing Fos-ir is sensitive to the stimulus. A number of reports have described the pattern of Fos-ir induced by female chemosensory cues (Dudley and Moss 1999; Halem et al. 1999, 2001; Pankevich et al. 2006; Tubbiola and Wysocki 1997). Virtually all of the brain regions shown in Figure 1 show increased Fos-ir after exposure to female chemosensory cues, as would be expected. The connection between the chemosensory responsive circuitry and the hypothalamic-pituitary-gonadal axis is unclear in the mouse. In the hamster, electrical stimulation of the vomeronasal organ increases Fos-ir in GnRH neurons (Meredith and Fewell 2001), demonstrating the existence of neural connections between the VNO and GnRH neurons. Future work will need to define the details of the anatomical connections in mice.

Effects on Social Preferences

Preference for Categories of Social Stimuli

In addition to using odor cues to differentiate among individuals, mice display strong preferences for odor cues associated with certain classes of stimulus animals. For example, female mice use odor cues to identify and avoid parasite-infected males (Kavaliers and Colwell 1995). Male mice prefer the odors of estrous over nonestrous females (Kavaliers and Kinsella 1995). Estrous (but not anestrous) female mice prefer odor cues from dominant males over subordinate males (Mossman and Drickamer 1996). Together, these few examples show that a great deal of information can be gathered by the olfactory system and that mice exhibit strong preferences based on this information.

Pheromonal versus Other Chemosensory Cues

Chemosensory cues exert profound effects on behavior via pheromonal effects. They also influence behavior in mice through mechanisms that may not clearly be pheromonal in nature. Adult male mice typically prefer to investigate estrous female stimulus animals as opposed to anestrous females or males (Bakker et al. 1996, 2002; Pankevich et al. 2006; Pierman et al. 2006; Wersinger et al. 2004), and periestrous female mice prefer to investigate male stimulus animals rather than female stimulus animals. At other times during their cycle, mice have no preference. In addition to

exhibiting preferences for animals, mice display preferences for chemosensory cues alone (e.g., soiled bedding or urine). Are these effects pheromonal? Certainly odor cues from conspecifics alter the behavior or physiology of the receivers. Is the primary function of these cues to communicate with conspecifics? This is difficult to test in many instances. Estrous female bedding has a distinctive chemical signature. Did the compounds emanating from estrous females evolve as a signal to males or did males evolve a mechanism to detect compounds that primarily function to support reproductive physiology but allow the male to predict receptivity in the female? At a practical level and a mechanistic level, this distinction is less important than if one is attempting to define pheromonal versus nonpheromonal effects.

Neural Basis of Social Preference

For an animal to exhibit a preference for one animal over another, several processes must be intact. First, the animal must be able to discriminate between the sexes. Mice with lesions of the VNO, but not the main olfactory epithelium, are able to differentiate male odor cues from female odor cues (Pankevich et al. 2004). Thus, discrimination appears to rely heavily on the main olfactory system in the mouse. However, the *preference* for odor cues of one sex over the other requires the VNO (Pankevich et al. 2004). A male mouse with a functional VNO prefers urine from an estrous female compared to water or urine from a male. If VNO function has been disrupted, the mouse still prefers urine over water, but the preference for estrous female urine over male urine is abolished. An animal without a functional vomeronasal organ that is allowed to physically interact with stimulus animals (anesthetized or awake) prefers to spend time near an estrous female rather than a castrated male. It seems that the subject relies on odor cues processed by the VNO in the absence of the stimulus animal (i.e., when only urine is present) but not when the stimulus animal with its other sensory cues (i.e., auditory, tactile, and visual) is available in close proximity.

What is the mechanism by which odor cues alter odor preferences? There are several theoretical possibilities. One hypothesis that has been tested is that odor cues activate the mesolimbic dopamine system, which has been implicated in reward. A phenomenon termed conditioned place preference (well described by Paredes in this issue) is an effective way to test whether or not animals find a stimulus rewarding. There are many variations on this paradigm. The basic idea is to teach the animal to associate a certain location with a rewarding stimulus; the more rewarding the stimulus, the stronger the animal's preference for the location associated with it. Pankevich and colleagues (2004) used this paradigm to estimate the reward value of an estrous female mouse for male mice with either an intact or lesioned VNO. They used a large three-chambered box, in which one chamber was black with a smooth floor, another was white with a rough floor, and the middle, neutral chamber was gray with a smooth floor. The subjects were exposed to an estrous female in one of the lateral chambers every other day for 10 days, and on alternate days were placed in the contralateral chamber. After the final conditioning trial, the subjects were allowed to freely explore all three chambers. Males with an intact VNO spent more time in the chamber in which they had been exposed to an estrous female than in the other chamber; males with a lesioned VNO failed to exhibit this preference. This suggests that the lack of an intact VNO diminishes the reward value of an estrous female. When subjects with a functional VNO were exposed to the chamber that had been paired with an estrous female (but was empty during this exposure), there were more Fos-ir neurons in the AOB, the VTA, the nucleus accumbens (shell), and areas in the VNO projection pathway than in similarly exposed males with a lesioned VNO.

These findings suggest two important things. First, chemosensory cues need not be present for activity in the VNO circuit to be altered. Second, this conditioned neural response requires inputs from the VNO. It has also been shown that urine odors alone induce Fos-ir in the nucleus accumbens of males with an intact, but not lesioned, VNO. Behaviorally, animals with or without an intact VNO investigate urine odors more than water or nonsocial odors. Together, these findings clearly indicate that the VNO mediates the reward value of social stimuli to a large extent. Since males without an intact VNO nevertheless investigate urine cues more than water or nonsocial odors, some reward value is likely conveyed by other chemosensory systems. Another study provides further supporting evidence for this hypothesis by demonstrating that Fos-ir is increased in the nucleus accumbens of females after their exposure to male chemosensory cues (Moncho-Bogani et al. 2005).

Another paper (Martinez-Hernandez et al. 2006), however, provides evidence that the answer is not simple. The ventral tegmental area (VTA) contains the cell bodies of the neurons that constitute the reward pathways. Lesions of the VTA eliminate the preference for sugar typically exhibited by mice. But these lesions failed to affect the innate preference that female mice display toward male chemosensory cues. This strongly suggests that taste preferences and social odor preferences do not share a dependence on the VTA. Future work is necessary to determine the extent to which social odors act through the reward system or activate the reward circuitry using a pathway independent of the VTA.

Preference for Individuals

Mice exhibit preferences for some individuals based not only on sex but also on other factors. In addition to gauging sex from chemosensory cues, they appear to use these cues to ascertain other information about a conspecific. This information is, in turn, integrated with other information to influence the mouse's behavior. For example, mice have strong social and mating preferences based on major

histocompatability complex (MHC) proteins (Brennan 2004; Brennan and Kendrick 2006; Jordan and Bruford 1998; Spehr et al. 2006a), which can indicate genetic relatedness. Many (but not all) studies report that mice express preferences for sexual partners with an MHC profile different from their own. The adaptive significance of MHC preferences in mice has yet to be demonstrated. One hypothesis is that MHC complement is a marker of the genetic relationship between two animals. Since close inbreeding has strong selective pressure against it, natural selection would favor mice that prefer animals with MHC complements different from theirs over animals with identical MHC complements and that the former would have greater success than the latter. MHC proteins appear to affect the activity of both the main and vomeronasal systems in the mouse (Leinders-Zufall et al. 2004; Spehr et al. 2006a; Wysocki et al. 2004).

Conclusion

For a vast majority of animals chemosensory cues are the primary mechanism for social communication. Often this communication not only transmits information but also directs alterations in a conspecific's physiology and behavior. Here we have briefly reviewed the involvement of chemosensory cues in the mediation of reproductive physiology in the mouse (although many of the concepts elucidated by this model are applicable to other species). In addition to suggesting ways to optimize housing conditions in the laboratory, we believe the information in this article can help to advance scientific understanding of the evolution of social behavior in mammals.

We have described some of the specific effects of chemosensory cues on behavior and physiology, but much remains unknown. More work is needed to fully understand these effects, isolate the exact cues, determine the contribution of each chemosensory system, and define the complete neural mechanisms. Each of these questions has been explored and answers are emerging but a complete picture has yet to be realized.

Acknowledgments

We thank Jennifer Temple for her helpful comments on the manuscript.

References

Alvarez P, Wendelken L, Eichenbaum H. 2002. Hippocampal formation lesions impair performance in an odor-odor association task independently of spatial context. Neurobiol Learn Mem 78:470-476.

Bakker J, Honda S, Harada N, Balthazart J. 2002. Sexual partner preference requires a functional aromatase (cyp19) gene in male mice. Horm Behav 42:158-171.

Bakker J, Van Ophemert J, Slob AK. 1996. Sexual differentiation of odor and partner preference in the rat. Physiol Behav 60:489-494.

Bellringer JF, Pratt HP, Keverne EB. 1980. Involvement of the vomeronasal organ and prolactin in pheromonal induction of delayed implantation in mice. J Reprod Fertil 59:223-228.

Bialy M, Kaczmarek L. 1996. c-Fos expression as a tool to search for the neurobiological base of the sexual behaviour of males. Acta Neurobiol Exp (Wars) 56:567-577.

Breer H, Fleischer J, Strotmann J. 2006. The sense of smell: Multiple olfactory subsystems. Cell Mol Life Sci 63:1465-1475.

Brennan P, Kaba H, Keverne EB. 1990. Olfactory recognition: A simple memory system. Science 250:1223-1226.

Brennan PA. 2004. The nose knows who's who: Chemosensory individuality and mate recognition in mice. Horm Behav 46:231-240.

Brennan PA, Kendrick KM. 2006. Mammalian social odours: Attraction and individual recognition. Philos T Roy Soc B 361:2061-2078.

Brennan PA, Kendrick KM, Keverne EB. 1995. Neurotransmitter release in the accessory olfactory bulb during and after the formation of an olfactory memory in mice. Neuroscience 69:1075-1086.

Brennan PA, Peele P. 2003. Towards an understanding of the pregnancy-blocking urinary chemosignals of mice. Biochem Soc Trans 31:152-155.

Brennan PA, Schellinck HM, de la Riva C, Kendrick KM, Keverne EB. 1998. Changes in neurotransmitter release in the main olfactory bulb following an olfactory conditioning procedure in mice. Neuroscience 87:583-590.

Bronson FH, Maruniak JA. 1976. Differential effects of male stimuli on follicle-stimulating hormone, luteinizing hormone, and prolactin secretion in prepubertal female mice. Endocrinology 98:1101-1108.

Bruce HM. 1959. An exteroceptive block to pregnancy in the mouse. Nature 184:105.

Canteras NS, Simerly RB, Swanson LW. 1992a. Connections of the posterior nucleus of the amygdala. J Comp Neurol 324:143-179.

Canteras NS, Simerly RB, Swanson LW. 1992b. Projections of the ventral premammillary nucleus. J Comp Neurol 324:195-212.

Canteras NS, Simerly RB, Swanson LW. 1995. Organization of projections from the medial nucleus of the amygdala: A PHAL study in the rat. J Comp Neurol 360:213-245.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ. 2002. Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

Champlin AK. 1971. Suppression of oestrus in grouped mice: The effects of various densities and the possible nature of the stimulus. J Reprod Fertil 27:233-241.

Coolen LM, Wood RI. 1998. Bidirectional connections of the medial amygdaloid nucleus in the Syrian hamster brain: Simultaneous anterograde and retrograde tract tracing. J Comp Neurol 399:189-209.

Coppola DM, Vandenbergh JG. 1985. Effect of density, duration of grouping and age of urine stimulus on the puberty delay pheromone in female mice. J Reprod Fertil 73:517-522.

Coquelin A, Clancy AN, Macrides F, Noble EP, Gorski RA. 1984. Pheromonally induced release of luteinizing hormone in male mice: Involvement of the vomeronasal system. J Neurosci 4:2230-2236.

Dalal SJ, Estep JS, Valentin-Bon IE, Jerse AE. 2001. Standardization of the Whitten Effect to induce susceptibility to *Neisseria gonorrhoeae* in female mice. Contemp Top Lab Anim 40:13-17.

de Catanzaro D, Zacharias R, Muir C. 1996. Disruption of early pregnancy by direct and indirect exposure to novel males in mice: Comparison of influences of preputialectomized and intact males. J Reprod Fertil 106:269-274.

de Catanzaro D, Wyngaarden P, Griffiths J, Ham M, Hancox J, Brain D. 1995. Interactions of contact, odor cues, and androgens in strange-male-induced early pregnancy disruptions in mice (*Mus musculus*). J Comp Psychol 109:115-122.

Dominic CJ. 1965. The origin of the pheromones causing pregnancy block in mice. J Reprod Fertil 10:469-472.

Dominic CJ. 1966a. Block to pseudopregnancy in mice caused by exposure to male urine. Experientia 22:534-535.

Dominic CJ. 1966b. The inhibition of the olfactory block to pregnancy in mice by the administration of exogenous progesterone. Naturwissenschaften 53:310-311.

Dominic CJ. 1966c. Observations on the reproductive pheromones of mice. II. Neuro-endocrine mechanisms involved in the olfactory block to pregnancy. J Reprod Fertil 11:415-421.
Dorries KM, Adkins-Regan E, Halpern BP. 1997. Sensitivity and behavioral responses to the pheromone androstenone are not mediated by the vomeronasal organ in domestic pigs. Brain Behav Evol 49:53-62.
Dragunow M, Faull R. 1989. The use of c-fos as a metabolic marker in neuronal pathway tracing. J Neurosci Methods 29:261-265.
Drickamer LC. 1977. Delay of sexual maturation in female house mice by exposure to grouped females or urine from grouped females. J Reprod Fertil 51:77-81.
Drickamer LC. 1983a. Chemosignal effects on puberty in young female mice: Urine from pregnant and lactating females. Dev Psychobiol 16:207-217.
Drickamer LC. 1983b. Male acceleration of puberty in female mice (*Mus musculus*). J Comp Psychol 97:191-200.
Drickamer LC. 1984. Acceleration of puberty in female mice by a urinary chemosignal from pregnant or lactating females: Timing and duration of stimulation. Dev Psychobiol 17:451-455.
Drickamer LC. 1986a. Effects of urine from females in oestrus on puberty in female mice. J Reprod Fertil 77:613-622.
Drickamer LC. 1986b. Peripheral anosmia affects puberty-influencing chemosignals in mice: Donors and recipients. Physiol Behav 37:741-746.
Drickamer LC. 1992. Pre- and postweaning excretion of puberty-influencing chemosignals in house mice. Dev Psychobiol 25:1-16.
Drickamer LC. 1999. Pregnancy in female house mice exposed to urinary chemosignals from other females. J Reprod Fertil 115:233-241.
Drickamer LC, Assmann SM. 1981. Acceleration and delay of puberty in female housemice: Methods of delivery of the urinary stimulus. Dev Psychobiol 14:487-497.
Dudley CA, Moss RL. 1999. Activation of an anatomically distinct subpopulation of accessory olfactory bulb neurons by chemosensory stimulation. Neuroscience 91:1549-1556.
Dulac C, Torello AT. 2003. Molecular detection of pheromone signals in mammals: From genes to behaviour. Nat Rev Neurosci 4:551-562.
Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW. 1999. Associative processes in addiction and reward: The role of amygdala-ventral striatal subsystems. Ann N Y Acad Sci 877:412-438.
Fanselow MS, LeDoux JE. 1999. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.
Finn CA, Keen PM. 1963. The induction of deciduomata in the rat. J Embryol Exp Morphol 11:673-682.
Haberly LB. 2001. Parallel-distributed processing in olfactory cortex: New insights from morphological and physiological analysis of neuronal circuitry. Chem Senses 26:551-576.
Halem HA, Cherry JA, Baum MJ. 1999. Vomeronasal neuroepithelium and forebrain Fos responses to male pheromones in male and female mice. J Neurobiol 39:249-263.
Halem HA, Cherry JA, Baum MJ. 2001. Central forebrain Fos responses to familiar male odours are attenuated in recently mated female mice. Eur J Neurosci 13:389-399.
Halpern M, Jia C, Shapiro LS. 1998. Segregated pathways in the vomeronasal system. Microsc Res Tech 41:519-529.
Harris JA. 1998. Using c-fos as a neural marker of pain. Brain Res Bull 45:1-8.
Herrada G, Dulac C. 1997. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 90:763-773.
Hoffman GE, Smith MS, Verbalis JG. 1993. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol 14:173-213.
Hoppe PC. 1975. Genetic and endocrine studies of the pregnancy-blocking pheromone of mice. J Reprod Fertil 45:109-115.
Jemiolo B, Andreolini F, Xie TM, Wiesler D, Novotny M. 1989. Puberty-affecting synthetic analogs of urinary chemosignals in the house mouse, *Mus domesticus*. Physiol Behav 46:293-298.
Jemiolo B, Novotny M. 1994. Inhibition of sexual maturation in juvenile female and male mice by a chemosignal of female origin. Physiol Behav 55:519-522.
Johnston RE, Bronson F. 1982. Endocrine control of female mouse odors that elicit luteinizing hormone surges and attraction in males. Biol Reprod 27:1174-1180.
Jordan WC, Bruford MW. 1998. New perspectives on mate choice and the MHC. Heredity 81 (Pt 3):239-245.
Kaba H, Keverne EB. 1988. The effect of microinfusions of drugs into the accessory olfactory bulb on the olfactory block to pregnancy. Neuroscience 25:1007-1011.
Kaba H, Rosser A, Keverne B. 1989. Neural basis of olfactory memory in the context of pregnancy block. Neuroscience 32:657-662.
Kaneko N, Debski EA, Wilson MC, Whitten WK. 1980. Puberty acceleration in mice. II. Evidence that the vomeronasal organ is a receptor for the primer pheromone in male mouse urine. Biol Reprod 22:873-878.
Karlson P, Luscher M. 1959. Pheromones: A new term for a class of biologically active substances. Nature 183:55-56.
Kavaliers M, Colwell DD. 1995a. Discrimination by female mice between the odours of parasitized and non-parasitized males. Proc Biol Sci 261:31-35.
Kavaliers M, Kinsella DM. 1995b. Male preference for the odors of estrous female mice is reduced by the neurosteroid pregnenolone sulfate. Brain Res 682:222-226.
Kelliher KR. 2007. The combined role of the main olfactory and vomeronasal systems in social communication in mammals. Horm Behav 52:561-570.
Kelliher KR, Spehr M, Li XH, Zufall F, Leinders-Zufall T. 2006. Pheromonal recognition memory induced by TRPC2-independent vomeronasal sensing. Eur J Neurosci 23:3385-3390.
Keverne EB, de la Riva C. 1982. Pheromones in mice: Reciprocal interaction between the nose and brain. Nature 296:148-150.
Kevetter GA, Winans SS. 1981a. Connections of the corticomedial amygdala in the golden hamster. I. Efferents of the "vomeronasal amygdala". J Comp Neurol 197:81-98.
Kevetter GA, Winans SS. 1981b. Connections of the corticomedial amygdala in the golden hamster. II. Efferents of the "olfactory amygdala". J Comp Neurol 197:99-111.
Kippin TE, Cain SW, Pfaus JG. 2003. Estrous odors and sexually conditioned neutral odors activate separate neural pathways in the male rat. Neuroscience 117:971-979.
Krettek JE, Price JL. 1978a. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J Comp Neurol 178:225-254.
Krettek JE, Price JL. 1978b. A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. J Comp Neurol 178:255-280.
Leinders-Zufall T, Brennan P, Widmayer P, Chandramani P, Maul-Pavicic A, Jager M, Xiao-Hong L, Breer H, Zufall F, Boehm T. 2004. MHC class I peptides as chemosensory signals in the vomeronasal organ. Science 306:1033-1037.
Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, Zufall F. 2000. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature 405:792-796.
Leise EM. 1990. Modular construction of nervous systems: A basic principle of design for invertebrates and vertebrates. Brain Res Brain Res Rev 15:1-23.
Licht G, Meredith M. 1987. Convergence of main and accessory olfactory pathways onto single neurons in the hamster amygdala. Exp Brain Res 69:7-18.
Lomas DE, Keverne EB. 1982. Role of the vomeronasal organ and prolactin in the acceleration of puberty in female mice. J Reprod Fertil 66:101-107.
Ma W, Miao Z, Novotny MV. 1998. Role of the adrenal gland and adrenal-mediated chemosignals in suppression of estrus in the house mouse: The Lee-Boot effect revisited. Biol Reprod 59:1317-1320.
Marchlewska-Koj A. 1977. Pregnancy block elicited by urinary proteins of male mice. Biol Reprod 17:729-732.

Marchlewska-Koj A. 1981. Pregnancy block elicited by male urinary peptides in mice. J Reprod Fertil 61:221-224.
Martinez-Hernandez J, Lanuza E, Martinez-Garcia F. 2006. Selective dopaminergic lesions of the ventral tegmental area impair preference for sucrose but not for male sexual pheromones in female mice. Eur J Neurosci 24:885-893.
Martinez-Marcos A, Halpern M. 1999. Differential centrifugal afferents to the anterior and posterior accessory olfactory bulb. Neuroreport 10:2011-2015.
Maruniak JA, Bronson FH. 1976. Gonadotropic responses of male mice to female urine. Endocrinology 99:963-969.
Massey A, Vandenbergh JG. 1980. Puberty delay by a urinary cue from female house mice in feral populations. Science 209:821-822.
Massey A, Vandenbergh JG. 1981. Puberty acceleration by a urinary cue from male mice in feral populations. Biol Reprod 24:523-527.
Matsumoto S, Hull JJ, Ohnishi A, Moto K, Fonagy A. 2007. Molecular mechanisms underlying sex pheromone production in the silkmoth, *Bombyx mori*: Characterization of the molecular components involved in bombykol biosynthesis. J Insect Physiol 53:752-759.
Meredith M, Fewell G. 2001. Vomeronasal organ: Electrical stimulation activates Fos in mating pathways and in GnRH neurons. Brain Res 922:87-94.
Moncho-Bogani J, Martinez-Garcia F, Novejarque A, Lanuza E. 2005. Attraction to sexual pheromones and associated odorants in female mice involves activation of the reward system and basolateral amygdala. Eur J Neurosci 21:2186-2198.
Mori K, Nagao H, Yoshihara Y. 1999. The olfactory bulb: Coding and processing of odor molecule information. Science 286:711-715.
Mossman CA, Drickamer LC. 1996. Odor preferences of female house mice (*Mus domesticus*) in seminatural enclosures. J Comp Psychol 110:131-138.
Nishimura K, Utsumi K, Yuhara M, Fujitani Y, Iritani A. 1989. Identification of puberty-accelerating pheromones in male mouse urine. J Exp Zool 251:300-305.
Novotny MV, Jemiolo B, Wiesler D, Ma W, Harvey S, Xu F, Xie TM, Carmack M. 1999a. A unique urinary constituent, 6-hydroxy-6-methyl-3-heptanone, is a pheromone that accelerates puberty in female mice. Chem Biol 6:377-383.
Novotny MV, Ma W, Wiesler D, Zidek L. 1999b. Positive identification of the puberty-accelerating pheromone of the house mouse: The volatile ligands associating with the major urinary protein. Proc Biol Sci 266:2017-2022.
Ojima H, Mori K, Kishi K. 1984. The trajectory of mitral cell axons in the rabbit olfactory cortex revealed by intracellular HRP injection. J Comp Neurol 230:77-87.
Pandey SD, Pandey SC. 1988. Male-induced puberty acceleration in young female wild mice: Hormonal regulation and source of pheromonal cue. Acta Physiol Hung 72:165-170.
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 24:9451-9457.
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 120:925-936.
Paredes RG. 2008. Evaluating the neurobiology of sexual reward. ILAR J 50:15-27.
Peele P, Salazar I, Mimmack M, Keverne EB, Brennan PA. 2003. Low molecular weight constituents of male mouse urine mediate the pregnancy block effect and convey information about the identity of the mating male. Eur J Neurosci 18:622-628.
Perrot-Sinal TS, Heale VR, Ossenkopp KP, Kavaliers M. 1996. Sexually dimorphic aspects of spontaneous activity in meadow voles (*Microtus pennsylvanicus*): Effects of exposure to fox odor. Behav Neurosci 110:1126-1132.
Pierman S, Douhard Q, Balthazart J, Baum MJ, Bakker J. 2006. Attraction thresholds and sex discrimination of urinary odorants in male and female aromatase knockout (ArKO) mice. Horm Behav 49:96-104.
Price MA, Vandenbergh JG. 1992. Analysis of puberty-accelerating pheromones. J Exp Zool 264:42-45.
Rajendren G, Dominic CJ. 1987. The male-induced pregnancy block (the Bruce effect) in mice: Re-evaluation of the ability of exogenous progesterone in preventing implantation failure. Exp Clin Endocrinol 89:188-196.
Rajendren G, Dominic CJ. 1988a. Effect of cyproterone acetate on the pregnancy-blocking ability of male mice and the possible chemical nature of the pheromone. J Reprod Fertil 84:387-392.
Rajendren G, Dominic CJ. 1988b. The male-induced implantation failure (the Bruce effect) in laboratory mice: Investigations on luteal failure in pregnancy-blocked females. Exp Clin Endocrinol 92:13-19.
Regnier FE, Law JH. 1968. Insect pheromones. J Lipid Res 9:541-551.
Rekwot PI, Ogwu D, Oyedipe EO, Sekoni VO. 2001. The role of pheromones and biostimulation in animal reproduction. Anim Reprod Sci 65:157-170.
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 79:1245-1255.
Reynolds J, Keverne EB. 1979. The accessory olfactory system and its role in the pheromonally mediated suppression of oestrus in grouped mice. J Reprod Fertil 57:31-35.
Ryan KD, Schwartz NB. 1977. Grouped female mice: Demonstration of pseudopregnancy. Biol Reprod 17:578-583.
Salazar I, Brennan PA. 2001. Retrograde labelling of mitral/tufted cells in the mouse accessory olfactory bulb following local injections of the lipophilic tracer DiI into the vomeronasal amygdala. Brain Res 896:198-203.
Scalia F, Winans SS. 1975. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. J Comp Neurol 161:31-55.
Scharmann W, Wolff D. 1980. Production of timed pregnant mice by utilization of the Whitten effect and a simple cage system. Lab Anim Sci 30:206-208.
Schoenbaum G, Eichenbaum H. 1995. Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. J Neurophysiol 74:733-750.
Schoenbaum G, Chiba AA, Gallagher M. 1999. Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci 19:1876-1884.
Schoenbaum G, Nugent SL, Saddoris MP, Setlow B. 2002. Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. Neuroreport 13:885-890.
Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. 2003. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron 39:855-867.
Scott JW. 1981. Electrophysiological identification of mitral and tufted cells and distributions of their axons in olfactory system of the rat. J Neurophysiol 46:918-31.
Selway R, Keverne EB. 1990. Hippocampal lesions are without effect on olfactory memory formation in the context of pregnancy block. Physiol Behav 47:249-252.
Setlow B, Holland PC, Gallagher M. 2002. Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. Behav Neurosci 116:267-275.
Shepherd GM. 1992. Toward a consensus working model for olfactory transduction. Soc Gen Physiol Ser 47:19-37.
Shipley MT, Ennis M. 1996. Functional organization of olfactory system. J Neurobiol 30:123-176.
Signoret JP. 1970. Reproductive behaviour of pigs. J Reprod Fertil Suppl 11:Suppl 11:105+.
Spehr M, Kelliher KR, Li XH, Boehm T, Leinders-Zufall T, Zufall F. 2006a. Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. J Neurosci 26:1961-1970.
Spehr M, Spehr J, Ukhanov K, Kelliher KR, Leinders-Zufall T, Zufall F. 2006b. Parallel processing of social signals by the mammalian main and accessory olfactory systems. Cell Mol Life Sci 63:1476-1484.
Thom MD, Stockley P, Jury F, Ollier WE, Beynon RJ, Hurst JL. 2008. The direct assessment of genetic heterozygosity through scent in the mouse. Curr Biol 18:619-623.

Thompson RR, Moore FL. 2000. Vasotocin stimulates appetitive responses to the visual and pheromonal stimuli used by male roughskin newts during courtship. Horm Behav 38:75-85.

Tirindelli R, Mucignat-Caretta C, Ryba NJ. 1998. Molecular aspects of pheromonal communication via the vomeronasal organ of mammals. Trends Neurosci 21:482-486.

Tubbiola ML, Wysocki CJ. 1997. FOS immunoreactivity after exposure to conspecific or heterospecific urine: Where are chemosensory cues sorted? Physiol Behav 62:867-870.

Van Der Lee S, Boot LM. 1955. Spontaneous pseudopregnancy in mice. Acta Physiol Pharm N 4:442-444.

Vandenbergh JG. 1973. Acceleration and inhibition of puberty in female mice by pheromones. J Reprod Fertil Suppl 19:411-419.

Vandenbergh JG, Whitsett JM, Lombardi JR. 1975. Partial isolation of a pheromone accelerating puberty in female mice. J Reprod Fertil 43:515-523.

Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. Cell 79:981-991.

Wersinger SR, Kelliher KR, Zufall F, Lolait SJ, O'Carroll AM, Young WS 3rd. 2004. Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task. Horm Behav 46:638-645.

Wersinger SR, Temple JL, Caldwell HK, Young WS 3rd. 2008. Inactivation of the oxytocin and the vasopressin (Avp) 1b receptor genes, but not the Avp 1a receptor gene, differentially impairs the Bruce effect in laboratory mice (*Mus musculus*). Endocrinology 149:116-121.

Whitten WK. 1958. Modification of the oestrous cycle of the mouse by external stimuli associated with the male: Changes in the oestrous cycle determined by vaginal smears. J Endocrinol 17:307-313.

Whitten WK. 1959. Occurrence of anoestrus in mice caged in groups. J Endocrinol 18:102-107.

Whitten WK, Bronson FH, Greenstein JA. 1968. Estrus-inducing pheromone of male mice: Transport by movement of air. Science 161:584-585.

Winans SS, Scalia F. 1970. Amygdaloid nucleus: New afferent input from the vomeronasal organ. Science 170:330-332.

Wysocki CJ. 1979. Neurobehavioral evidence for the involvement of the vomeronasal system in mammalian reproduction. Neurosci Biobehav Rev 3:301-341.

Wysocki CJ, Lepri JJ. 1991. Consequences of removing the vomeronasal organ. J Steroid Biochem Mol Biol 39:661-669.

Wysocki CJ, Yamazaki K, Curran M, Wysocki LM, Beauchamp GK. 2004. Mice (*Mus musculus*) lacking a vomeronasal organ can discriminate MHC-determined odortypes. Horm Behav 46:241-246.

Yokoi M, Mori K, Nakanishi S. 1995. Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. Proc Natl Acad Sci U S A 92:3371-3375.

Zacharias R, de Catanzaro D, Muir C. 2000. Novel male mice disrupt pregnancy despite removal of vesicular-coagulating and preputial glands. Physiol Behav 68:285-290.