From Pheromones to Behavior

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Tirindelli R, Dibattista M, Pifferi S, Menini A. From Pheromones to Behavior. Physiol Rev 89: 921–956, 2009; doi:10.1152/physrev.00037.2008.—In recent years, considerable progress has been achieved in the comprehension of the profound effects of pheromones on reproductive physiology and behavior. Pheromones have been classified as molecules released by individuals and responsible for the elicitation of specific behavioral expressions in members of the same species. These signaling molecules, often chemically unrelated, are contained in body fluids like urine, sweat, specialized exocrine glands, and mucus secretions of genitals. The standard view of pheromone sensing was based on the assumption that most mammals have two separated olfactory systems with different functional roles: the main olfactory system for recognizing conventional odorant molecules and the vomeronasal system specifically dedicated to the detection of pheromones. However, recent studies have reexamined this traditional interpretation showing that both the main olfactory and the vomeronasal systems are actively involved in pheromonal communication. The current knowledge on the behavioral, physiological, and molecular aspects of pheromone detection in mammals is discussed in this review.

I. INTRODUCTION

Chemosensation is one of the sensory modalities that animals have evolved to analyze the chemical properties of the external world, enabling the detection and the discrimination of a very high number of molecules with different structures. Substances carrying a chemical message among animals are termed "semiochemicals," from...
the Greek “semeion” (sign). An important class of semiochemicals is constituted by pheromones. The term pheromone, based on the Greek "pherein" (to carry or transfer) and "hormon" (to stimulate or excite), was introduced in 1959 by the entomologists Peter Karlson and Martin Lüscher (151) to identify specific biologically active substances “which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behavior or a developmental process.” In the same year, the first insect pheromone bombykol, released by the female silkworm to attract mates, was characterized by Butenandt et al. (43). The use of pheromones among insects has been extensively investigated, and interested readers may consult several reviews on the subject (4, 111, 130, 379).

Another class of semiochemicals is constituted by allelomones: substances produced by members of one species that influence the behavior of members of another species (for a review, see Ref. 333). Although in this review we will mainly discuss the effects elicited by pheromones in mammals, we will also briefly mention some examples of allelomonal communication (5, 107, 108, 109).

In most mammals, the nasal cavity contains at least two major sensory systems that may be involved in the detection of pheromones: the vomeronasal and the main olfactory systems. Evidence indicates that some behaviors are mediated exclusively by one or by a complementary action of both systems. For example, Bruce (37) reported that, when female mice that have been recently inseminated are exposed to urine of a male different from the one they mated with, the pregnancy is interrupted and the female returns to estrus. This effect depends on a functional vomeronasal system (15). Differently, the nipple search in rabbit pups is one of the typical examples of behavior strictly dependent on an intact main olfactory system (119). In other instances, behavioral responses are mediated by both the vomeronasal and the main olfactory systems. This is the case of female hamsters, whose ultrasonic calling during estrus is abolished by the inactivation of either the vomeronasal or the main olfactory system (145). In recent years, the discovery of distinct pheromone receptor families coupled to a complex network of signal transduction pathways, together with advances in molecular genetics and functional electrophysiological and imaging techniques, have greatly advanced our understanding of the physiological role of pheromones.

II. MAMMALIAN PHEROMONES

A. Pheromones in Various Animal Orders

Pheromones have a very large chemical variability (Table 1), and therefore they are preferably grouped according to their functions rather than to their structural determinants. For example, trace pheromones allow attracting a conspecific, while alarm pheromones alert other conspecifics about an external challenge and can evoke aggressive behaviors.

A first classification groups intraspecific signaling molecules in releaser and primer pheromones (239). Releaser pheromones lead to individual recognition and evoke short-latency behavioral responses in the conspecific like in aggressive attacks, or mating, or territory marking. Conversely, primer pheromones induce delayed responses that are commonly mediated through the activation of the neuroendocrine system. Thus, through the emission of chemical cues, an individual forces the hormonal balance of a receiver, thus modulating its reproductive status.

In mammals, pheromones have been reported to be involved in a variety of behavioral effects also in unrelated species and, surprisingly, some pheromonal molecules are shared, with different purposes, between species.

1. Proboscidea

Despite their size, which would obviously preclude extensive behavioral studies, Asian and African elephants have been largely investigated for their pheromonal responses by the late Bets Rasmussen. During musth, a periodic condition in which mature males are characterized by highly aggressive behavior, elephants produce large quantities of a specific pheromone, frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane), that is released in temporal gland secretions, urine, and breath (307). Adult males are mostly indifferent to frontalin, whereas subadult males are highly reactive, often exhibiting repulsion or avoidance. Female chemosensory responses to frontalin vary with the hormonal status. Females in the follicular phase are the most responsive and often demonstrate mating-related behaviors subsequent to frontalin stimulation (304). Furthermore, female Asian elephants excrete a urinary pheromone, (Z)-7-dodecen-1-yl acetate, to signal to males their readiness to mate (305). Interestingly, females of more than 100 species of insects use the same compound as part of their pheromone blend to attract insect males. The finding that the same molecule may be shared by unrelated species for a different pheromonal purpose is only apparently surprising, owing to the fact that structural constraints are often dictated by common metabolic pathways.

2. Marsupialia

In marsupials, the gray short-tailed opossum (Monodelphis domestica) communicates by scent marking. The male opossum possesses a prominent suprasternal scent gland, whose extracts strongly attract female opossums (131), stimulating the estrus in anestrous females via the vomeronasal system.
3. Soricomorpha (Insectivores)

In musk shrew (*Suncus murinus*), virgin females exposed to cages recently vacated by an adult male receive mounts from males significantly sooner than females exposed to cages soiled by a castrated male, by another female, or to a clean cage (310).

### Table 1. Table of pheromones

<table>
<thead>
<tr>
<th>Pheromone</th>
<th>Origin</th>
<th>Organ</th>
<th>Target</th>
<th>Cellular/Biochemical Response</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,5-Dimethyl pyrazine</td>
<td>Mouse female urine</td>
<td>VNO</td>
<td>V1R neurons</td>
<td>IP$_3$ production in the VNO (377)</td>
<td>Suppression of estrus cycle (Lee Boot-effect) (219, 281)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td><em>Electrical response and calcium influx in VNO neurons (187)</em></td>
<td></td>
</tr>
<tr>
<td>2-Sec-butyl4,5-dihydrothiazole (BT)</td>
<td>Mouse male urine</td>
<td>VNO</td>
<td>V1R neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (187)</em></td>
<td>Estrus induction and synchronization (Whitten effect) (138)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Protooncogene expression in the AOB (32, 95)</em></td>
<td>Puberty acceleration (Vandenbergh effect) (266, 283)</td>
</tr>
<tr>
<td>2,3-Dehydro-exo-brevicomin (DB)</td>
<td>Mouse male urine</td>
<td>VNO</td>
<td>V1R neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (187)</em></td>
<td>Estrus induction and synchronization (Whitten effect) (138)</td>
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<td></td>
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<td><em>Protooncogene expression in the AOB (32, 95)</em></td>
<td>Puberty acceleration (Vandenbergh effect) (266, 283)</td>
</tr>
<tr>
<td>α- and β-Farnesene</td>
<td>Mouse preputial glands</td>
<td>VNO</td>
<td>V1R neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (187)</em></td>
<td>Puberty acceleration (Vandenbergh effect) (219)</td>
</tr>
<tr>
<td>6-Hydroxy-16-methyl-3-heptanone</td>
<td>Mouse male urine</td>
<td>VNO</td>
<td>V1ra and V1rb (V1Rs) neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (61, 187)</em></td>
<td>Estrus extension (137)</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>Mouse urine</td>
<td>VNO</td>
<td>V1R neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (187)</em></td>
<td>Suppression of estrus cycle (Lee Boot-effect) (281)</td>
</tr>
<tr>
<td>α-Pentyl acetate</td>
<td>Mouse urine</td>
<td>VNO</td>
<td>V1ra and V1rb (V1Rs) neurons</td>
<td><em>Electrical response in VNO neurons (61)</em></td>
<td>Estrus acceleration and vaginal opening (276, 301)</td>
</tr>
<tr>
<td>Isobutylamine</td>
<td>Mouse urine</td>
<td>VNO</td>
<td>V1ra and V1rb (V1Rs) neurons</td>
<td><em>Electrical response in VNO neurons (61)</em></td>
<td>Attractiveness to females (2)</td>
</tr>
<tr>
<td>3-Amino-s-triazole (similar to BT)</td>
<td>Mouse male urine</td>
<td>?</td>
<td>?</td>
<td></td>
<td>Attractiveness to females (2)</td>
</tr>
<tr>
<td>4-Ethyl phenol</td>
<td>Mouse male urine</td>
<td>?</td>
<td>?</td>
<td></td>
<td>Attractiveness to females (2)</td>
</tr>
<tr>
<td>4-Ethyl-2,7-dimethyl octane (similar to DB)</td>
<td>Mouse male urine</td>
<td>?</td>
<td>?</td>
<td></td>
<td>Repulsion to mouse (territorial marking?) (2)</td>
</tr>
<tr>
<td></td>
<td>Mouse male urine</td>
<td>?</td>
<td>?</td>
<td></td>
<td>Attractiveness to females (2)</td>
</tr>
<tr>
<td>3-Cyclohexene-1-methanolMajor urinary proteins (MUPs)</td>
<td>Mouse male urine</td>
<td>?</td>
<td>?</td>
<td></td>
<td>Puberty acceleration (Vandenbergh effect) (266, 283)</td>
</tr>
<tr>
<td></td>
<td>Mouse urine</td>
<td>VNO</td>
<td>V2R neurons</td>
<td><em>Electrical response and calcium influx in VNO neurons (47, 163)</em></td>
<td>Individual recognition (49, 123, 375)</td>
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<td><em>In vitro survival and proliferation of VNO neurons (408)</em></td>
<td>Internmale aggressiveness (398)</td>
</tr>
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<td></td>
<td><em>Electrical response and calcium influx in MOE and VNO neurons (334)</em></td>
<td>Attractiveness to individuals of a different mouse strain (398)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td><em>Single-cell recording in VNO neurons (156, 187)</em></td>
<td>Pregnancy block (Bruce effect)</td>
</tr>
<tr>
<td>MHC class I peptides</td>
<td>Mouse urine</td>
<td>VNO</td>
<td>V2R neurons</td>
<td><em>Electrical response and calcium influx in MOE and VNO neurons (334)</em></td>
<td>Internmale aggressiveness (398)</td>
</tr>
<tr>
<td>(SYFPEITHIE, AAPDINREFT)</td>
<td></td>
<td></td>
<td></td>
<td><em>Single-cell recording in VNO neurons (156, 187)</em></td>
<td>Pregnancy block (Bruce effect)</td>
</tr>
<tr>
<td>Exocrine gland secreting peptides</td>
<td>Extraorbital lacrimal glands</td>
<td>VNO</td>
<td>V2R neurons</td>
<td><em>Electrical response in VNO neurons (162, 163)</em></td>
<td>?</td>
</tr>
<tr>
<td>o2-Urinary proteins (o2u)</td>
<td>Rat urine</td>
<td>VNO</td>
<td>V2R neurons</td>
<td><em>G protein activation and IP$_3$ production in the VNO (176, 321)</em></td>
<td>?</td>
</tr>
<tr>
<td>Dodecyl-propionate Aphrodisin</td>
<td>Rat preputial gland</td>
<td>?</td>
<td>?</td>
<td><em>IP$_3$ production in the VNO and c-fos expression in AOB neurons (136, 177)</em></td>
<td>Ano-genital licking (36)</td>
</tr>
<tr>
<td></td>
<td>Hamster vaginal glands</td>
<td>VNO</td>
<td>?</td>
<td></td>
<td>Copulatory behavior in male (356)</td>
</tr>
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<td></td>
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<td></td>
<td><em>IP$_3$ production in the VNO and c-fos expression in AOB neurons (136, 177)</em></td>
<td>Attraction and oral grasping in pups (335)</td>
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<td><em>IP$_3$ production in the VNO and c-fos expression in AOB neurons (136, 177)</em></td>
<td>Mating-associated behaviors in males (306)</td>
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<td>Repulsion, attractiveness, and sexual behavior depending on the racemic ratio (92, 304)</td>
</tr>
</tbody>
</table>

VNO, vomeronasal organ; MOE, main olfactory epithelium; AOB, accessory olfactory bulb. Reference numbers are given in parentheses.
In tree shrew (*Tupaia belangeri*), both males and females mark their surroundings with urine and skin gland secretions. Males mark more frequently than females in the absence of conspecific scent; moreover, male scent stimulates marking in females. Interestingly, increasing amounts of male scent result in a corresponding increase in marking by females (114), suggesting that, in the wild, pheromones may not exert an all-or-none effect but rather act in a dose-specific fashion.

4. Carnivora and Ungulata

Both carnivores (dogs and cats) and ungulates commonly mark their territory with urine and display a specific behavior called “flehmen” (67, 88, 391). In the flehmen reaction, animals, after the physical contact with a conspecific or with scents emanating from its excretions (urine or feces), lift the head, draw back their lips, and push the tongue towards the anterior region of the palate in a manner that makes them appear to be “grimacing.” It is commonly believed that this attitude would allow a faster transfer of pheromones into the olfactory organs.

The saliva of the adult male pig contains a mixture of steroids (boar odor). When a boar becomes aggressive or sexually aroused, it produces copious amount of saliva whose pungent odor is attractive to estrus females (287) and facilitates the display of the estrous female’s mating posture (352). The major component of the boar odor is androstenone, a sexually dimorphic steroid that elicits both behavioral responses (65). Curiously, androstenone has been hypothesized to be a human pheromone as well (18, 96).

5. Primata

Primates scarcely rely on chemical communication; however, the ring-tailed lemur (*Lemur catta*) exhibits the most highly developed olfactory system among all species belonging to this order (75, 83, 338). *Lemur catta* possesses a suite of specialized scent glands and a variety of scent-marking displays (147, 258, 338). Both sexes have apocrine, located on the wrists, and sebaceous gland fields in their genital regions and adopt distinctive handstand postures to deposit glandular secretions on substrates. Males mix the glandular secretions and then deposit this mixture via “wrist marking.” They also impregnate their tail fur with their own secretions and then waft their tail at opponents during characteristic “stink fights” (147).

There is nowadays a general consensus for the existence of a chemical communication in humans, although there is still a great controversy about the existence, extent, and nature of human pheromones. Among the best and first known effects attributed to human pheromones is the synchronization of menstrual cycle (237, 238, 367) and the dimorphic activation of brain areas (332). Exposure to odorless axillary’s secretions from females in the late follicular and ovulatory phase of the menstrual cycle, respectively, shortens and lengthens the menstrual cycles of recipient females, accelerating or delaying the luteinizing hormone (LH) surge (367). Women smelling an androgen-like compound activate the preoptic and ventromedial nuclei of the hypothalamus in contrast to men who activate the paraventricular and dorsomedial nuclei hypothalamus when smelling an estrogen-like substance (332). Although a robust hypothalamic response is commonly observed with ordinary odorants, such a dramatic dimorphic difference can be interpreted as a hormonal response to pheromone stimulation.

The apocrine gland secretion of the axilla in combination with the microbial flora is probably the most relevant source of candidate compounds for human pheromones, which consists of a blend of molecules characterizing the individual body odor (14). However, salivary and seminal secretions and urine represent other reservoirs of putative human pheromones. The male sweat and salivary progestrone derivative androstadienone (4,16-androstadien-3-one) (AND) has been indicated as a putative human pheromone influencing brain activity (20, 331, 332), endocrine levels (406), physiological arousal, context-dependent mood, and sexual orientation (132, 133, 212, 232, 300). Woman urine contains the estrogen like steroid estradiol-3,5(10),16-tetraen-3-ol (EST) that also appears to be a good candidate compound for human pheromones (20, 132, 331, 332).

In studies of brain activity by positron emission tomography, as above reported, smelling AND and EST activates regions primarily incorporating the sexually dimorphic nuclei of the anterior hypothalamus (332), and this activation is differentiated with respect to sex and compound. Very interestingly, homosexual males process AND similarly to heterosexual women rather than to heterosexual men (20). Conversely, lesbian women process AND stimuli as common odorants and, when smelling EST, they partly share activation of the anterior hypothalamus with heterosexual men (20). These observations indicate the involvement of the anterior hypothalamus in physiological processes related to sexual orientation/preference in humans.

6. Rodentia

The paradigmatic model for mammalian pheromone investigations involves laboratory species, among which rodents and especially mice are the most employed, because they are easy to handle, have extremely well-developed olfactory systems, and offer an almost unlimited possibility to act on behavioral responses by genetic manipulation. In these species, several effects have been carefully described, and the mechanisms of their action have even been dissected at a molecular level.
The vaginal secretion and saliva of female golden hamsters (*Mesocricetus auratus*) contain an aphrodisiac substance that facilitates the mating behavior of the male (223, 356). This pheromonal effect has been clearly demonstrated in assays that measure the mounting behavior of a male in the presence of an anesthetized male hamster (defined as surrogate female) scented with the stimuli (222). Evidence shows that biologically active compounds were present in the proteinaceous fraction, whereas aqueous fractions, organic extracts, and distillates containing volatile compounds did not exert detectable effects. After purification, pheromonal activity was attributed to a 17-kDa soluble glycosylated protein appropriately termed aphrodisin (356). The complete primary structure of aphrodisin shows that this protein belongs to the lipocalin superfamily. A prominent feature of almost all lipocalins is the presence of a hydrophobic pocket that accommodates endogenous molecules with a relatively high lipophilic coefficient. The biochemically purified aphrodisin carries natural molecules that are contained in the vaginal secretions (33, 34) and that may be responsible for the pheromonal effect observed in males (136). Biochemical and immunohistochemical data suggest that aphrodisin may act through G protein-coupled receptors that are expressed in the vomeronasal system (136, 177).

Fossorial mammals, like the mole-rat, are noted for sensory specializations, such as exquisite tactile senses (45, 60). Although suppression of mole-rat reproduction in females is thought to be mediated by behavioral, rather than pheromonal, cues from the breeding females (360), the chemosensory systems retain demonstrable functions of scent marking in common nesting and toilet loci (359) that have been associated with pheromonal communication.

In rats, several pheromones are used in different contexts as territory marking (309), alarm (1), and maternal behaviors (188, 254). The chemically characterized rat pheromones include compounds that are produced by pup’s preputial glands and urine. A particular pattern of rat maternal behavior is the specific licking and cleaning of pup’s anogenital area, an action crucial to pup survival, as nonlicked pups cannot defecate and run into death. Thus dodecyl propionate, a compound isolated from pups’ preputial glands, can sustain pups’ anogenital licking by dams and therefore pups’ survival (36).

The odors emanating from physically stressed rodents are recognized by conspecifics (220, 386). Once the chemical signal is perceived, the receiver undergoes significant changes. For example, lymphocyte T-killers are suppressed, whereas B-cells proliferate (57), rats develop hyperthermia (160), c-Fos protein expression is increased in the mitral cell layer of the accessory olfactory bulb (164), reproductive behavior and sexual maturation initiate (389a), and social hierarchy may be modified (140). These bodily compounds, defined as alarm pheromones, signal alert to the presence of a potential danger, promoting dispersion and the adoption of defensive actions in the group (400). Usually, mice escape from places in which the odors from the urine of a physically stressed conspecific are present (418). Among other ketones, 2-heptanone is detectable in the mouse and rat urine (97, 342). In rat, 2-heptanone was found to be increased in urine of stressed subjects and to induce immobility in a recipient conspecific forced to swim (97).

Several other urinary compounds have been indicated as activators of distinct anatomical regions of the pheromonal pathway in rats (176, 321, 372); however, none of the effects exerted by these substances is reportedly linked to obvious behavioral responses.

Mouse ecology is largely triggered by pheromonal signals, which have been investigated for a long time, and therefore, mice represent the world-wide animal model to study intraspecies communication. In the last decade, an enormous burden of data has been collected and, how it is often the case, there is yet an open and sometimes contrasting debate on the specific properties of the different signaling molecules.

Early studies have demonstrated a number of incontrovertible effects of primer pheromones in mice. It is known that grouped female mice modify or suppress their estrous cycle (Lee-Boot effect) (387), while male urine can restore and synchronize the estrus cycle of noncycling females (Whitten effect) (401) or accelerate puberty onset in females (Vandenbergh effect) (388). In addition, the exposure of a recently mated female mouse to a male, different from the stud, prevents implantation of fertilized eggs (Bruce effect) (38), implying that the stud or its individual odor must be memorized at the moment of mating to be recognized later. These pheromonal effects are commonly believed to be mediated by stimuli present in urine that act via the vomeronasal system.

Signaling pheromones also account for many behavioral responses in the mouse. For example, male as well as lactating female aggressiveness towards an intruder male are both believed to be triggered by molecules that are present in male urine.

**B. Major Urinary Proteins and Major Histocompatibility Complex Molecules**

Male mouse urine contains an unusually high quantity of proteins, called major urinary proteins (MUPs). MUPs form a large family of highly homologous androgen-dependent proteins that are synthesized in the liver and excreted with urine (46, 208). MUPs and the rat hortologs α2 urinary globulins belong to the lipocalin superfamily, and accordingly, they bind and release small (volatile) pheromones that are also produced in an androgen-dependent fashion (9, 23). Although MUPs are also ex-
pressed in exocrine glands as mammary, parotid, sublingual, submaxillary, lachrymal, nasal, and in modified sebaceous glands like preputial and perianal glands (344), their biological effects have been exclusively demonstrated when these proteins were purified from urine or added to urine that does not possess pheromonal activity (i.e., of a prepubertal or castrated mouse) (123, 266).

MUPs have been reported to act either as primer or releaser pheromones; however, some of their effects appear contradictory. This is in part due to the tight binding of MUPs with volatile pheromones that have been proven to be directly implicated in estrus synchronization (138), attractiveness to females (139), intermale aggression (280), induction of estrus (α- and β-farnesene) (219), puberty acceleration in females (283), and territory marking (21, 122). The advantage of such a pheromone-protein complex may include concentration and slow release of chemosignals, stabilization of labile pheromones, or delivery to the reception sites. Common chromatographic procedures for purification of MUPs are ineffectual to remove these pheromones, and solvent extraction of volatile pheromones appears the only way to obtain the native apoproteins. Therefore, in literature, the term of MUPs commonly defines the protein with the bound pheromones.

While a general consensus exists about the source, male urine, that releases the pheromonal stimuli responsible for the puberty acceleration (Van denbergh effect), a debate is currently open about the molecules that determine this behavioral response. Evidence suggests that MUPs and not the bound pheromones are effective in accelerating the puberty onset in females. These results are supported by the biological activity shown by MUPs when stripped of its pheromones and by a short synthetic hexapeptide homologous to the NH2-terminal sequence of the volatile pheromones and by a short synthetic hexapeptide homologous to the NH2-terminal sequence of the volatile pheromones (297). Conclusions from a different laboratory, performing similar but not identical experiments, seem, however, to argue against this hypothesis (283).

It has been also demonstrated that the combinatorial diversity of urinary MUPs among wild mice (a male can produce up to 12 variants from the polymorphic MUP family, that includes a total of 21 functional isoforms) is virtually as great as for molecules of the major histocompatibility complex (MHC) (see below) (48, 123). However, although MHC has been long regarded as the primary determinant for individual recognition via bodily odors, this evidence has now been challenged by recent studies in which it appears that recognition, at least in the wild, is instead strictly and exclusively dependent on the MUPs variant profile contained in each individual urine (49). Very intriguing is the observation that recognition requires the physical contact with the donor urine, thus discarding the hypothesis of this effect being exclusively attributed to the volatile pheromones released from MUPs (49).

Thom et al. (375) indicated that female mice use MUPs as a specific genetic marker to identify and preferentially associate with heterozygous males. The primary function of MUPs is in signaling social information through scent, including individual genetic identity. Mice are highly sensitive to differences in the fixed patterns of multiple MUP isoforms expressed by each individual and also avoid inbreeding with close relatives that share the same MUP type as themselves. Heterozygosis assessment, in contrast, most likely involves recognition of the greater diversity of MUP isoforms expressed by heterozygous animals.

Other biological effects of MUPs include attractiveness to females and repulsion to males (265), modification of light-avoidance behavior in both males and females (263, 264), the aggressive reactions of males towards adult and newborn mice (264, 267), and intermale aggressiveness (47). Mate choice and parent-offspring interactions have been demonstrated to be influenced by MHC genotype B (411). For example, house mice avoid mating with individuals that are genetically similar at the MHC locus. Mice are able to recognize MHC-similar individuals through specific odorant cues. Moreover, females avoid mating with males carrying MHC genes of their foster family, supporting a familial imprinting hypothesis (288). Mate preference for MHC-dissimilar individuals can be adaptive as it would increase offspring MHC heterozygosity, with beneficial influences on offspring viability through increased resistance to infectious disease or avoidance of inbreeding effects (180, 297).

The polymorphism of MHC molecules is reflected into structurally diverse binding sites, such that different MHC binds to distinct peptides. Like other membrane-bound proteins, these MHC molecules are anchored in the lipid bilayer. However, it has been demonstrated that the peptide-binding pocket releases its peptide in the extracellular fluid when the MHC is cleaved from the cell. Thus peptides or their fragments are constitutively excreted in urine and other bodily secretions (358) that in turn can be used for interindividual communication (357).

C. Exocrine Gland-Secreting Peptides

Mice secrete a family of peptides of different length called exocrine gland-secreting peptides (ESPs) from extraorbital, Harderian and submaxillary glands into tear, nasal mucus, or saliva. The ESP family consists of 38 members in mice and 10 in rats, while it is absent from the human genome.

Moreover, at least two of these peptides, produced by the extraorbital lacrimal gland, are gender specific: ESP1 is exclusively secreted by males (162), while ESP36 is female specific (163). Despite the fact that these peptides are recognized by systems dedicated to pheromone per-
ception, until now there were no clues regarding their physiological function.

III. CHEMOSENSORIAL SYSTEMS DETECTING PHEROMONES

It has been believed for a long time that two chemosensory systems, the main olfactory and the vomeronasal system, were responsible for different functions. The main olfactory system was considered to be responsible for recognizing the conventional volatile odorant molecules, whereas the vomeronasal system was thought to be tuned for sensing pheromones. Recent studies have demonstrated that both chemosensory systems, together with additional olfactory organs, are involved in pheromone detection. In these systems, peripheral chemosensory neurons located in the nasal cavity express distinct families of receptors that are believed to bind pheromones and trigger a cascade of molecular and electrical events that, ultimately, influence some aspects of the social behavior of the individual.

Although the main olfactory and vomeronasal systems share a similar histological organization, they also display relevant differences with regard to the receptor repertoire that they express and to the connections to specific central areas (Fig. 1). Each system possesses primary sensory neurons that send axons to second-order neurons (mitral cells) in specific regions of the main olfactory bulb (main olfactory system) or of the accessory olfactory bulb (vomeronasal system). The mitral cells of the main olfactory bulb project to several higher centers including the piriform cortex and the cortical amygdala. Instead, projections from the accessory olfactory bulb only reach the medial amygdala and the posterior-medial part of the cortical amygdala. From here fibers terminate in the hypothalamus either directly or via the bed nucleus of the stria terminals.

In the following sections we summarize how the various sensory epithelia are functionally organized. For more exhaustive anatomical description, readers may consult reviews specifically dedicated to these subjects (66, 229).

A. Vomeronasal Epithelium

The vomeronasal epithelium is part of the vomeronasal organ (VNO) of Jacobson, a tubular structure en-cased in a protective bony capsule located at the base of the nasal septum (68, 134) (Fig. 2). Given its recessed nature, the vomeronasal epithelium cannot be reached by the airstream that regularly flows through the nasal cavity. Thus, to target the vomeronasal epithelium, molecules dissolved in the nasal mucus must be sucked into the lumen of the organ. Lateral to the lumen are blood vessels and sinuses, innervated by the autonomic nervous system, that induces vasodilation and vasoconstriction, thereby producing a pulplike action for stimulus access to the lumen (72, 109, 244, 248, 249, 328). In ungulates, as described in section IV A4, the "flehmen" behavior is thought to be associated with promoting stimulus access to the vomeronasal organ (268).

Norepinephrine accumulation has been measured in the vomeronasal organ of mice after exposure to pheromonal cues (419). It is possible that this hormonal release alters the vascular tone, or changes the glandular secretions, thus increasing the receptivity of the sensory epithelium in particular behavioral contexts. The functioning of the vomeronasal organ is thus not simply a passive event, but it can be actively modulated.

In the mouse, the vomeronasal lumen is lined by a pseudostratified epithelium composed of three types of cells: sensory neurons, supporting cells, and basal cells. The basal stem cells are located both along the basal membrane of the sensory epithelium and at the boundary with the nonsensory epithelium (85). Supporting cells are found in the uppermost superficial layer of the sensory epithelium, while vomeronasal neurons form two overlapping populations, basal and apical, that are further described in detail in sections IV and V.

In the mouse, the vomeronasal sensory epithelium increases from birth to puberty and becomes fully developed and completely active at 2 mo after birth (117). The sensory neurons, on the mucosal side, bear dendrites and apical microvilli, whereas, on the opposite side, their axons cross the basal membrane and merge together, forming vomeronasal nerves that run between the paired olfactory bulbs and enter the accessory olfactory bulb at the posterior dorsal side of the main olfactory bulb (see Figs. 2 and 3).

The vomeronasal sensory neurons take origin from the olfactory placode together with gonadotropin-releasing hormone (GnRH, also known as LH-RH or luteinizing hormone-releasing hormone) neurons and \( \gamma \)-aminobutyric acid (GABA)-containing neurons (405). The GnRH

FIG. 1. A schematic diagram showing the anatomical pathways of the rodent vomeronasal and main olfactory systems.
neurons traverse the developing accessory olfactory bulb and migrate to the mediobasal hypothalamus. The vomeronasal sensory neurons maintain a functional relationship with the hypothalamic areas in the adult mammal, influencing neuroendocrine function and behavior.

Do humans have a vomeronasal organ? The presence of a vomeronasal organ in human embryos, containing bipolar neurons similar to developing vomeronasal neurons of other species, was clearly demonstrated (24, 25, 166, 240). However, the vomeronasal structure becomes more simplified later in development (24), and several reports showed that in human adults it becomes a blind-ended diverticulum in the septal mucosa (246, 383). Moreover, the epithelium lining the vomeronasal organ in human adults is different from that of other species and from the olfactory or respiratory epithelium (259, 366). Several studies showed that vomeronasal cells were not stained by an antibody against the olfactory marker protein (259, 366, 383, 403), a protein abundantly expressed in mature neurons in the olfactory and vomeronasal epithelia of other species (41, 144). In addition, Trotier et al. (383) did not identify vomeronasal nerve bundles using a
specific antibody against the S-100 protein, a characteristic axonal marker (7). Therefore, even if vomeronasal cells were chemosensitive, they would not have any obvious way to communicate with the brain. Therefore, the development of human vomeronasal structures seems to be limited to the embryonic stage, when they possibly play a role in the migration of GnRH-secreting neurons toward the brain (383; for review, see Ref. 246).

B. Olfactory Epithelium

New evidence suggests that some pheromones are detected by the main olfactory epithelium, a structure specifically dedicated to sensing volatile molecules, usually named general odorants. The main olfactory epithelium lines cartilaginous protuberances in the posterior nasal cavity (Fig. 2), called turbinates, and is a pseudostratified epithelium composed, similarly to the vomeronasal epithelium, of three main types of cells: olfactory sensory neurons, supporting cells, and basal cells. The olfactory sensory neurons are bipolar neurons with a single ciliated dendrite extending to the mucosal surface. Cilia are the site of primary transduction for odorants and, possibly, for pheromones. Although most olfactory sensory neurons have the same type of transduction cascade, it has been recently discovered that subsets of olfactory neurons express different types of transduction molecules (see sect. vB2) (reviewed in Refs. 31, 217, 269). Axons of sensory neurons are segregated in the epithelium and, after crossing the basal membrane, bundle together to form the fascicles of the olfactory nerves. Axons project to distinct domains of the main olfactory bulb as further described in section V.

In the mouse, the olfactory system is functionally established during the prenatal phase as the first axons contact the telencephalic vesicle around embryonic day 13 (59, 320). Conversely, axonal targeting to specific domains within the main olfactory bulb occurs as early as embryonic day 15.5 (59).

In the mouse, both vomeronasal and olfactory sensory neurons undergo a continuous renewal process throughout adult life, and their rate of neurogenesis is also regulated by environmental factors (11, 59, 90, 91, 402). Readers interested in the neurogenesis of olfactory and vomeronasal sensory neurons may consult some recent reviews on the subject (101, 205).

The human olfactory epithelial region is a small area (1–5 cm²) covering part of the superior turbinate, septum, and roof of the nasal cavity. The transition boundary between olfactory and respiratory epithelium is not as sharp as in other mammals; instead, there is an irregular zone where olfactory and respiratory cells intermingle. Moreover, patches of respiratory epithelium have been identified in purely olfactory areas (262).

C. Other Olfactory Organs

1. Septal organ of Masera

The septal organ of Masera (SO) (35, 311) is a small island of olfactory epithelium within the nasal mucosa and is located on each side of the septum, posterior to the nasopalatine duct that connects the oral to the nasal cavity (Fig. 2). The anatomical organization of the septal organ appears similar to that of the main olfactory epithelium, although its neuroepithelium appears thinner (218, 396). Furthermore, sensory neurons of the septal organ are provided with cilia that bear shorter dendrites and larger dendritic knobs than those of the main olfactory epithelium (218); their axons project to a subset of glomeruli in the posterior, ventromedial main olfactory bulb (Fig. 3). Interestingly, some of these glomeruli exclusively receive input from the septal organ sensory neurons, while others are also innervated by fibers originating from the main olfactory epithelium (191). The anterior position of the septal organ, its vicinity to the nasopalatine duct, and the wide distribution in many mammalian species suggest that this organ may be functionally specialized for the early detection of biologically relevant molecules as those, for example, resulting from licking behavior.

2. Grueneberg ganglion

The Grueneberg ganglion is located bilaterally in the anterodorsal area of the nasal cavity in the corners formed by the septum and the nasal roof of several mammalian species (93a) (Fig. 2). Differently from other chemosensory organs, neurons of the Grueneberg ganglion cluster in small groups (30, 79, 80, 317). Only two types of
cells have been identified in the Grueneberg ganglion: glial cells and ciliated neurons (30). Each neuron has a single axon that fasciculates with others into nerve bundles that project to ~10 glomeruli in the main olfactory bulb (Fig. 3). These glomeruli are a subpopulation of the necklace glomeruli. Since necklace glomeruli are active during the suckling behavior of the pup, it was suggested that the Grueneberg ganglion may sense maternal pheromones (82, 173, 317). However, a very recent study has rejected this hypothesis, suggesting instead that alarm pheromones, volatile molecules released by animals to alert conspecifics about danger, are the likely substrates of the Grueneberg ganglion neurons. These results are discussed in more detail in section vB.

IV. PUTATIVE PHEROMONE RECEPTORS

A. Vomeronasal Receptors

In the vomeronasal organ of rodents, detection of pheromones is mediated by two anatomically independent pathways that originate from chemosensory neurons of distinct compartments, basal and apical, of the neuroepithelium. The organization of the vomeronasal epithelium of rodents in apical and basal neurons was initially based on the specific expression pattern of marker molecules (261, 340, 341). More recently, exhaustive data established that also distinct transduction components characterize these two subsets of chemosensory neurons. The G protein subunit Gαi2 was found to enrich the apical neurons, whereas Gαo was identified in the basal ones (19, 143). These important findings are credited to have addressed the efforts towards the search of receptors that could specifically couple to G proteins. Thus two large families of vomeronasal G protein-coupled receptors, named V1R and V2R, have been discovered, and their expression pattern was extensively analyzed. Moreover, other putative receptors as the major histocompatibility complex class Ib molecules (H2-Mv) have been identified in the vomeronasal organ, and their expression was demonstrated to be tightly linked to that of V2Rs.

1. V1Rs

The first family of vomeronasal receptors, V1Rs, was discovered with elegant experiments of differential screening, based on the assumption that the genetic profile of two vomeronasal neurons expressing Gαi2 only differs for the receptor RNA that they express (71, 285). It was also evident that V1R expression is restricted to the apical neurons of the vomeronasal organ which express Gαi2 (71, 285). From the structural point of view, V1Rs are related to group I of G protein-coupled receptors and, in the mouse, represent a strikingly expanded receptor family compared with other species (313, 346, 415, 422). The gene tree of functional V1Rs in placental mammals, based on the mouse repertoire, is split into 12 clades (a-l) (313). All rat V1R receptor genes, except for one, are distributed within 10 of these 12 clades (a-g and j-l). Thus the mouse h and i clades represent an instructive example of species specificity, exclusively including mouse sequences. Given that one rat pseudogene is placed in each of the h and i subfamilies, it is likely that the absence of rat functional genes in these clades is the result of deletion in this species and of postspeciation expansion in the mouse lineage.

V1Rs in the mouse account for almost 200 potentially functional receptors and are highly expressed in vomeronasal neurons, at least at mRNA level. The mouse has a larger V1R repertoire than the rat (Fig. 5).

In the mouse V1R phylogenetic tree, clades share from 15 to 40% of their amino acid residues between each other, with intrafamily identities varying between 40 and 99%. High sequence variability is found in transmembrane domain 2, in intracellular loop 1, and in extracellular loops 2 and 3, while transmembrane domain 3 is highly conserved. A few amino acid residues mostly located between transmembrane domains 5 and 6, and a potential glycosylation in extracellular loop 2, are common features to virtually all members of the 12 V1R clades, while specific amino acid signatures are characteristic of each of the 12 families (313).

![Diagram of the mouse vomeronasal receptor families.](https://physrev.physiology.org/)

**Fig. 4.** The mouse vomeronasal receptor families. Top: phylogenetic tree of V1Rs showing 12 phylogenetically isolated subfamilies. Bottom: phylogenetic tree of the 4 V2R families showing their clades. Numbers of receptors for each clade and family are shown in red. [Modified from Silvotti et al. (355).]
V1R genes are scattered in regions of many chromosomes, where members of a given family are found almost invariably clustered. It has been recently reported that V1R clusterization may not simply reflect gene expansion but may be actively linked to the mechanisms underlying the tight regulation of receptors’ expression. V1R expression is, indeed, monoallelic and monogenic, both of these being tools that achieve the expression of one receptor type per cell, thus contributing to the narrow tuning of the vomeronasal neurons (318).

At present, some vertebrate genomes of species of different orders have been extensively scrutinized for the presence of V1Rs (Fig. 5). Interestingly, V1R genes were found in all species except in chicken and chimpanzee, in which the presence of a functional vomeronasal organ has not been consistently demonstrated. The number of functional V1R genes drops dramatically from rodents to other species. The human genome contains several V1R pseudogenes, only five human V1R genes that have an intact open reading frame, and at least one V1R that is transcribed in the olfactory epithelium. The chimpanzee genome has no obvious functional V1R genes but a large collection of pseudogenes. This is surprising if we consider that the massive loss of V1R genes in chimpanzee
appears even greater than for the human repertoire, in which V1R genes have been identified. However, the most surprising observation is that the dog only possesses eight functional V1R genes spread among four clades. This finding is unexpected because the dog is traditionally renowned to have evolved an extraordinary well-developed olfactory system and a complex social behavior (346). Although it is plausible that V1Rs emerged as dominant receptors for triggering specific rodent behaviors (multiple losses of ancestral V1R genes in carnivores and artiodactyls and gains of many new genes by gene duplication in rodents), it cannot be alternatively ruled out that a loss of dog V1Rs is due to the breeding and domestication processes of this species. Therefore, diversification into subfamilies probably occurred prior to the rodent-dog-primate split early in the mammalian evolution (415).

The question as to whether V1Rs are exclusively expressed in the vomeronasal epithelium or also in other tissues is still controversial. Karunadasa et al. (152) reported the expression of V1RRL RNA in some cells scattered within the main olfactory epithelium of embryonic and postnatal mouse and suggested that pheromone detection may also take place in this structure. V1R-homolog RNAs have also been identified in the main olfactory epithelium of goats and humans (316, 394). However, caution must be taken before raising any hypothesis as, until now, there are no clues that a functional V1R is expressed at a detectable level in the main olfactory epithelium.

2. V2Rs

A second family of vomeronasal receptors, V2Rs, was discovered in 1997 (110, 234, 323) and, in mouse, it accounts for ~120 potentially functional members. V2Rs belong to group III G protein-coupled receptors and are characterized by a long NH$_2$-terminal domain. This region has been demonstrated to represent the binding domain in most receptors of group III (for review, see Ref. 293). V2Rs are strictly coexpressed with the G protein subunit Go$_4$ in the basal neurons of the vomeronasal organ.

In the mouse, intact V2Rs can be grouped in four distinct families (A-D) according to sequence homology (Fig. 4). Together, receptors of families A, B, and D represent 95% of all V2Rs. Their expression pattern strongly suggests that these receptors are expressed by mutual exclusion in the vomeronasal organ. A phylogenetic analysis shows that family C V2Rs (also known as V2R2 family) are very divergent from those of families A, B, and D (3, 231, 323), and these differences are also functional (354). This family is also noteworthy for the high sequence conservation between species. Moreover, compared with other V2R pseudogenes, family C pseudogenes have one or very few inactivating mutations (also in the human pseudogenes), suggesting a relatively recent loss of function.

In rodents, this receptor superfamily is largely expanded and, in contrast to V1Rs, it appears completely degenerated in primates, dogs, and cows (Fig. 5). In the dog, the lack of a sizable V1R and V2R family does not probably reflect a reduction in the pheromonal relevance in triggering intraspecific behavior, but rather a general decline of the vomeronasal system, a hypothesis that is supported by anatomical data (63). In contrast, in the opossum, V2Rs are abundantly represented (Fig. 5). Thus it appears that the evolution of opossum and rodent V2Rs genes parallel that of the V1R ones in these species, which expanded independently. Moreover, most opossum and rodent V2R and V1R genes arose by duplication (rodent V2R and V1R genes are found in genomic clusters, and closely related V2R genes tend to reside near one another in the genome) after these lineages diverged. This receptor diversification and the consequent detection of additional pheromones may have resulted in a selective advantage.

The expression pattern of V2Rs makes them different from V1Rs and odorant receptors in that each basal neuron expresses two V2Rs, one of which is a member of family C (231). Moreover, there is evidence for a combinatorial scheme for the detection of chemosensory cues in the basal neurons of the vomeronasal organ. Recent data indicate the existence of multimolecular complexes in individual basal neurons, constituted by MHC molecules, by family A, B, or D and family C V2Rs (355). For example, neurons expressing family A receptors of the highly represented clades I, III, and IV almost exclusively express the family C receptor Vmn2r2, whereas neurons expressing clade VIII receptors specifically express the family C receptor Vmn2r1. Interestingly, coexpression of Vmn2r1 and Vmn2r2 with family A V2Rs seems to involve whole clades rather than individual receptors. Thus a peculiar modality of expression subsists in the basal layer of the vomeronasal organ, where two family receptors are mutually exclusively expressed in the same basal neurons.

It has been suggested that V2Rs bind MHC peptides (185) and MUPs (47), as further discussed in section VA. A V2R receptor, V2R83, is also expressed outside the vomeronasal organ, in a large subset of neurons of the Grueneberg ganglion (79), and it is a good candidate receptor for alarm pheromones (30).

At present, however, direct evidence that V2Rs are directly involved in pheromone signaling is still missing. At least 50% of the basal vomeronasal neurons express another multigene family representing nonclassical class I major histocompatibility complex (H2-Mv) genes. Each of the nine identified H2-Mv genes is present in a subset of vomeronasal neurons, and multiple H2-Mv genes can be coexpressed in the same neuron (127, 207). H2-Mv molecules are also coexpressed in a combinatorial
manner with receptors of specific subfamily of V2Rs (127, 207). It has been initially proposed that H2-Mv may function as indispensable escort molecules in the transport of V2Rs to the plasma membrane (207). However, this chaferone-like function is not generalized to all V2Rs (127, 354), and moreover, it is likely to be exclusively restricted to the mouse or rat as the opossum genome contains a hundred functional V2R genes and no H2-Mv genes (346).

3. Ligands of vomeronasal receptors

Although an initial report provided clues on the possibility that V1Rs could be expressed in a heterologous system (99), the problem of expression later became the major barrier to the identification of ligands for vomeronasal receptors, as well as for odorant receptors (for reviews, see Refs. 224, 256, 312). Some information about ligands for V1Rs was obtained from physiological approaches to the problem. Results from calcium imaging experiments on slices of the mouse vomeronasal organ indicated that some molecules credited as pheromones stimulated specific vomeronasal neurons that, for their topographic position in the vomeronasal organ, were likely to express the V1R repertoire (187). Furthermore, these authors reasoned that, if neurons responding to these pheromones indeed reflected a V1R-dependent activity, V1Rs must be narrowly tuned to bind one or very few pheromones. This contrasts with the broad selectivity of olfactory sensory neurons that can be stimulated by a large array of odorants. Further and more direct investigations on the V1R ligand specificity lead to the conclusion that V1Rs are indeed qualified as pheromone receptors: Del Punta et al. (61) succeeded to delete a large genomic region containing ~16 V1R genes of subfamily a and b, including the receptor V1rb2. The behavior of these mutant animals was also thoroughly investigated showing remarkable deficits (see sect. VII). Moreover, electrophysiological responses of vomeronasal neurons to some pheromones, including 6-hydroxyl-6-methyl-3-heptanone, 354), and moreover, it is likely to be exclusively restricted to the mouse or rat as the opossum genome contains a hundred functional V2R genes and no H2-Mv genes (346).

To deorphanize V1Rs and study their binding properties, it is necessary to develop an efficient bioassay. At present, it is only plausible to assume that V1Rs are vomeronasal receptors narrowly tuned to bind small volatile pheromones, possibly through a hydrophobic pocket located in their transmembrane region. Nevertheless, achievements in this direction have been very recently accomplished by Shirokova and colleagues who managed to deorphanize the human V1Rs employing NH2-terminally tagged receptors expressed in the cell line HeLa/Olf (350). It appears that, in sharp contrast to V1Rs, each human recombinant V1R receptor responds to several aliphatic alcohols or aldehydes via the olfactory G protein Gaolf (350). Unfortunately, a pheromonal function of human V1R ligands has yet to be demonstrated.

4. Formyl peptide receptors

Recently, a group of receptors belonging to the family of formyl peptide receptors (FPRs) has been exclusively identified in the vomeronasal organ (310a). FPRs are G protein-coupled receptors expressed in all mammals, with three genes present in the human genome and seven in the mouse genome (249a). At least two of the mouse FPRs have been reportedly involved in immune and inflammation responses being expressed in granulocytes, monocytes, and macrophages (183a). An in situ analysis of the vomeronasal organ of the mouse indicates that five FPRs are expressed in a small subset of chemosensory neurons of the apical layer. Antibody staining reveals that FPRs are featured by a subcellular distribution similar to that of V2R receptors including, besides the microvillar surface, the dendrite and somata. Although Fpr expression is restricted to the apical layer and, as well as V1Rs, adopts monogenic transcription, no coexpression is evident with receptors of this large family, suggesting that FPRs characterize a new and specific class of chemosensory neurons. Experiments based on calcium imaging on the whole vomeronasal epithelium and in dissociated chemosensory neurons establish that indeed FPR agonists, such as some microbial, antimicrobial, and viral peptides, stimulate neuronal subsets with a nonidentical affinity profile. These findings indirectly prove that these receptors play a role in offering a barrier against immunological diseases, or perhaps also in pheromonal communication itself, given that formyl peptides have been detected in urine (52a) and that, in the olfactory and vomeronasal epithelium, two FPR isotypes appear to be expressed in the glandular but not chemosensory compartment (310a).

B. Odorant Receptors

Odorant receptor genes were discovered in 1991 by Buck and Axel (39). The vast majority of the olfactory
neurons express only 1 of the 1,000 odorant receptor genes (51, 226, 273, 308, 370, 389). Neurons expressing different receptor types are usually found in one of four partially overlapping zones of the olfactory epithelium, within each of which a given odorant receptor appears to be randomly distributed (128, 251, 308, 369, 389).

An olfactory sensory neuron typically responds to more than one type of odorant molecules, and a given odorant can activate neurons with different specificity (70, 77, 226, 351). For the discrimination and perception of odorants, the odorant receptor family seems to employ a combinatorial scheme, with one single odorant molecule activating several types of receptors and each receptor sensing different types of odorants, thereby producing a unique combination of receptors activated by each odorant molecule. This combinatorial scheme allows the olfactory system to recognize an enormous number of odorants (40, 256).

Odorant receptor genes have been identified in regions outside the main olfactory epithelium as the septal organ and the vomeronasal epithelium. About 50 odorant receptor genes are expressed in the mouse septal organ (150, 378). Moreover, a subset of 44 odorant receptor genes is also expressed in the vomeronasal epithelium (190), although their functional role has not been established.

At present, we do not possess evidence that odorant receptors can also function as pheromone receptors. However, intriguingly, recent experiments linked the expression of a subclass of odorant receptors with stereotyped behavioral expressions in the mouse, thus suggesting that odorant receptors can convey information both about the quality of an odorant and the individual that has emitted it (169).

For additional information about odorant receptors, the reader is referred to several reviews that extensively discuss this subject (86, 224, 225, 256).

C. Trace Amine-Associated Receptors

In 2006, Liberles and Buck (194) reported the expression of a second class of chemosensory receptors in the mouse olfactory epithelium. These receptors are the “trace amine-associated receptors” (TAARs) (27, 192, 203), a family of G protein-coupled receptors discovered in 2001 (27, 42) (for review, see Ref. 425). TAARs are unrelated to odorant receptors but have similarities with receptors for biogenic amines, such as serotonin and dopamine receptors. The TAAR gene family includes 15 members in the mouse (Fig. 5), and each of them, except for TAAR1, is expressed in the olfactory epithelium (194). Moreover, the level of expression of TAAR genes is similar to that of odorant receptors, although TAARs and odorant receptors do not appear to be coexpressed in the same neurons, suggesting that subsets of neurons exist in the olfactory epithelium that are not exclusively dedicated to the detection of conventional odorants. By the expression of olfactory TAARs in heterologous cell systems it was demonstrated that at least four of them (mTAAR3, mTAAR4, mTAAR5, mTAAR7f) bind small-molecule amines and that each of these receptors recognizes a specific set of ligands, among which are β-phenylethylamine, isoamylamine, and trimethylamine that are natural components of the mouse urine. β-Phenylethylamine, a ligand for mTAAR4, is contained in human and rodent urine, where its concentration rises during stressful situations (93, 286, 361). Isoamylamine and trimethylamine bind to mTAAR3 and mTAAR5, respectively, and are present at higher concentrations in male compared with female urine (84, 276). Noteworthy, isoamylamine has been reported to act as a pheromone accelerating puberty onset in female mice (276). Therefore, stimulation of mTAARs seems to affect the capacity of discriminating gender and social status of an individual. These findings strongly contribute to confirm that the main olfactory epithelium is involved in the elicitation of physiological responses and innate behaviors.

In the mouse, TAARs are also expressed in subsets of neurons of the Grueneberg ganglion (80). As in the main olfactory epithelium, also in the Grueneberg ganglion, distinct TAAR subtypes are expressed in nonoverlapping subsets of neurons that do not coexpress the vomeronasal receptors V2Rs. Since the number of neurons expressing TAARs and V2Rs in the Grueneberg ganglion is much higher during the perinatal stage than in the adult, it was suggested that these neurons may be important for interactions between pups and their mother (80).

TAARs have also been identified in the genome of many species including humans and fish, with the number of putatively functional TAAR genes greatly varying among species (105) (Fig. 5).

D. Olfactory-Specific Guanylyl Cyclase Type D Receptor

A small subset of olfactory sensory neurons localized in the dorsal region of the main olfactory epithelium expresses a specific subtype of the transmembrane receptor guanylyl cyclase, named guanylyl cyclase type D (GC-D) (81, 149). The GC-D receptor is encoded by one of the seven guanylyl cyclase genes with whom it shares a similar topology: all receptor guanylyl cyclases retain an NH2-terminal ligand binding region, a single transmembrane loop, and a COOH terminus that includes the catalytic domain (81, 296). Specific ligands have been identified for some guanylyl cyclases, but not for GC-D yet; however, potential ligands for this receptor have been recently assessed. In the mouse, for example, neurons...
expressing GC-D respond to dilute urine and to the intestinal peptide hormones uroguanylyn and guanylin, while neurons in GC-D knockouts are unresponsive to these compounds (186). Given their localization in the enteric tract, uroguanylyn and guanylin are supposed to contribute to the maintenance of salt and water homeostasis or to the detection of cues related to hunger, satiety, or thirst (186). GC-D neurons also express carbonic anhydrase II and respond to CO₂ stimuli (118). It is not clear if there is a possible interplay between these two chemosensory responses.

Despite its exquisite expression in the main olfactory epithelium, GC-D appears to be a pseudogene in species lacking a functional vomeronasal organ such as humans, apes, Old World and New World monkeys, and tarsier. This suggests that a functional GC-D gene was lost early in primate evolution and that chemical detection in most primate species is unlikely to involve GC-D (416).

V. PHEROMONE TRANSDUCTION

Distinct neuronal subsets of the olfactory organs express specific types of receptors, second messenger systems, and ion channels to transform the pheromone binding into electrical signals.

The electrical activity of chemosensory neurons in response to stimuli can be measured with a number of approaches, including recording of the field potential, of the firing activity with an array of extracellular electrodes and by patch-clamp technique at the single-cell level. Nonetheless, calcium imaging either in dissociated cells or epithelium slices is often employed as well as the metabolic activity of stimulated neurons is frequently monitored upon pheromonal stimulation by evaluating the expression of the early protooncogenes.

A. Vomeronal Sensory Neurons

1. Signal transduction molecules

The detailed signal transduction cascade in the vomeronasal organ is still largely unknown, although it has been clearly demonstrated that pheromone stimuli cause membrane depolarization and increase the action potential firing rate in vomeronasal sensory neurons (28, 53, 61, 68, 115, 124, 125, 185, 187, 211, 363, 374). As described in previous sections, the sensory epithelium of the vomeronasal organ presents two subsets of neurons: apical and basal. Apical neurons express members of the VIR family of vomeronasal receptor genes together with $\text{G}_{\alpha_2}$, whereas basal neurons express V2R receptors and $\text{G}_{\alpha_i}$ (19, 102, 142, 143, 321, 348, 371, 398). The anatomical and perhaps functional segregation in the vomeronasal epithelium is likely to be a prerogative of species as rats, mice, hamsters, opossum, and platypus that possess functional V2R genes (Fig. 5).

Krieger et al. (176) reported that whole male urine induces inositol 1,4,5-trisphosphate (IP₃) production via the activation of $\text{G}_{\alpha_o}$ as well as $\text{G}_{\alpha_i}$, suggesting a role for both G protein subtypes in transducing pheromonal responses mediated by urinary components. Moreover, according to these authors, the activation of $\text{G}_{\alpha_o}$ and $\text{G}_{\alpha_i}$ subtypes in the vomeronasal epithelium appears to be caused by two structurally different classes of molecules. Whereas $\text{G}_{\alpha_o}$ activation was only observed upon stimulation with lipophilic volatile components, $\text{G}_{\alpha_i}$ activation was elicited by the rat major urinary protein, α₂-globulin. A confirmatory role of these G proteins in pheromone transduction issues from behavioral (see sect. vii) and anatomical studies performed on $\text{G}_{\alpha_2}$ and $\text{G}_{\alpha_i}$ negative mutant mice (278, 373): both mouse lines show a remarkable reduction in the size of the neuronal layers where $\text{G}_{\alpha_o}$ and $\text{G}_{\alpha_2}$ were originally expressed, indicating that G protein-mediated activity is required for survival of vomeronasal sensory neurons.

Since PLC stimulation and IP₃ production appear to be predominantly mediated by the release of the $\beta\gamma$ complex of the heterotrimeric G proteins, $\text{G}_{\alpha_o}$ and $\text{G}_{\alpha_i}$ (56), it has been proposed that $\beta\gamma$ may play an important role in the transduction process of the vomeronasal epithelium. One $\beta$-subunit (Gβ2) and two $\gamma$-subunits (Gγ2 and Gγ8) have been described in the vomeronasal epithelium (321, 322, 380). Gγ2 immunopositive neurons are exclusively localized in the apical layer, whereas Gγ8 neurons are preferentially restricted to the basal one. Gβ2, on the other hand, seems to be active in both neuronal subsets (Fig. 6). Biochemical studies reveal that IP₃ formation induced by stimulation of membrane preparations with volatile urinary compounds was selectively blocked by an anti-Gβ2 antibody, whereas second messenger production induced by stimulation with major urinary proteins was inhibited by preincubation of the vomeronasal membranes with an antibody recognizing Gγ8 (321). Thus it appears that the $\text{G}_{\alpha_i}\beta\gamma$ complex may control PLC activation by proteinaceous pheromones, whereas $\text{G}_{\alpha_i}\beta\gamma\gamma$ neurons respond to urinary volatile compounds.

Other lines of evidence indicate that, at least in some vomeronasal sensory neurons, transduction is mediated by a phosphatidylinositol signaling pathway. Some studies showed that electrical responses, IP₃ production, and calcium entry upon pheromonal stimulation (53, 176, 177, 215, 330, 397) are specifically blocked by the phospholipase C inhibitor U73122 (115, 363). Recently, Thompson et al. (377) demonstrated that compounds known to induce pregnancy block (Bruce effect), such as MHC peptides and male urine, also increased the IP₃ formation, confirming a major role of the phosphatidylinositol pathway in the vomeronasal signal transduction.
The ion channel TRPC2, a member of the superfamily of transient receptor potential channels (390, 412), is expressed in the microvilli of both apical and basal neurons (197, 241, 271) and plays an important role in the transduction process of some, but not all, vomeronasal sensory neurons. Interesting to note, TRPC2 is unique among TRP channels in that a functional gene has been lost from the Old World monkey and human genomes (198, 420). Proposed mechanisms for the activation of the TRPC2 channel in the vomeronasal neurons are similar to those involved in the signaling cascade of *Drosophila* photoreceptors (103, 250): pheromones trigger the activa-
Gene knockout and heterozygous mice show that these molecules play a key role in the biological response to pheromones. For example, mice lacking the gene for calmodulin (CaM) show a decreased response to pheromones (362).

Interestingly, Liman (195) showed that a cation channel activated by a high concentration of Ca$^{2+}$ is expressed in hamster vomeronasal sensory neurons. This channel is permeant to both Na$^{+}$ and K$^{+}$ and is blocked by ATP and cAMP, sharing many features with the TRPM4 ion channel (275). These properties make this channel a good candidate to be directly involved in sensory transduction or in amplifying a primary sensory response. At present, the molecular nature of this channel and its relation with TRPC2 or with IP$_3$ production are not yet resolved.

Some components of the cAMP signaling cascade have been identified in vomeronasal sensory neurons. These include adenylyl cyclase types 2 (19), 4, 5 (184), and 6 (184, 319); the subunit CNGA4 of the olfactory cyclic nucleotide-gated channel (19, 184); and phosphodiesterase types 1 and 4 (50, 183), suggesting that cAMP may play a physiological role in vomeronasal sensory neurons. Indeed, some pheromones induce a reduction of the cAMP level in the gartner snake (215) and in rodents (215, 319, 424). However, the decrease in cAMP occurs with a longer delay with respect to pheromone stimulation (319). Possible targets for modulatory actions by cAMP include the Ca$^{2+}$-activated cation channel (195) and the recently characterized hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels (64).

It is intriguing that a subpopulation of apical sensory neurons expresses members of the odorant receptor gene family, together with Gq and TRPC2 (190). Since some conventional odors are detected by the vomeronasal epithelium (329, 382, 409), it was suggested that vomeronasal neurons expressing odorant receptors might be those that respond to odors (190).

Following the initial transduction events, when the membrane potential of vomeronasal sensory neurons reaches threshold, action potentials are generated and propagate along the axons to the accessory olfactory bulb. Interestingly, most vomeronasal neurons fire tonically for several seconds, without sign of adaptation, when small current injections (64, 196, 347, 384) or dilute urine are applied for up to 2–3 s (211, 421). However, in one study, longer stimulations of 10–60 s caused spike-frequency adaptation (384), and in addition, a recent work suggested that sensory adaptation in vomeronasal sensory neurons requires the influx of Ca$^{2+}$ and is mediated by calmodulin (362).

2. Responses to urinary pheromones

Using an array of extracellular electrodes, Holy et al. (115) simultaneously recorded the activity of a large number of mouse vomeronasal neurons in response to dilute urine. Neurons responded to urine with an increase in the firing frequency that remained approximately unchanged if the stimulus was applied for as long as 100 s, indicating that the response of these neurons do not adapt to prolonged stimulus exposure. Furthermore, they identified distinct and nonoverlapping subsets of neurons that were selectively activated by substances contained in female or male urine; in contrast, about half of the vomeronasal neurons detected stimuli that were independent of sex.
Thus these findings establish the capacity of the vomeronasal organ to identify differences between sexes.

A recent study by He et al. (106) further stressed the concept of the vomeronasal organ being subtly dedicated to the identification of conspecifics. These authors quantified the number of neurons responding to individual urine of either sex and of different strains in slices of the vomeronasal epithelium of mice genetically modified to express a calcium indicator. It was observed that only a very small subpopulation of neurons (1–3%) exclusively responded to urine samples from all individuals of the same sex. The gender-specific neurons were evenly distributed both in the apical and basal layers of the epithelium and did not differ in males or females, consistent with the little dimorphism generally observed at the level of receptor expression in the vomeronasal epithelium. Interestingly, neurons specific to female urine showed different activation patterns depending on the phase of the estrus cycle; in contrast, urine from castrated mice failed to elicit a response in male-specific neurons. Furthermore, information about individuals and strains is not encoded by specialized neurons but, rather, by the combinatorial activation of vomeronasal neurons, as no urine samples from different mice of the same sex elicited the same pattern of neuronal activity. Thus the information contained in the mouse urine about gender is encoded by dedicated neurons; strains and individuals are recognized by a combinatorial code of neuronal activation (106), with the obvious advantage to discriminate a large population of individuals with a restricted repertoire of receptors.

He et al. (106) also compared responses elicited by synthetic strain-specific MHC class I peptides with those induced by urine of the same strain and found that neurons activated by urine and by the peptides did not overlap. Furthermore, the same authors (106) pointed out that only fragments, but not the peptides, of the MHC complexes are detectable in urine (358). This result strongly contrasts with what was observed by Leinders-Zufall et al. (185), who identified a specific subset of basal neurons highly sensitive to MHC class I peptides and to urine from mice of the relevant haplotype.

Other studies investigated the response of the vomeronasal epithelium to specific urinary components, such as MUPs, that represent another class of candidates for strain recognition, as well as for heterozygosity signals (375). Kimoto et al. (163) found that a recombinant isoform of MUPs elicits electrical responses in the vomeronasal epithelium, and Chamero et al. (47) showed that native and recombinant MUPs evoke calcium influx in sensory neurons expressing the G protein subunit $\alpha_o$. Since $\alpha_o$ coexpresses with V2Rs, it is likely that MUPs directly activate a response in vomeronasal sensory neurons by binding to a subset of these receptors (47). In sharp contrast to these observation, Holekamp et al. (113), by using a new microscopical technique that allows an exceptionally low-noise imaging of large neuronal populations, unexpectedly found that responses to dilute urine are predominantly, and perhaps exclusively, generated by neurons in the apical layer, where V1Rs are expressed.

Leinders-Zufall et al. (187) showed that some volatile hydrophobic pheromones, known to be tightly bound to the hydrophobic pocket of MUPs, produced a calcium increase in neurons of slice preparations of the vomeronasal organ. The distribution pattern of the activated neurons by any of these molecules reflects a localization that can be associated with the expression of the vomeronasal receptors V1Rs (187), and the percentage of neurons responding to an individual pheromone (0.2–3%) matches the proportion of neurons expressing one type of V1R. Therefore, when volatile molecules are released by MUPs, in the external environment or in the nasal cavity, they are likely to bind and activate V1Rs.

Urine contains compounds whose physiological role is largely unknown. Nodari et al. (277) applied chromatographic fractions of female mouse urine to isolated vomeronasal epithelia mounted on a multielectrode array and measured the spiking activity of neurons (277). Increase in the firing rate was associated with compounds with homogeneous chemical properties that, after further purifications, appeared to correspond to sulfated steroids. Sulfatase-treated urine extracts lost $>80\%$ of their activity, indicating that sulfated compounds are the predominant ligands for vomeronasal neurons in female urine. Interestingly, a collection of 31 synthetic sulfated steroids triggered responses 30-fold more frequently than did a similarly sized stimulus set containing the majority of all previously reported ligands. Vomeronasal neurons seemed to have a high tuning specificity for sulfated steroids, since each neuron responded only to one or to a few structurally similar compounds, but collectively, neurons detected all major classes of sulfated steroids. Some of these compounds may be indicators of a status of stress. Indeed, the concentration of two sulfated glucocorticoids in urine increased about threefold in animals subjected to stress compared with unstressed controls, indicating that information about the status of stress of these mice is encoded in urine and could be detected by vomeronasal sensory neurons (277).

### 3. Responses to secretory peptides

Kimoto et al. (163) showed that mouse recombinant exocrine gland-secreting peptides (see sect. III C) evoked electrical activity in the vomeronasal but not in the olfactory epithelium. Interestingly, when the electrical responses of individual neurons to ESP1 were recorded with a multielectrode array, only a small percentage of neurons, 1.6%, responded to ESP1 (163). This result is consistent with the possibility that ESP1 is recognized by one
type of receptor only, most likely by a specific V2R, as ESP1
induced c-fos production in vomeronasal neurons expressing
V2Rp5, a V2R receptor of the family A, clade III (98).

B. Olfactory Sensory Neurons

1. The cAMP transduction Neurons

In contrast to a common belief, some sensory neu-
rons of the main olfactory epithelium respond to phero-
mones (364, 395). A large majority of olfactory sensory
neurons express a member of the odorant receptor family
that is coupled to a signal transduction pathway mediated
by cAMP (Fig. 6). The binding of ligands to the odorant
receptor activates the G protein subunit Goαolf which
stimulates adenylyl cyclase-3 activity producing an in-
crease in cAMP concentration. cAMP causes the opening
of the ciliary cyclic nucleotide-gated channels composed
of three subunits: CNGA2, CNGA4, and CNGB1b (for
reviews, see Refs. 29, 153, 292). These channels allow
an influx of Na+ and Ca2+ inside the cilia. Ca2+-activated Cl−
channels are then activated in turn and, due to the unusu-
ally high intracellular Cl− concentration, allow an outflow
of Cl−, contributing to the inward current (167, 179). As a
result of the odorant binding to receptors, the olfactory
sensory neuron depolarizes. The CNG channel allows
Ca2+ entry not only for excitatory but also for inhibitory
purposes. The complex Ca2+-calmodulin activates a phos-
phodiesterase (PDE1C2) that hydrolyzes cAMP and, im-
portantly, produces a negative-feedback effect on the
CNG channel itself, that has been shown to mediate ol-
factory adaptation (178, 242).

When the depolarization following the activation of
the transduction cascade reaches the threshold for activa-
tion of action potentials, they will travel along the axon
to the main olfactory bulb. Differently from vomeronasal
sensory neurons, a maintained current injection will only
elicit a short burst of action potentials in olfactory sen-
sory neurons, instead of a tonic firing (196).

For more details about the cAMP transduction cascade
in olfactory sensory neurons, the reader may consult pre-
vious comprehensive reviews (40, 168, 235, 242, 292, 337).

2. Neuronal subsets

The subset of olfactory sensory neurons expressing
TAARs also express Goαolf, and therefore, it is possible
that they use a signal transduction pathway coupled to
adenylyl cyclase and cAMP (194), although additional
components of the transduction pathway in these neu-
rons are still unknown.

The ion channel TRPM5 is expressed in another sub-
set of olfactory sensory neurons that coexpress the
CNGA2 channel, and some components of the PLC path-
way, such as PLC-β2 and the G protein γ13 subunit (202).

Since TRPM5 channels are directly activated by intracel-
ular Ca2+ (112, 204, 299, 423), it is tempting to speculate
that Ca2+ entry through CNG channels could gate TRPM5
channels.

Neurons expressing TRPM5 are located in the vent-
rolateral zones of the olfactory epithelium and project to
domains of the ventral region of the main olfactory bulb,
an area responsive to urine and other putative phero-
mones (199–201, 336, 409). Unfortunately, electrical re-
 sponses from individual olfactory sensory neurons ex-
pressing TRPM5 have not yet been investigated.

As previously mentioned (see sect. wD), another sub-
set of neurons in the main olfactory epithelium expresses
compounds typical of a cGMP instead of a cAMP sec-
ond messenger system, namely, the receptor guanylyl
cyclase type D (GC-D), the cGMP phosphodiesterase
PDE2 (149), and the cyclic nucleotide-gated channel
CNGA3, that was originally discovered in cone photore-
ceptors (417). These neurons are located in the central
area of the olfactory epithelium and project to an anatom-
ically distinct group of interconnected glomeruli, the
necklace glomeruli, that have been implicated in the suck-
ling response of mammals (149). Leinders-Zufall et al.
(186) showed that neurons expressing GC-D can be acti-
vated by the peptide hormones uroguanylin and guanylin
and by natural urine stimuli, while Hu et al. (118) demon-
strated that these neurons may be involved in the detec-
tion of carbon dioxide. In both studies, GC-D neurons
were shown to use a cGMP signaling cascade (118, 186).

A subset of neurons in the main olfactory epithelium
of humans and mice expresses some vomeronasal recep-
tors of the V1R family (152, 313, 315). Ligands correspond-
ing to aliphatic alcohols or aldehydes have been identified
for human V1Rs (350); however, it is still unresolved
whether neurons expressing these receptors project their
axons to the main or the accessory olfactory bulb.

A small population of neurons in the main olfactory
epithelium expresses the TRPC2 channel. At present,
there are no clues whether these neurons have chemosen-
sory properties, but this finding is certainly relevant
and must be seriously taken into account when compar-
ing the behavioral effects produced by the genetic dele-
tions of the TRPC2 channel with those of the surgical
removal of the vomeronasal organ (P. Mombaert, per-
sonal communication).

Early microscopical and immunological studies have
revealed the existence of a novel cell type in the olfactory
epithelium that is morphologically different from chemosen-
sory neurons or supporting cells (44, 148, 260). The
main feature of these cells is represented by the microvilli
that they bear on the mucosal side. It now appears that
olfactory microvillar cells do not represent a homoge-
neous population of cells but rather they include different
neuronal subsets expressing distinct signal transduction
molecules (6, 74, 201). It is very unlikely that these cells
are true neurons as they do not seem to project their long foot process to the olfactory bulb (74, 201). However, although their role is enigmatic, a possible chemosensory function for microvillar cells has been reported (74).

3. Responses of the main olfactory epithelium to pheromones

Wang et al. (395) showed that milk and urinary pheromones that are responsible for puberty delay aggressiveness in the mouse (279, 281) and evoke an electrical response from the main olfactory epithelium, as measured by field potential recordings. Moreover, when the same experimental approach was adapted to mice in which the adenyl cyclase type 3 gene had been knocked out, no electrical response was recorded, indicating that receptors responding to these compounds are likely to be coupled to the cAMP signaling pathway. Since behavioral deficits are also evident in adenyl cyclase type 3 mutant mice, it can be envisaged that a cAMP-mediated pheromone signaling may play an important role in the olfactory epithelium.

MHC peptides can also be detected by sensory neurons of the main olfactory epithelium via the cAMP signaling cascade, as shown by Spehr et al. (364). Two MHC peptide ligands, SYFPEITHI and AAPDNRETF, elicited an electrical response, measured by field potential recordings, in the main olfactory epithelium at a concentration near $10^{-11}$ M (364). Mutant mice lacking the CNGA2 gene failed to display electrical responses when stimulated with both peptides, suggesting that a subset of olfactory neurons expressing CNGA2 are actively involved in the detection of pheromones besides conventional odors.

In contrast to that, Lin et al. (200) have demonstrated that at least two pheromones, 2-heptanone and dimethylpyrazine, stimulate the main olfactory epithelium without the intervention of the CNGA2 channel, suggesting that pheromone detection in the olfactory epithelium relies on multiple transduction pathways.

C. Grueneberg Ganglion

The transduction pathways in the Grueneberg ganglion are still largely uncharacterized. Fleischer and colleagues showed that the majority of neurons express the vomeronasal receptor V2R83 (V2R2 family), together with the G protein $G_{i1}$ (79, 80), while a distinct subpopulation of $G_{i1}$-expressing neurons also expresses TAAR receptors (80). Another study investigated whether neuronal subsets of the Grueneberg ganglion express transduction components similar to those of GC-D neurons, based on the observation that both types of neurons project to the necklace domain of the main olfactory bulb (Fig. 3). Fleischer et al. (78) reported that a subset of Grueneberg ganglion neurons expressing the V2R83 receptor expresses also the GC-D receptor and the cGMP-stimulated phosphodiesterase PDE2A.

Brechbuhl et al. (30) recently uncovered at least one of the functional roles of the Grueneberg ganglion. Indeed, a subpopulation of neurons was shown to respond to the yet unidentified “alarm pheromones,” a group of volatile compounds that are released by animals subjected to stress. Lesions of this organ completely abolish the so-called freezing reaction, a typical fear response elicited by alarm pheromones. Curiously, in this study, lesions of the Grueneberg ganglion did not affect mother-pup recognition (30), a behavior that was believed to be mediated by this organ (118, 173, 317).

VI. SIGNALING FROM SENSORY EPITHELIA TO THE BRAIN

A. Main and Accessory Olfactory Bulbs

1. Anatomical organization

The main olfactory bulb is the primary target region of the olfactory neurons, whereas vomeronasal sensory neurons project to a well-defined dorsal and posterior region of the olfactory bulb, namely, the accessory olfactory bulb. The main olfactory bulb exists in all vertebrates, except for aquatic mammals. In contrast, the accessory olfactory bulb is absent in adult Old World monkeys, apes, and humans (for more information, see Table 1 in Ref. 240). In rodents, both the main and the accessory olfactory bulbs have a largely similar laminar organization consisting of a superficial nerve layer formed by the axonal projections of the chemosensory neurons, the glomerular layer that represents the first-order synaptic region between sensory neurons and mitral cell dendrites, and the external and internal plexiform layers that are regions where soma of, respectively, mitral/tufted and granule cells reside (for review, see Ref. 240; see also Ref. 182 for suggested changes to the nomenclature of the accessory olfactory bulb). In the main olfactory bulb, glomeruli are quite well anatomically separated, encapsulated by periglomerular cells, and rather uniform in size (~50 μm diameter in mice). Conversely, in the accessory olfactory bulb, these synaptic structures are very diffusely organized, surrounded by a small number of periglomerular cells, and highly variable in size (10–30 μm diameter in mice). In the main olfactory bulb, mitral/tufted cells form a distinct monolayer, whereas in the accessory olfactory bulb they are much less organized. An important difference between mitral/tufted cells of the two olfactory systems is their arborization: in the main olfactory bulb these neurons bear only one apical dendrite, whereas in the accessory olfactory bulb mitral/tufted cells are equipped with up to six apical dendrites, each entering a different glomerulus (Fig. 7; for review, see Ref. 240).

2. Topographic projections

Axons of all the olfactory sensory neurons expressing a particular odorant receptor converge to only two glomeruli
in the main olfactory bulb (257, 308, 389). This has been experimentally demonstrated at a single-neuron resolution by using transgenic mice in which the expression of a given odorant receptor was linked to that of the green fluorescent protein, thus allowing the visualization of the axonal network (255, 256, 381). In mice there are ~2,000 glomeruli, and their localization is roughly conserved among individuals. Therefore, the olfactory bulb is topographically organized, with each glomerulus representing a single type of odorant receptor (Fig. 7C).

**Fig. 7.** Main and accessory olfactory bulbs. A and B show the general organization of the main olfactory bulb (MOB) and of the accessory olfactory bulb (AOB) in the adult rat from Nissl-stained specimens. A: each mitral cell (MC) of the main olfactory bulb has a single apical dendrite projecting to a single glomerulus and several lateral dendrites that contact granule cell dendrites. B: each mitral cell (here indicated as large principal cell, LPC) of the accessory olfactory bulb has several apical dendrites projecting to multiple glomeruli. EPL, external plexiform layer; MC, mitral cells; EGC, external granule cell; asterisk, tufted cell axon; IGC, internal granule cell dendrite; S, superficial centrifugal fiber; LOT, lateral olfactory tract. [From Larriva-Sahd (182).] C and D: schematic topographical representations of sensory projections in the main olfactory (C) and vomeronasal (D) systems. C: in the main olfactory system, olfactory sensory neurons expressing a given odorant receptor project to the same glomerulus (or glomerular pair). Three populations of olfactory sensory neurons, each expressing one different type of odorant receptor, are represented in different colors. D: in the vomeronasal system, sensory neurons that express the same vomeronasal receptor project to multiple small glomeruli. Six populations of vomeronasal sensory neurons, each expressing one different type of vomeronasal receptor, are represented in different colors.
In rodents, V1Rs and V2Rs are individually expressed in vomeronasal sensory neurons, and each neuron expressing a given vomeronasal receptor (or vomeronasal receptor pair) sends its axonal projection toward the anterior (V1Rs) or posterior (V2Rs) accessory olfactory bulb (100, 143, 414). Gene targeting approaches were used to visualize the topographical map of each targeted receptor in the mouse accessory olfactory bulb (16, 62, 314). When a fluorescent marker protein was inserted downstream of a vomeronasal receptor gene, the resulting visualized axonal projections of neurons expressing that specific receptor showed that several glomeruli in subregions of the anterior or posterior accessory olfactory bulb received inputs. A more accurate analysis indicated that axons from neurons expressing the same receptor coalesce into 10–30 glomeruli and that a single glomerulus receives input from neurons expressing different receptor types (Fig. 7D). Moreover, the capacity to converge and to form these glomeruli depends on the possibility to express a specific vomeronasal receptor gene, suggesting that the receptor proteins themselves play a role in axonal wiring to the bulb (16, 314). In fact, vomeronasal neurons expressing a nonfunctional (truncated) V1R gene no longer converge in the accessory olfactory bulb. This phenotype, also observed for odorant receptors (343), could be possibly due to the inability of the truncated gene to prevent the expression of other V1Rs, resulting in a population of fluorescently tagged neurons randomly expressing different V1Rs. Indeed, Roppolo et al. (318) showed that the expression of a nonfunctional V1R allele allows the coexpression of other V1R genes. Although no V1R expression has been reported in other subcellular compartments besides the projecting dendrite, it is possible to envisage that V1Rs may direct axon guidance, perhaps through a mechanism that involves their expression in the growth cones.

Wagner et al. (393) generated transgenic mouse lines in which projections from various populations of neurons expressing distinct V1Rs were differentially labeled with fluorescent proteins. They found a glomerular map divided in multiple nonoverlapping domains, with each domain clustering glomeruli formed by neurons expressing V1Rs of the same subfamily, randomly intermingled (393). Moreover, these authors (393) found that a mitral cell is not only connected to glomeruli innervated by neurons expressing the same V1R (62) but also to those associated with receptors of the same subfamily. Therefore, in the anterior accessory olfactory bulb, the spatial map originated from vomeronasal organ inputs seems to rely on receptor subfamilies, rather than individual receptors as in the main olfactory bulb. This wiring scheme may help to discriminate molecules with highly similar chemical structure at different ratios in a pheromonal blend.

In both the main and accessory olfactory systems, mitral cells are activated by primary sensory neurons through glutamatergic synapses, but the chemical information is further processed by the activity of inhibitory interneurons as well as periglomerular and granule cells (141, 210, 339) that contact mitral cells via glutamatergic dendrodendritic synapses. These consist of an excitatory glutamatergic input from mitral cells to granule cells, inducing the release of GABA from granule cells, that in turn provides inhibition to the mitral cells (141, 182, 303). For more information, the reader may consult previous comprehensive reviews (339, 345, 385).

3. Encoding pheromonal information in the main and accessory olfactory bulbs

To simultaneously examine the responses to pheromones and odorants in the mouse main and accessory olfactory bulbs, Xu et al. (409) used high-resolution functional magnetic resonance imaging (fMRI), a very powerful technique that can show dynamic responses in both entire olfactory bulbs to various stimuli in the same animals. Mouse urine activated restricted areas in both olfactory bulbs, eliciting signals mainly in the ventral region of the main olfactory bulb and in the anterior part of the main accessory bulb. Since the anterior region of the mouse accessory olfactory bulb receives input from V1R-expressing vomeronasal sensory neurons, this study is in agreement with recent results from Holekamp et al. (113) who suggested that urine mainly activates V1Rs (see sect. vA2).

The encoding of pheromonal information in the mitral cells of the accessory olfactory bulb has been investigated by recording responses in awake mice using a miniature motorized drive to move microelectrodes and record the activity of single neurons (213, 214). Male mice were allowed to investigate conspecifics differing by sex, genetic makeup, and hormonal status. Interestingly, an increase in mitral cell activity occurred after test animals directly contacted and actively investigated the head and the anogenital regions of the stimulus animal. The recorded mitral cell responses were found to be specific for sex and strain of the stimulus mouse. In addition, mitral cells exhibited both highly specific excitatory and inhibitory responses, these latter likely originating from the inhibitory interneuron activity. Therefore, the activity of mitral cells appears to be tuned by the combination of sex and genetic makeup (213, 214), and therefore, information about sex and strain is already integrated in the accessory olfactory bulb.

Several studies have revealed that the exposure to urinary components from opposite-sex conspecifics increased the number of c-fos-immunoreactive mitral and granule and periglomerular cells in the accessory olfactory bulb (116, 126, 228, 295, 410). Volatile urinary odors from male as well as female mice also stimulate c-fos expression in distinct clusters of glomeruli in the main olfactory bulb in both sexes (228, 270).
Lin et al. (199) recorded the electrical activity in the accessory olfactory bulb in response to urine of different mouse strains. A subset of mitral cells was found to be very selective, responding only to male urine. Moreover, by combining electrophysiology and gas chromatographic separation of male mouse urine components, these authors identified a male specific compound, methyl-thiomethanethiol (MTMT), that was indeed responsible for this effect. Interestingly, mitral cells responding to MTMT were not stimulated by any other urinary compound, allowing the appellation of “specialist” cells. Furthermore, behavioral experiments also established the biological activity of this molecule, as female mice developed interest and attraction towards castrated mouse urine after the addition of synthetic MTMT.

B. Signaling Beyond the Olfactory Bulbs

In the main olfactory bulb, mitral/tufted cells directly transmit signals from glomeruli to pyramidal neurons in the olfactory cortex, without a thalamic relay. The primary olfactory cortex, defined as the ensemble of brain regions that receive direct input from the olfactory bulb, is composed of several anatomically distinct areas: the piriform cortex, the olfactory tubercle, the anterior olfactory nucleus, the cortical amygdala, and the entorhinal cortex. In turn, most of these cortical regions project back to the main olfactory bulb and to other areas of the brain, including the thalamus, the hypothalamus, the hippocampus, the frontal cortex, and the orbitofrontal cortex (Fig. 8; reviewed in Ref. 349). Anatomical studies and genetic approaches indicate that the topographical organization of odorant information in the olfactory bulb is not reflected in the olfactory cortex. Instead, odorant receptors seem to be mapped to multiple, discrete clusters of cortical neurons.

Mitral cells of the accessory olfactory bulb project to areas of the limbic system: the medial amygdala and the posteromedial cortical amygdala, which are also called “vomeronasal amygdala,” the accessory olfactory tract, and the bed nucleus of the stria terminalis (Fig. 8) (272, 334, 392).

![Diagram of central pathways](image-url)
Although vomeronasal sensory neurons expressing V1Rs or V2Rs, respectively, project to distinct areas of the accessory olfactory bulb, this segregation, at least in some species, does not seem to be completely maintained in the amygdalic regions, where clusters of neurons may combine signal information originating from both families of vomeronasal receptors (230, 252, 253, 327, 392).

In essence, the majority of these studies provide evidence that, excluding areas of convergence, the two vomeronasal pathways project to specific areas of the amygdala and hypothalamus, thus suggesting a partial functional separation (252, 253).

In the vomeronasal system, projections to the hypothalamus from the limbic regions are directed to the ventromedial and the medial preoptic areas, which are involved in reproductive and social behaviors (158, 159, 289).

The medial amygdala receives direct input from the accessory olfactory bulb, but also from the main olfactory bulb. However, other rostral basal telencephalic regions seem to play a relevant role in the integration of signals originating from both the vomeronasal and the main olfactory epithelia.

C. Pheromone-Activated Hormonal Systems

Recent experiments have revolutionized the common beliefs about the control of hormonal responses by pheromones (26, 413). The effects of pheromones on the neuroendocrine status are mediated by a group of neurons in the hypothalamus, which constitutes the endocrine control center (for reviews, see Refs. 89, 245, 353). These neurons secrete the GnRH, also known as LH-RH, which in turn stimulates the anterior pituitary gland to release two gonadotrophins: the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). Both hormones control the development and the function of the gonads in males and females, although with different effects.

A genetic approach has been used to visualize the GnRH neuronal network and, surprisingly, GnRH neurons were found to be connected with areas relaying information from the main olfactory system, in contrast to the traditional view that only ascribes connections with GnRH neurons from the vomeronasal system (26, 413).

Boehm et al. (26) identified neurons that synapse with GnRH by using a genetic transneuronal tracer, barley lectin, a glycoprotein that is transferred across synapses. The barley lectin gene was positioned under the control of the GnRH promoter so that only GnRH neurons would express this peptide. In another study, Yoon et al. (413) used a different experimental approach to anatomically trace the afferent pathways to GnRH neurons in the hypothalamus. Only neurons expressing GnRH were infected with a modified pseudo-rabies virus and green fluorescent protein reporter. The genetically controlled viral tracing allowed the identification of a major projection pathway from the main olfactory system.

Both studies (26, 413) came to the conclusion that the small pool of GnRH cells (800 neurons) was surprisingly synaptically connected to some tens of thousand neurons in ~50 diverse brain areas, including the main olfactory ones, suggesting that GnRH neurons may influence a large variety of brain functions.

Interestingly, Yoon et al. (413) reported the absence of synaptic connections between the vomeronasal pathway and GnRH neurons. This could be due to different experimental approaches used in the two studies or, perhaps, presynaptic inputs from the vomeronasal pathway identified in one study (26) might derive from a subset of GnRH neurons distinct from those targeted in the other study (413). However, the observations of Yoon et al. (413) do not refute previous neuroanatomical tracing data indicating that the vomeronasal pathway projects to the hypothalamic area where GnRH neurons are located. Indeed, Yoon et al. (413) confirmed that neurons in this area receive inputs from the vomeronasal pathway. It could be speculated that the connections with GnRH are, at least in part, mediated by nonsynaptic (e.g., hormonal or neuromodulatory) mechanisms.

As a confirmation of these observations, stimulation of male mice with urinary pheromones induced the activation of neurons in the anterior cortical amygdala as well as in the dorsal and ventral endopiriform nucleus. Therefore, signals in response to pheromonal cues also occur via the main olfactory epithelium and are transmitted to GnRH neurons through the olfactory cortex. Moreover, feedback loops have been identified so that GnRH neurons could influence both odorant and pheromone signaling.

VII. PHEROMONE-MEDIATED BEHAVIORS

From results presented in the previous sections, it now appears evident that both the vomeronasal and the main olfactory systems may be activated by chemicals that are known to produce pheromonal effects. The involvement of the vomeronasal and main olfactory systems on specific behaviors is usually investigated by inactivating one or both systems. In the past, lesions were made only by surgical removal of the vomeronasal organ (VNOx) or by chemical ablation of the main olfactory epithelium (MOEx) (Table 2). The introduction of a molecular genetic approach allowed the deletion of specific transduction molecules, although it must be taken into account that the knowledge of genes involved in transduction in each olfactory organ is still incomplete, and therefore, results of behavioral experiments from knockout animals must be interpreted very cautiously.
Here, we will discuss experimental results mainly related to sexual and aggressive behaviors and will try to interpret to what extent the vomeronasal and/or the main olfactory systems appear to be involved. It is very important to note that behavioral expressions largely vary among species; moreover, responses to pheromones cannot be simply described as innate or stereotyped, but may also depend on contextual situations and can be modified by some forms of learning and memory.

### TABLE 2. Effects of surgical and genetic ablations of olfactory and vomeronasal systems

<table>
<thead>
<tr>
<th>Species</th>
<th>VNX</th>
<th>VGENX</th>
<th>OEX</th>
<th>OGENX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>↓ Scent marking (290)</td>
<td></td>
<td>↓ Partner preference (10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Male copulatory behavior (55, 76, 298)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Lordosis behavior (221)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Individual recognition (365)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>↓ Stress-induced hyperthermia (165)</td>
<td>↓ Sex discrimination (trpc2) (193, 284, 368)</td>
<td>↓ Copulatory behavior (ac-3) (305)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal aggression (170)</td>
<td>↓ Intramale aggressiveness (trpc2; gol2) (278, 368)</td>
<td>↓ Sex discrimination (154)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Male induced sexual receptivity (302)</td>
<td>↓ Maternal aggression (trpc2; vIra-vIrb; gol2) (61, 161, 193, 278)</td>
<td>↓ Intramale aggressiveness (ac-3) (306)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Nursing behavior (36)</td>
<td>↓ Intermale aggressiveness (54, 233)</td>
<td>↓ Individual recognition (cnga2) (364)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Sexual arousal (325)</td>
<td>↓ Lactating behavior (trpc2) (161)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Lordosis behavior (326)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>↓ Lordosis behavior to male mount (155)</td>
<td>↓ Sex discrimination (trpc2) (193, 284, 368)</td>
<td>↓ Copulatory behavior (ac-3) (305)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Sperm motility (175)</td>
<td>↓ Intramale aggressiveness (trpc2; gol2) (278, 368)</td>
<td>↓ Sex discrimination (154)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Urine marking (181, 233)</td>
<td>↓ Maternal aggression (trpc2; vIra-vIrb; gol2) (61, 161, 193, 278)</td>
<td>↓ Intramale aggressiveness (ac-3) (306)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Intermale aggressiveness (54, 233)</td>
<td>↓ Lactating behavior (trpc2) (161)</td>
<td>↓ Individual recognition (cnga2) (364)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Marking</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>↓ Copulatory behavior (54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Puberty onset (299)</td>
<td>↓ Copulatory behavior (vIra-vIrb) (61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Strain recognition (206)</td>
<td>↓ Marking (trpc2)(193)</td>
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<tr>
<td></td>
<td>↓ Pregnancy block (156, 206, 216)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>↓ Sexual investigation (135)</td>
<td></td>
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<tr>
<td></td>
<td>Male-like sexual behavior in females (trpc2) (161)</td>
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<tr>
<td>Ferret</td>
<td>↓ Sexual investigation</td>
<td></td>
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<tr>
<td></td>
<td>(404)</td>
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<td></td>
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</tr>
<tr>
<td>Lemur</td>
<td>↓ Copulatory behavior (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Intramale aggressiveness (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opossum</td>
<td>↓ Male-mediated induction of estrus (131)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prairie vole</td>
<td>↓ Intramale aggressiveness (399)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Pairing (189)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>↑ Maternal behavior (87)</td>
<td></td>
<td>↓ Suckling behavior</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
<td>↑ Maternal behavior (52)</td>
<td>↓ Catnip reaction (104)</td>
</tr>
</tbody>
</table>

Reference numbers are given in parentheses.

### A. Male Behaviors

#### 1. Male sexual behavior

Sexual behavior has been intensively approached by many studies in the last 20 years, particularly in rodents. In male mice, the typical mating sequence consists of olfactory exploration of estrus females followed by multiple episodes of ultrasound vocalization, mounting and intromission, and eventually ejaculation. All these parameters can be evaluated in a typical test experiment (120, 121).
The genetic ablation of the TRPC2 channel greatly impairs some features of the sexual behavior. Indeed, although male mice mate normally with females, they also show an indiscriminate courtship and mounting behavior toward females (161, 193, 368). A similar situation was observed in VNOx male mice (161). Furthermore, individual recognition, through the discrimination of nonvolatile odorants, is also affected in VNOx mice (155, 284). On the other hand, the simultaneous deletion of a group of V1R receptors results in a significant decrease of male copulatory behavior (61), although this latter appears normal in Goα2 mutants, suggesting that apical neurons are likely not to be of relevant importance for mating motivation. All these findings reveal that the mouse vomeronasal organ is indeed involved in individual recognition and reproductive activity, but that signaling through the vomeronasal organ is not required for initiating sexual behavior.

The chemical ablation of the main olfactory system (via nasal irrigation with zinc sulfate) produces a striking reduction in male reproductive behavior (227). Furthermore, the genetic deletion of the CNGA2 channel (227), or of adenylyl cyclase type 3 (395), entirely abolishes male mating performances producing severe deficiencies in each step involved in mating: olfactory exploration, mounting, and intromission, compared with normal mice. Thus the main olfactory system seems to play an essential role for male mouse mating behavior.

In rats, individual recognition and sexual arousal is only temporary or moderately depressed by lesions of the vomeronasal organ (22, 325) as well as the overall copulatory behaviors appear to be slightly or partially affected (171, 324, 325).

Sexual behavior in male hamsters is totally abolished by bilateral removal of the olfactory bulbs, an operation that eliminates sensory input from both the olfactory and the vomeronasal systems (298). The destruction of the main olfactory epithelium did not impair male hamster mating behavior, whereas the peripheral deafferentation of the vomeronasal system produces severe sexual behavior deficits in approximately one-third of the treated animals. Combined deafferentation of both the vomeronasal and the olfactory systems completely abolishes copulation in all experimental animals (298). Mating behavior alterations appear after removal of the vomeronasal organ in sexually inexperienced males, and this effect is reversed by intracerebroventricular injections of GnRH (76, 247), indicating that GnRH acts as an intermediate in the facilitation of mating behavior by vomeronasal sensory input. However, sexual experience prior to the removal of the vomeronasal organ mitigates the effect of the lesion on sexual behavior, indicating that behavioral responses are influenced both by VNOx and by sexual experience (291). In this species, other ways of communication as scent or flank marking are likely to be primarily mediated by the main olfactory system (145).

2. Male aggressiveness

In mice, male to male territorial aggression is a remarkable innate social behavior that is triggered by compounds present in urine (see sect. II, Table 1). In a common assay employed to test male aggressiveness, a male intruder is introduced in a cage containing a singly housed male resident. After a period of olfactory investigation, to allow individual recognition, the resident mouse initiates a series of attacks against the intruder. The number and duration of the attacks and the latency to the first attack represent standard parameters to evaluate the aggressive behavior (58, 233).

The surgical ablation of the vomeronasal organ has been long known to nearly abolish aggressiveness in male mice and prairie voles (12, 54, 55, 233, 399, 407), an effect that is recapitulated in mutant mice bearing the deletion of the gene encoding TRPC2 (161, 193, 368). Indeed, male mice bearing the genetic ablation of the TRPC2 channel fail to display aggression toward male intruders (193, 368), a behavioral effect that, in an attenuated form, is also evident in mice lacking Goα2 (278).

The main olfactory system also plays a very important role in male aggressiveness. Indeed, mice that either have undergone the chemical ablation of the olfactory epithelium (via nasal irrigation with zinc sulfate) (154) or have been genetically mutated in the genes encoding the odorant transduction molecules adenylyl cyclase type 3 and CNGA2 (227, 395) show abnormal sexual and aggressive behaviors (227).

Thus both the olfactory and the vomeronasal systems appear to be necessary for eliciting male aggressiveness.

B. Female Behaviors

1. Female sexual behavior

In females of different species, sexual behavior is characterized by ultrasonic vocalization and lordosis elicited by lumbosacral tactile stimulation (174, 294).

In mice, the genetic ablation of the TRPC2 channel did not alter female sexual receptivity to males but, surprisingly, caused a female sexual behavior similar to that of males, i.e., females attempted to copulate as males (161). Kimchi et al. (161) also showed that similar sexual behaviors were displayed by females in which the vomeronasal organ was surgically removed, in contrast to a previous study that described a reduced sexual receptivity in VNOx females (155). The chemical ablation of the main olfactory epithelium (with zinc sulfate) reduces sexual behavior in female mice (154).

In female rats, both chemical ablation of the main olfactory epithelium and vomeronasal organ removal af-
fect sexual receptivity and the male-induced release of GnRH (17, 302).

In female hamsters, the removal of the vomeronasal organ disrupts the elicitation of lordosis by lumbosacral tactile stimulation, an effect reversed by injection of GnRH (221).

2. Maternal aggressive behavior

Female mice are normally not aggressive towards intruders, but during lactation, they violently attack non-resident males. This type of maternal aggressive behavior has been shown to be dependent on chemosensory cues (13). The surgical removal of the vomeronasal organ affects aggression of lactating females (13, 161), whereas it has little or no effect on other maternal behavior parameters as nest building or pup retrieval (13). The genetic ablation of the TRPC2 channel (161, 193), the Gaq subunit (278), or a subfamily of V1R receptors (61), also results in a low expression of maternal aggression, suggesting that this type of behavior is possibly linked to the apical subset of chemosensory neurons.

VIII. CONCLUSIONS AND FUTURE CHALLENGES

Although we are only beginning to understand the overlapping role of the main olfactory and vomeronasal systems for pheromone sensing, there is now overwhelming evidence that the main olfactory system is involved in a variety of important physiological mechanisms underlying pheromonal communication.

In the early 1970s, different studies developed a model that led to the so-called dual olfactory hypothesis, sustained by the observation that efferent and afferent connections of the main and accessory olfactory bulb project in nonoverlapping areas of the brain, according to which the olfactory and vomeronasal systems were organized as parallel anatomical axes subserving different functions. Recent anatomical and functional studies have indicated that this distinction is not as strict as commonly thought (229). There seems to be, in fact, areas of large convergence in the anterior medial amygdala. Moreover, in the rostral basal telencephalon, both inputs converged in areas that were classically categorized as exclusively vomeronasal or olfactory. As for the anatomical observation, behavioral and biochemical studies seem to point to a complementary role of the two systems. For example, the functional ablation of the olfactory and vomeronasal organs leads to similar behavioral changes. Chemosensory neurons sensitive to pheromone have been identified in both subsystems. Not last, humans as well as other species in which the vomeronasal system is absent can probably detect pheromones via the main olfactory epithelium. It is therefore possible that the two systems may have developed independently for the detection of pheromones, and only the main olfactory epithelium has further specialized also for the perception of conventional odors. Proof of this hypothesis will require additional studies especially in species in which olfaction is not a primary sensory process as humans, for example; however, it is interesting to note that part of the main olfactory central pathways seem to converge in regions that control the sexual status of the individual.

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