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Chemosensory cues from the lacrimal and preputial glands stimulate production of IP₃ in the vomeronasal organ and aggression in male mice

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Abstract

The social and reproductive behaviors of most mammals are modulated by chemosensory cues. The perception of some of these cues is mediated by the vomeronasal organ, which is a cartilage-encased elongated organ associated with the vomer bone in the rostral nasal cavity. Several studies have shown that chemosensory cues are present in urine, seminal fluid or vaginal secretions but only a few studies have focused on exocrine glands as a source of chemosensory cues. Here we show that chemosensory cues present in two exocrine glands, the preputial gland located at the caudal region, and the lacrimal gland located at the rostral region are capable of stimulating aggression in male mice. We further show that these extracts can stimulate the production of inositol-(1,4,5)-trisphosphate in the vomeronasal organ.

Keywords

Pheromones; VNO; Exocrine Glands; Harderian Gland; Lacrimal Gland; IP₃

1. Introduction

Most mammals use chemical signals (pheromones) to coordinate reproductive and social behaviors. These chemical signals can be classified into two categories: those with short term effects on the behavior of the recipient (signaling pheromones), and those with long term effects on the physiology of the recipient (priming pheromones) [1]. For example, signaling pheromones in urine or glandular secretions play a role in the initiation of copulatory behavior and aggression [2], whereas priming pheromones are responsible for puberty acceleration [3, 4,5,6,7], and reproductive activation [8,9]. Although the perception of signaling pheromones may be mediated by the main olfactory system, the physiological effects of most priming pheromones are initiated in the vomeronasal organ (VNO). Recent evidence has shown that the detection of pheromones can rely on both the main olfactory system and the vomeronasal system [10,11]. The VNOs are paired, cartilage-encased elongated organs associated with the vomer bone in the rostral nasal cavity. The VNO contains a lumen which communicates via a duct with the oral or nasal cavity [12,13]. Therefore chemical stimuli in urine and glandular secretions of conspecifics can act upon the dendritic microvilli of bipolar chemosensory neurons in the VNO.

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Molecular evidence has led to the isolation of two independent families of vomeronasal receptor genes (VR), known as V1Rs [14], and V2Rs [15,16,17] that encode putative pheromone receptors. V1Rs and V2Rs are expressed by distinct subpopulations of VNO neurons. These populations are non-overlapping and individual VNO neurons express only one receptor gene [14,15,16,17]. Neurons lining the apical layer of the VNO neuroepithelium express V1Rs [14] whereas neurons in the basal layer express V2Rs [15,16,17]. The neurons expressing V1Rs also express the alpha subunit of $G\alpha_{i2}$ and project to the anterior region of the accessory olfactory bulb (AOB), whereas the neurons expressing V2Rs also express the alpha subunit of $G\alpha_O$ and project to the posterior regions of the AOB [18,19,20]. The expression of two types of pheromone receptors supports the idea that they might be involved in different types of chemosensory information.

Most of the studies based on identifying the ligands for pheromone receptors has focused on chemicals present in urine, seminal fluid and vaginal secretions. However recent studies by Luo et al. [21] found that in their recording sessions, the first interactions between animals involved investigating the facial and mouth areas. They found that test animals investigated the face and head of stimulus animals more frequently and for longer time periods than the anogenital areas. These rostral areas also evoked the most robust responses by neurons in the AOB of behaving mice [21]. From these experiments they suggested that chemicals from glands found in the facial area such as the Harderian glands and the submaxillary salivary glands are capable of stimulating the VNO. A more recent study by Kimoto et al., [22] demonstrated that a peptide from the lacrimal gland of a male mouse was able to stimulate V2R –expressing vomeronasal sensory neurons in the female mouse. These studies suggest that exocrine compounds found in the facial region of the mouse are capable of stimulating the VNO. We therefore decided to explore whether other exocrine glands located at the caudal or rostral region can stimulate the VNO via the production of IP_3 . Furthermore, we wanted to determine the behavioral role elicited by these exocrine compounds.

2. Methods & Materials

2.1. Animals

CD-1 mice were originally obtained from Charles River Laboratories (Kingston, NY) and maintained in a breeding colony in the Department of Biological Sciences at Alabama State University. Animals were housed in Institutional Animal Care and Use Committee (IACUC) inspected and approved facilities and cared for according to the NIH Guide for Care and Use of Laboratory Animals. Mice were kept in Nalgene cages 26 cm × 21 cm × 14 cm, at 25°C room temperature and a 12/12 hour light/dark cycle. Food and water were provided *ad libitum*.

2.2. Membrane Preparation

Male mice VNO's were dissected from their crevices in the nasal cavity, removed from the cartilaginous capsule and frozen on dry ice. The tissues were minced with a razor blade, crushed with a Teflon pestle and subjected to sonication for 2–5 min in ice-cold phosphate-buffered saline (PBS) in a Branson bath sonicator. The resulting suspension was layered on a 45% (w/w) sucrose solution and centrifuged at 4°C for 30 min at 40,000 r.p.m. in a Beckman SW55Ti rotor. The membrane fraction was collected and centrifuged as before for 15 min to pellet the membranes. The membranes were resuspended in 100 μ l of ice-cold PBS. Protein concentration was then determined according to the method of Lowry *et al.* [23]. The procedure used for the preparation of microvillar membranes is modeled after well established methods for harvesting olfactory cilia from olfactory neuroepithelium [24,25]. The sonication of membranes results not only in the detachment of vomeronasal microvilli but also in the detachment of microvilli from sustentacular cells and plasma membrane fragments from other

components of the neuroepithelium. Electron microscopic examination of these preparations revealed vesicles, axonemal structures devoid of a plasma membrane, and axonemal structures associated with membrane fragments [24,25]. The membrane preparation we refer to as “microvillar membranes” is, therefore, likely to contain contaminants derived from other components of the VNO, including microvillar membranes from supporting cells, and it is difficult to estimate the purity of the preparation precisely. However, our preparation appears to be sufficiently enriched in chemosensory membranes for the purpose of our studies [26, 27,28,29].

2.3. Exocrine Glandular Extract Preparation

The preputial gland, lacrimal gland, and the Harderian gland were dissected from adult male CD-1 mice. Preputial glands are located in front of the genitals and are thought to produce pheromones. The lacrimal glands are paired exocrine glands that sit alongside the eyeball within the orbit, nestled in the lacrimal fossa of the frontal bone and secrete lacrimal fluid. The Harderian glands are located near the eyes and produce a reddish-brown discharge when the animals are stressed. The glands from five adult male mice were dissected free and placed in ice-cold PBS. They were homogenized, centrifuged and aliquoted for storage. For our studies we used 20 μ l which is estimated to be equal to one gland as was done in previous studies by Ma *et al.*, (1999). Male urine was collected from adult males, pooled, spun for 5 min at 5,000 g and decanted on the day of use.

2.4. Second Messenger Assay

For IP₃ assays, reactions were incubated for 1min at 37°C in 25 mM Tris-acetate buffer pH 7.2, 5 mM Mg-acetate, 1 mM DTT, 0.5 mM ATP, 0.1 mM CaCl₂, 0.1 mg/ml bovine serum albumin, 10 μ M GTP, and 20 μ g VNO membrane protein. 20 μ l of stimulant, which is equivalent to 10% of the reaction volume, was used. Reactions were terminated by the addition of 1M trichloroacetic acid. IP₃ was measured with a kit from Perkin Elmer, Inc. (Boston, MA) according to the manufacturer’s instructions and is based on displacement of [³H] IP₃ from a specific IP₃ binding protein. Differences between experimental and control animals were analyzed by analysis of variance (ANOVA).

2.5. Behavioral Studies

In order to evaluate if the exocrine extracts induced aggression in males, we monitored the ability of males to initiate aggressive displays in response to pheromones using the resident-intruder paradigm. No conditioning or training period is required in this assay as it relies on innate and stereotyped behaviors initiated by chemosensory cues. After an isolated resident male mouse has established the cage as its territory, a dummy mouse to which 20 μ l of a glandular extract or PBS had been applied was placed in the cage. The latency to sniff, duration of sniffs, and the number of attacks (bites) were recorded during a 5 min test period. Behavioral tests were conducted in an isolated, darkened room using a 40 watt red bulb for visibility. To facilitate the delivery of only one gland’s components, a dummy mouse was constructed using the toe portion of a white cotton sock. Each construct was stuffed with cotton, shaped in a characteristic manner resembling a mouse, and sewn closed. Several sizes were made in order to be size matched with the resident mouse at the time of presentation. Extracts were applied in a rostral or caudal location and the dummy mouse placed in the opposing corner with the rostral end towards the resident male. The dummy mouse was chosen over castrate males for the following reasons: 1) although castrate males produce no androgen dependent pheromones, other chemosensory cues are present such as the MHC class I peptides, ESP1, and possible others as yet unidentified, 2) lacrimal and Harderian glands have not previously been shown to be androgen dependent, 3) the use of the dummy mouse allowed the application of chemical cues from one specific source without influence from other venues, and 4) use of the dummy

mouse is not that different than using a cotton swab to introduce single odorants in experiments for habituation-dishabituation studies. Experiments using castrated mice were performed as follow-up studies to the previous experiments. These experiments were identical to the dummy mouse experiments. Although the numerical values increased the overall trends remained the same (Data not included). Differences between experimental and control animals were analyzed by analysis of variance (ANOVA).

3. RESULTS

3.1. Preputial and Lacrimal Gland Extracts Stimulate the Production of IP₃ in the Male Vomeronasal Organ

Relatively little information is available about the role of exocrine glands producing chemicals that affect the vomeronasal organ. To study compounds produced by exocrine glands that stimulate the vomeronasal organ, we focused our attention to the preputial gland (PG), lacrimal gland (LG), and the Harderian gland (HG). These exocrine glands, two of which are located at the rostral region of the mouse (HG and LG) and one (PG) located at the caudal region of the male mouse, were selected due to behavioral responses observed in mouse social behaviors [21]. When mice are put into a cage they spend a significant amount of time investigating the caudal and rostral region of each other. This suggests that there might be chemical signals produced in these areas that are passed from one animal to the other. Since several experiments have focused on the role of urine as a source of pheromones, we decided to focus our attention on the exocrine glands located in these regions. Incubation of male VNO membrane preparations with extracts from the preputial gland and the lacrimal gland of male mice resulted in a significant increase in the production of IP₃ ($P < 0.05$), whereas incubation of male VNO preparations with the Harderian gland was not significant (Figure 1). In this experiment we used male urine as our positive control and PBS as our negative control. These data suggest that there are compounds that are being produced in the preputial and lacrimal gland that are capable of stimulating the VNO.

3.2. Preputial and Lacrimal Gland Extracts Stimulate Aggression in Male Mice

Resident mice typically sniff and attack intruder males. In order to determine if the gland extracts from the preputial, lacrimal and Harderian gland stimulate male-male aggression, we introduced a dummy mouse swabbed in the exocrine gland extract. We found that when the resident males were exposed to a dummy mouse swabbed in male urine, preputial extract or lacrimal extract, the latency to sniff decreased significantly ($P < 0.05$) (Figure 2), whereas the duration of sniffs (Figure 3), and attacks (Figure 4) significantly increased ($P < 0.05$). Exposure to extracts from the Harderian gland had no effect on the latency to sniff, duration of sniffs or attacks.

4. Discussion

Despite the importance of the VNO for reproduction and social behaviors of mammals, little information is known about the identity and source of the chemosensory cues and their transduction cascades. This is unlike the reptilian system where the vomeronasal system has been well characterized. In snakes the vomeronasal system provides the predominant chemosensory input. In the garter snake a defined polypeptide purified from the secretions of earthworms, the snake's prey, stimulates phospholipase C and inhibits adenylate cyclase [36, 37]. In the turtle, an inositol trisphosphate activated conductance has been observed in patch clamp studies on vomeronasal receptor cells upon intracellular injection of IP₃ [38]. Studies on identifying the source and identity of mammalian pheromones on the other hand have been few. In hamsters a protein named aphrodisin from female vaginal discharge has been identified that acts on the vomeronasal organ of male hamsters and induces copulation [39,40]. In mice,

recent studies show that MHC peptides excreted in male urine are capable of kin recognition and pregnancy block [41]. Other compounds in urine such as 2-heptanone and 2, 5 dimethylpyrazine have also been shown to stimulate the vomeronasal organ. Most of the documented studies have focused on identifying chemicals from vaginal discharge, urine, and seminal fluid.

In our studies, we have been able to show that incubation of male VNO membranes with extracts from the lacrimal gland and the preputial gland stimulate the production of IP₃ in the male VNO (Figure 1). Both these glands are exocrine glands but the lacrimal gland is located in the rostral region of the mouse whereas the preputial gland is located in the caudal region of male mice. Interestingly, we observed that although stimulation with preputial gland and lacrimal gland were significantly higher than the basal levels, stimulation by urine was also significantly higher than stimulation by the lacrimal gland and preputial gland. These results suggest that urine may contain a cocktail of pheromones that stimulate many different types of receptors and thus stimulate a higher amount of IP₃ production whereas the lacrimal and preputial gland may contain fewer compounds that stimulate fewer receptors. In order to determine whether another endocrine gland located in the rostral region is capable of stimulating the VNO we also incubated our VNO membranes with extracts from the Harderian gland. Incubation with extracts from the Harderian gland did not stimulate the production of IP₃ in VNO membranes (Figure 1). Thus it seems that the response of the male VNO to lacrimal gland extracts is specific and may coordinate a specific behavior.

Previous studies on the preputial gland have associated it with male/male aggression but the lacrimal gland and Harderian glands have not been correlated to any specific behaviors. The mouse preputial gland has been shown to release aggression-promoting pheromones to the urine [31,32,33]. Preputial hypertrophy results in male mice expressing dominant behaviors [34] whereas preputial atrophy results in male mice expressing subordinate behaviors [35]. These results suggest that preputial extracts may promote the acquisition and maintenance of social status. In male mice, the preputial gland has been shown to be involved in the release of aggression promoting pheromones to the urine [31,32,33]. Preputial activity depends on the circulating melanocortin, alpha-melanocyte-stimulating hormone (α -MSH) [39,40]. Melanocortin injection has been shown to release into the urine of male mice a chemical signal that stimulates attacking behavior from test mice when swabbed onto stimulus mice [44,45]. Deficiency in the melanocortin receptor MC5R which is highly expressed in the preputial gland has been shown to lead to reduced aggression and elevated defense toward wild type opponents [46,47] and a recent study has shown that decreased preputial pheromones contribute to the decreased aggressive responses of MC5R knock out mice [47]. Targeted deletion of the transient receptor potential channel 2 (TRP2) expressed in the VNO, attenuates the responsiveness of the VNO neurons to mouse urine and abolishes aggression [48,49]. Leypold et al., [48] examined aggression in TRP2^{-/-} animals, in a resident-intruder assay and found that after a period of vigorous olfactory exploration, wild-type resident mice would initiate vigorous attacks against the intruder. In experiments using sexually inexperienced control mice, they observed that sniffing was followed by biting attacks, chasing, violent tumbling, and fighting. The TRP2^{-/-} mice exhibited the same olfactory exploratory behavior as wild type mice, but rarely initiated biting attacks. These results suggest that the VNO is involved in initiating aggression in male mice [45] and our study shows that the lacrimal gland may be involved in producing chemical cues that initiate aggression.

Our behavioral studies show that lacrimal gland extracts and preputial gland extracts are capable of stimulating aggressive behaviors. We found that when the resident males were exposed to a dummy mouse swabbed with lacrimal extract or preputial extract, the latency to sniff decreased significantly ($P < 0.05$) (Figure 2), the duration of sniffs (Figure 3), and attacks (Figure 4) significantly increased ($P < 0.05$). These studies suggest that chemicals in the lacrimal

and preputial extracts are capable of stimulating spontaneous aggressive behaviors in male mice. In male mice, spontaneous aggression is elicited between two males and is thought to be influenced by pheromonal signals. This aggressive behavior may result in social dominance.

In summary, we have shown that chemosensory cues present in two exocrine glands, the preputial gland located at the caudal region, and the lacrimal gland located at the rostral region of the male mouse are capable of stimulating the vomeronasal organ via the production of IP₃. Furthermore we have shown that compounds from the lacrimal gland and the preputial gland stimulate aggression in male mice. Future experiments should lead to identifying chemicals in the lacrimal gland that stimulate aggression.

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References

1. Vanderbergh, JG. Pheromones and mammalian reproduction. In: Knobil, E.; Neill, D., editors. *Physiology of Reproduction*. 2. New York: Raven Press; 1994. p. 343-359.
2. Murphy MR. Sexual preferences of male hamsters: importance of preweaning and adult experience, vaginal secretion and olfactory or vomeronasal sensation. *Behav Neurol Biol* 1980;30:323-340.
3. Vanderbergh JG. Male odor accelerates female sexual maturation in mice. *Endocrinol* 1969;81:345-349.
4. Drickamer LC, Murphy RX. Female mouse maturation: effects of excreted and bladder urine from juvenile and adult males. *Dev Psychobiol* 1978;11:63-72. [PubMed: 564797]
5. Mucignat-Caretta C, Caretta F, Cavaggioni A. Acceleration of puberty onset in female mice by male urinary proteins. *J Physiol* 1995;482:517-522. [PubMed: 7473215]
6. Jemiolo B, Andreolini F, Xie T, Wiesler D, Novotny M. Puberty-affecting synthetic analogs of urinary chemosignals in the house mouse, *Mus domesticus*. *Physiol. Behav* 1989;46:293-298.
7. Hasler JF, Banks EM. The influence of mature males on sexual maturation in female collared lemmings (*Dicrostonyx groenlandicus*). *J. Reprod. Fertil* 1975;42:583-586.
8. Lepri JJ, Vandenbergh JG. Puberty in pine voles, *Microtus pinetorum*, and the influence of chemosignals on female reproduction. *Biol. Reprod* 1986;34:370-377.
9. Carter CS, Getz LL, Gavish L, McDermott JL, Arnold P. Male related pheromones and the activation of female reproduction in the prairie vole *Microtus ochrogaster*. *Biol. Reprod* 1980;23:1038-1045.
10. Boehm U, Zou Z, Buck LB. Feedback loops link odor and pheromone signaling with reproduction. *Cell* 2005;123:683-695. [PubMed: 16290036]
11. Yoon H, Enquist LW, Dulac C. Olfactory inputs to hypothalamic neurons controlling reproduction and fertility. *Cell* 2005;123:669-682. [PubMed: 16290037]
12. Meredith, M. Sensory physiology of pheromone communication. In: Vanderbergh, JG., editor. *Pheromones and reproduction in Mammals*. New York: Academic Press; 1983. p. 95-112.
13. Wysocki, CJ.; Meredith, M. The vomeronasal system. In: Finger, TE.; Silver, WL., editors. *Neurobiology of Taste and Smell*. New York: John Wiley & Sons; 1991. p. 125-150.
14. Duclac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 1995;83:195-206. [PubMed: 7585937]
15. Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell* 1997;90:763-770. [PubMed: 9288755]
16. Matsunami H, Buck L. A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* 1997;90:775-784. [PubMed: 9288756]
17. Ryba NJP, Tirindelli R. A new multigene family of putative pheromone receptors. *Neuron* 1997;19:371-379. [PubMed: 9292726]

18. Halpern M, Shapiro LS, Jia C. Differential localization of G proteins in the opossum vomeronasal system. *Brain Res* 1995;677:157–161. [PubMed: 7606461]
19. Wekesa KS, Anholt RRH. Differential expression of G proteins in the mouse olfactory system. *Brain Res* 1999;837:117–126. [PubMed: 10433994]
20. Rodriguez I, Feinstein P, Mombaerts P. Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. *Cell* 1999;97:199–208. [PubMed: 10219241]
21. Luo M, Fee MS, Katz LC. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. *Science* 2003;299:1196–1201. [PubMed: 12595684]
22. Kimoto H, Haga S, Sato K, Touhara K. Sex-specific peptides from exocrine glands stimulate mouse vomeronasal sensory neurons. *Nat* 2005;437:898–901.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement by Folin phenol reagent. *J Biol Chem* 1951;193:265–275. [PubMed: 14907713]
24. Anholt RRH, Aebi U, Snyder SH. A partially purified preparation of isolated chemosensory cilia from olfactory epithelium of the bullfrog, *Rana catesbiana*. *J Neurosci* 1986;6:1962–1969. [PubMed: 3525776]
25. Anholt, RRH. Preparation of olfactory cilia. In: Spielman, AL.; Brand, JG., editors. *Experimental Cell Biology of Taste and Olfaction, Current Techniques and Protocols*. New York; CRC Press: 1995. p. 163-168.
26. Wekesa KS, Anholt RRH. Pheromone regulated production of inositol-(1, 4, 5)-trisphosphate in the mammalian vomeronasal organ. *Endocrinol* 1997;138:3497–504.
27. Wekesa KS, Miller S, Napier A. Involvement of G(q/11) in signal transduction in the mammalian vomeronasal organ. *J Exp Biol* 2000;206:827–32. [PubMed: 12547937]
28. Thompson RN, Robertson BK, Napier A, Wekesa KS. Sex-specific responses to urinary chemicals by the mouse vomeronasal organ. *Chem Senses* 2004;29:749–54. [PubMed: 15574810]
29. Thompson RN, Napier A, Wekesa KS. Attenuation of the production of inositol 1,4,5-trisphosphate in the mouse vomeronasal organ by antibodies against the α q/11 subfamily of G-proteins. *Chem Senses* 2006;31:613–9. [PubMed: 16757571]
30. Ma W, Miao Z, Novotny MV. Induction of Estrus in Grouped Female Mice (*Mus domesticus*) by Synthetic Analogues of Preputial Gland Constituents. *Chem Senses* 1999;24:289–293. [PubMed: 10400447]
31. Jones RB, Nowell NW. Effects of preputial and coagulating gland secretions upon aggressive behaviour in male mice: a confirmation. *J Endocrinol* 1973;59:203–204. [PubMed: 4795918]
32. Novotny M, Harvey S, Jemiolo B, Alberts J. Synthetic pheromones that promote inter-male aggression in mice. *Proc Natl Acad Sci USA* 1985;82:2059–2061. [PubMed: 3856883]
33. Ingersoll DW, Morley KT, Benvenga M, Hands C. An accessory sex gland aggression-promoting chemosignal in male mice. *Behav Neurosci* 1986;100:777–782. [PubMed: 3778640]
34. Huckelbridge FH, Nowell NW, Wouters A. A relationship between social experience and preputial gland function in the albino mouse. *J Endocrinol* 1972;55:449–450. [PubMed: 4673881]
35. Brain PF, Simon VM, Martinez M. Ethopharmacological studies on the effects of antihormones on rodent agonistic behavior with especial emphasis on progesterone. *Neurosci Biobehav Rev* 1991;15:521–526. [PubMed: 1838800]
36. Jiang XC, Inouchi J, Wang D, Halpern M. Purification and characterization of a chemo-attractant from electric shock-induced earthworm secretion, its receptor binding, and signal transduction through the vomeronasal system of garter snakes. *J Biol Chem* 1990;265:8736–8744. [PubMed: 2160465]
37. Luo Y, Lu S, Chen P, Wang D, Halpern M. Identification of chemoattractant receptors and G-proteins in the vomeronasal system of garter snakes. *J Biol Chem* 1994;269:16867–16877. [PubMed: 7515886]
38. Taniguchi M, Kashiwayanagi M, Kurihara K. Intracellular injection of inositol-(1,4,5)-trisphosphate increases a conductance in membranes of turtle vomeronasal receptor neurons in the slice preparation. *Neurosci Lett* 1995;188:5–8. [PubMed: 7540275]
39. Clancy AN, Coquelin A, Macrides F, Gorski RA, Noble EP. Sexual behavior and aggression in male mice: involvement of the vomeronasal system. *J Neurosci* 1984;4:2222–2229. [PubMed: 6541245]

40. Singer AG, Macrides F, Clancy AN, Agosta WC. Purification and analysis of a proteinaceous aphrodisiac pheromone from hamster vaginal discharge. *J Biol Chem* 1986;261:13323–13326. [PubMed: 3759967]
41. Leinders-Zufall T, Brennan P, Widmayer P, Chandramani PS, Pavicic AM, Jager M, Li XH, Breer H, Zufall F, Boehm T. MHC class I peptides as chemosensory signals in the vomeronasal organ. *Science* 2004;306:1033–1037. [PubMed: 15528444]
42. Cooper MF, Bowden PE, Thody AJ, Shuster S. Proceedings: synergistic effects of testosterone and alpha-melanocyte-stimulating hormone on lipogenesis in rat preputial gland. *J Endocrinol* 1975;64:63.
43. Ebling FJ, Ebling E, Randall V, Skinner J. The synergistic action of alpha-melanocyte-stimulating hormone and testosterone on the sebaceous, prostate, preputial, Harderian and lachrymal glands, seminal vesicles and brown adipose tissue in the hypophysectomized castrated rat. *J Endocrinol* 1975;66:407–412. [PubMed: 1194815]
44. Nowell NW, Wouters A. Proceedings: release of aggression-promoting pheromone by male mice treated with alpha-melanocyte-stimulating hormone. *J Endocrinol* 1975;65:36–37.
45. Nowell NW, Thody AJ, Woodley R. The source of an aggression-promoting olfactory cue, released by alpha-melanocyte stimulating hormone, in the male mouse. *Peptides* 1980;1:69–72. [PubMed: 7195571]
46. Caldwell HK, Lepri JJ. Disruption of the fifth melanocortin receptor alters the urinary excretion of aggression-modifying pheromones in male house mice. *Chem Senses* 2002;27:91–94. [PubMed: 11751473]
47. Morgan C, Thomas RE, Cone RD. Melanocortin-5 receptor deficiency promotes defensive behavior in male mice. *Horm Behav* 2004;45:58–63. [PubMed: 14733892]
48. Leypold BG, Yu CR, Leinders-Zufall T, Kim MM, Zufall F, Axel R. Altered sexual social behaviors in *trp2* mutant mice. *PNAS* 2002;99:6376–6381. [PubMed: 11972034]
49. Stowers L, Holy TE, Meister M, Dulac C, Koentges G. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. *Science* 2002;295:1493–1500. [PubMed: 11823606]

Figure 1.

Preputial and lacrimal gland extracts from male mice stimulate the production of IP₃ in the male vomeronasal organ. Reactions were performed without stimulus, in the presence of 10% male urine, 10% preputial gland extract, 10% lacrimal gland extract or 10% Harderian gland extract. Significant stimulation compared to basal activity (PBS) is observed in the presence of male urine, preputial gland extract and lacrimal gland extract (* $P < 0.05$) and not in the presence of Harderian gland extract. The data points and error bars represent the averages and standard errors of at least ten independent experiments, each consisting of duplicate measurements.

Figure 2.

Male mice are highly responsive to extracts from male preputial and lacrimal gland. Resident males were exposed to a "dummy mouse" swabbed with PBS, male urine, preputial gland extract, lacrimal gland extract or Harderian gland extract for 5 min. The latency to approach the dummy mouse swabbed with preputial gland extract and lacrimal gland extract was significantly shorter than the one swabbed with PBS (* $P < 0.05$). The resident mice did not show any interest in the dummy mouse swabbed with Harderian gland extract nor PBS. The data points and error bars represent the averages and standard errors of at least ten independent experiments, each consisting of duplicate measurements.

Figure 3.

Male mice have higher duration of sniffs to extracts from male preputial and lacrimal gland. Resident males were exposed to a "dummy mouse" swabbed with PBS, male urine, preputial gland extract, lacrimal gland extract or Harderian gland extract for 5 min. The resident males sniffed the dummy mouse swabbed with male urine, preputial gland extract and lacrimal gland extract more frequently than the one with PBS or Harderian gland (* $P < 0.05$). The data points and error bars represent the averages and standard errors of at least ten independent experiments, each consisting of duplicate measurements.

Figure 4.

Male mice are more aggressive to extracts from preputial gland, lacrimal gland and male urine. Resident males were exposed to a "dummy mouse" swabbed with PBS, male urine, preputial gland extract, lacrimal gland extract or Harderian gland extract for 5 min. The resident mouse attacked the dummy mouse swabbed with preputial gland extract, or lacrimal gland, or male urine more than the control (PBS) (* $P < 0.05$). There was no significant difference in the frequency of attacks between the control dummy mouse and the dummy mouse swabbed with Harderian extract. The data points and error bars represent the averages and standard errors of at least ten independent experiments, each consisting of duplicate measurements.