Human Male Superiority in Olfactory Sensitivity to the Sperm Attractant Odorant Bourgeonal

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Abstract

Recent studies have shown that sperm chemotaxis critically involves the human olfactory receptor OR1D2, which is activated by the aromatic aldehyde bourgeonal. Given that both natural and sexual selection may act upon the expression of receptors, we hypothesized that human males are more sensitive than human females for bourgeonal. Using a 3-alternative forced-choice test procedure, olfactory detection thresholds were determined for a total of 500 subjects, 250 males, and 250 females between 18 and 40 years of age. We found that male subjects detected bourgeonal at significantly lower concentrations (mean value: 13 ppb) compared with female subjects (mean value: 26 ppb), whereas no such gender difference in olfactory sensitivity was found with helional, a structural analog of bourgeonal, and with n-pentyl acetate, an aliphatic ester, which were tested in parallel. Males and females did not differ in their frequency of specific anosmia for any of the 3 odorants. The frequency distributions of olfactory detection thresholds were monomodal with all 3 odorants in both genders. Olfactory detection thresholds did not differ significantly between pre- and postovulatory females with any of the 3 odorants. To the best of our knowledge, this is the first study ever to find a human male superiority in olfactory sensitivity. Single nucleotide polymorphisms and/or copy number variations in genes coding for olfactory receptors may be the proximate cause for our finding, whereas a gender difference in the behavioral relevance of bourgeonal may be the ultimate cause.

Key words: bourgeonal, gender differences, olfactory detection thresholds

Introduction

Gender differences in sensory or cognitive performance have held scientific interest since more than a century (Möbius 1900). In addition to the well-documented differences between men and women in verbal, spatial, and perceptual motor tasks (Kimura 1999), there is now evidence for gender differences in basic measures of performance in all sensory systems, including the olfactory, gustatory, visual, auditory, and somatosensory systems (Halpern 2000). With regard to olfaction, human gender differences in favor of females are a robust finding with odor identification, that is, the ability to correctly name or label a given odorant (Brand and Millot 2001). Gender differences in human olfactory sensitivity, in contrast, have often been studied but found for only some odorants. However, all studies that did report such differences in human olfactory sensitivity found, without an exception, a female superiority (Doty and Cameron 2009). Although the effects, when present, are usually not large, they raise the question as to what biological function a superior olfactory sensitivity of one of the genders may serve. Several lines of evidence from studies on nonhuman mammals suggest that differences in the behavioral relevance of odorants for the males and females of a given species might explain the observed gender differences in olfactory sensitivity, as is the case with sex pheromones. In pigs, for example, the volatile testosterone derivative 5-α-androst-16-en-3-one is secreted by males and elicits an immediate behavioral response, the mating stance, in females. Dorries et al. (1994) reported that adult female pigs are clearly more sensitive to 5-α-androst-16-en-3-one compared with male pigs, which makes sense as this sex pheromone, which is perceived via the main olfactory system and not via the vomeronasal system (Dorries et al. 1997), acts on the females. The markedly higher incidence of specific anosmia to 5-α-androst-16-en-3-one observed in adult human males compared with females suggests that gender-specific genotypic differences might, at least partly, account for this phenomenon (Wysoki and Beauchamp 1984).

The mammalian genome is coding for ≈1000 olfactory receptors. A small subset of these receptors is not only expressed in the olfactory epithelium but also in nonolfactory tissues...
including sperm cells (Feldmesser et al. 2006; De la Cruz et al. 2009). Recent studies have shown that the human olfactory receptor OR1D2 (formerly known as hOR17-4), which is activated by the aromatic aldehyde bourgeoinal, is critically involved in sperm chemotaxis (Spehr et al. 2003; Spehr, Schwane, Riffell, et al. 2004; Gakamsky et al. 2009). Due to its presumed function in the context of fertilization, this receptor, which is also expressed in the nasal epithelium (Spehr, Schwane, Heilmann, et al. 2004), is likely to be subjected to sexual selection raising the possibility of a gender difference in olfactory sensitivity to this odorant in favor of males.

It was therefore the aim of the present study to determine olfactory detection thresholds for the sperm attractant odorant bourgeoinal in a large sample of human males and females. Using the same subjects, we also assessed olfactory sensitivity for helional, a structural analog of bourgeoinal which has been shown not to activate OR1D2, and for \( n \)-pentyl acetate, an aliphatic ester for which previous studies failed to find gender differences in sensitivity. This allowed us to assess the odorant specificity of possible gender differences in olfactory sensitivity.

Materials and methods

Subjects

A total of 500 subjects, 250 males and 250 females between 18 and 40 years of age, participated. They were recruited from the student body and staff of Linköping University by personal contact, a printed advertisement on the University’s notice boards, and an electronic advertisement via the University’s intranet. The average age of the males was \( 22.7 \pm 2.9 \) years and that of the females was \( 22.5 \pm 2.7 \) years. None of the subjects had any history of olfactory dysfunction or suffered from an acute upper respiratory tract infection. Female subjects were asked for the phase of their menstrual cycle to assess possible effects of cycle phase on olfactory sensitivity.

The study reported here was performed as part of the master’s thesis project of Peter Olsson. The project was approved by the Institutional Review Board at the Department of Biology at Linköping University. All subjects were informed as to the aims of the study and provided written consent. The study was performed in accordance with the declaration of Helsinki/Hong Kong.

Odorants

The following 3 odorants were used: bourgeoinal (3-(4-tert-butylphenyl)-propanal, CAS\# 18127-01-0), helional (2-methyl-3-(3,4-methylenedioxyphenyl)-propanal, CAS\# 1205-17-0), and \( n \)-pentyl acetate (CAS\# 628-63-7). Bourgeoinal is an aromatic aldehyde, helional belongs to the same chemical class and is a structural analog to bourgeoinal, and \( n \)-pentyl acetate differs from bourgeoinal and helional as it does not share the same functional groups and is aliphatic rather than aromatic. Figure 1 shows the molecular structure of the odorants.

For each odorant, a geometric dilution series in 12 steps was prepared, starting with a stem solution of 1:10 for helional and bourgeoinal and a stem solution of 1:100 for \( n \)-pentyl acetate and progressing by a factor of 3. Odorless diethyl phthalate was used as the solvent. Stem dilutions were designated step 1 and subsequent dilutions step 2, 3, and so forth. Fresh dilutions were prepared on a regular basis following the initial preparations. All substances were of the highest available purity and were obtained from Firmenich (bourgeoinal), and Sigma-Aldrich (helional, \( n \)-pentyl acetate, and diethyl phthalate).

Experimental procedure

A 20-mL aliquot of each odorant was presented in a 250-mL high-density polyethylene squeeze bottle equipped with a flip-up spout. Bottles containing the pure diluent served as blanks. Subjects were instructed as to the manner of sampling and at the start of the session were allowed time to familiarize themselves with the bottles and the sampling technique. Care was taken that the spout was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to allow the stimulus to enter both nostrils.

Detection thresholds were determined using a 3-alternative forced-choice test procedure in which the subjects were presented with 3 randomly arranged bottles, 2 of which contained pure diluent and the third the stimulus (Laska and Hudson 1991; Laska and Teubner 1999; Laska 2004, 2010). In order to minimize adaptation effects, testing followed an ascending staircase procedure. Each bottle could be sampled twice per trial with an interstimulus interval of at least 10 s. Sampling duration was restricted to 1 second per presentation in order to minimize adaptation effects. Subjects were required to decide whether there was no difference between the bottles or identify one as containing the stimulus. In the case of “no difference”, testing proceeded to the next dilution step (with a higher concentration of the odorant); otherwise, the bottles were rearranged and the subject allowed to sample a second time. If both choices were correct, this was provisionally recorded as the threshold dilution. However, if these had been preceded by 1 correct and 1 incorrect choice, the previous dilution (with a lower concentration of the odorant) was again tested, and if both choices were then correct, this was taken as threshold. In this way, olfactory detection thresholds were determined.

![Figure 1](http://chemse.oxfordjournals.org) Molecular structure of the 3 odorants.
for each subject in the order $n$-pentyl acetate, helional, and bourgeonal.

**Data analysis**

Possible differences in olfactory sensitivity between male and female subjects as well as between pre- and postovulatory females were assessed using the Mann–Whitney $U$ test for independent samples. The Spearman rank correlation test was used to assess possible correlations between age and olfactory sensitivity. If not otherwise mentioned, data are reported as means ± standard deviations.

**Results**

**Olfactory sensitivity as a function of gender**

Figure 2 shows the mean olfactory detection thresholds for the 3 odorants tested, subdivided by male and female subjects. A statistically significant difference between the genders in their sensitivity for bourgeonal was found (Mann–Whitney, $P < 0.001$). On average, males detected bourgeonal at dilution step $5.82 ± 1.89$ and thus at a significantly lower concentration than the females ($5.34 ± 1.86$). In contrast, there was no statistically significant difference between the genders in their detection threshold for the 2 other odorants. For helional, the male and female detection thresholds were at dilution steps $3.26 ± 1.75$ and $3.22 ± 1.69$, respectively (Mann–Whitney, $P > 0.05$), and for $n$-pentyl acetate, the male and female detection thresholds were at dilution steps $5.82 ± 1.56$ and $5.58 ± 1.67$, respectively (Mann–Whitney, $P > 0.05$). Using published vapor pressure data and corresponding formulas (Weast 1987), these dilution steps correspond to the following gas phase concentrations (values for male subjects mentioned first): 13 and 26 ppb for bourgeonal, 480 and 520 ppb for helional, and 407 and 518 ppb for $n$-pentyl acetate, respectively.

**Frequency distribution of detection thresholds**

Figure 3 illustrates the frequency distribution of the olfactory detection threshold values for each of the 3 odorants subdivided by male and female subjects. All distributions are monomodal for both genders. The highest incidence...
of failure to detect the highest concentration (i.e., dilution step 1) of a given odorant occurred with helional. With this odorant, 10 (4% of 250) males and 11 (4.4% of 250) females failed to detect the highest concentration. With bourgeonal, 4 (1.6% of 250) males and 4 (1.6% of 250) females and with \( n \)-pentyl acetate only 1 female (0.4% of 250) failed to detect the highest concentration. Thus, the frequency of specific anosmia did not differ significantly between genders with any of the 3 odorants.

**Olfactory sensitivity as a function of cycle phase**

Out of 250, 227 females provided information as to their cycle phase at the day of testing. There was no significant difference between pre- and postovulatory females in their detection threshold for any of the 3 odorants (Mann–Whitney, \( P > 0.05 \) for all 3 odorants).

**Olfactory sensitivity as a function of age**

No statistically significant correlations between detection threshold values and age were found with any of the 3 odorants for any of the 2 genders (Spearman: \( r_s = -0.11 \), \( P > 0.05 \), with all 6 cases).

**Discussion**

The results of the present study demonstrate that male subjects detected bourgeonal at significantly lower concentrations compared with female subjects, whereas no such gender difference in olfactory sensitivity was found with helional and \( n \)-pentyl acetate. To the best of our knowledge, this is the first study ever to find a male superiority in olfactory sensitivity.

Experimental studies on gender differences in olfactory performance date back till the late 19th century (Toulouse and Vaschide 1899). Despite this long history, no study ever reported males being more sensitive to a given odorant than females. Among those studies that reported gender differences in olfactory sensitivity, females always displayed lower detection thresholds compared with males, for example, with citral (3,7-dimethyl-octa-2,6-dienal, Schneider and Wolf 1955), exaltolide (1,15-pentadecanolide, Koelega 1970), acetone (dimethyl ketone, Odeigh 1994), skatole and valeric acid (3-methyl indole and \( n \)-pentanoic acid, Jacob et al. 2003), iso-pentyl acetate, iso-pentanoic acid, \((R)(-)-\)carvone and 1,8-cineole (Menashe et al. 2007), and 2-methyl-3-mercapto-butanol (Chopra et al. 2008). It should be emphasized that the differences in mean threshold values between males and females reported in these studies usually span a factor of 2–3 and in no case exceed a factor of 10.

For a number of years, it was assumed that gender-specific differences in gonadal hormone levels might explain the observed differences in olfactory performance between males and females. However, studies on the effects of menstrual cycle variations, pregnancy, the administration of gonadal hormones, or of gonadectomy on olfactory sensitivity both in humans and in animal models yielded contradictory findings and suggest that the relationship between gonadal hormones and olfactory function is complex and explains neither the biological function that a superior olfactory sensitivity of one of the genders might serve nor the odorant specificity of gender differences as observed in the present as well as in several other studies (for a comprehensive review on this topic, see Doty and Cameron 2009).

Recent genetic studies suggest that 2 nonexclusive mechanisms might underlie the observed variation in olfactory capabilities among individual humans and, possibly, also between genders. Single nucleotide polymorphisms (SNPs), that is, single base substitutions, insertions, or deletions, are the most common type of genomic variation. They are believed to contribute to olfactory phenotype diversity (Hasin-Brumshtein et al. 2009). In line with this idea, Keller et al. (2007) found that the human olfactory receptor OR7D4, which responds to the putative human pheromones 5-\( \alpha \)-androst-16-en-3-one and androsta-4,16-dien-3-one, contains 2 SNPs that strongly affect sensitivity to these volatile steroids. Similarly, Menashe et al. (2007) reported that SNP variants in the human olfactory receptor OR11H7P show a strong correlation with sensitivity for isovaleric acid. Knape et al. (2008) found that the frequency distribution of the SNPs for the human olfactory receptor OR17-40 differed markedly between ethnic groups and, in some cases, between genders. This is, to the best of our knowledge, the first study demonstrating that SNPs found in olfactory receptor genes are not gender neutral and thus suggests that allelic variants might, at least partly, explain the results of the present study and of odorant-specific gender differences in olfactory sensitivity in general.

Copy number variations (CNVs) are DNA segments that are present in populations as alleles with variable copy numbers. Recent studies have shown that extensive CNVs are common in the human olfactory receptor gene family and thus provide another possibility for explaining variation in olfactory capabilities among humans (Nozawa et al. 2007). As it is reasonable to assume that individuals with a high number of copies for a given functional olfactory receptor gene also have an elevated expression rate of the corresponding olfactory receptor, this could lead to a higher sensitivity for the cognate ligand of this receptor compared with individuals with a low number of copies of the gene in question (Young et al. 2008). Based on findings from next-generation sequencing, Hasin-Brumshtein et al. (2009) conclude that both mechanisms, SNPs and CNVs, plausibly explain the well-documented phenotypic diversity of human olfaction, including phenomena such as specific anosmia, specific hyperosmia, or bimodal distributions of sensitivity for a given odorant.

Thus, our finding of a monomodal distribution of sensitivity for bourgeonal is not trivial given that several odorants,
including some for which a female superiority in detection thresholds have been reported, such as the putative human pheromones 4,16-androstadien-3-one (Lundström et al. 2003) and 5α-androst-16-en-3-one (Labows and Wysocki 1984), exaltolide and musk ambrette (2-tert-butyl-4,6-dinitro-5-methylanisole, Kalms and Seedburgh 1975), as well as acetone (Odeigah 1994) and methanethiol (Lison et al. 1980) were found to display bimodal distributions of sensitivity.

Although SNPs and CNVs are likely to underlie individual and, possibly, gender differences in olfactory performance, the question as to the biological function of such differences remains to be answered. Several studies suggest that differences in the behavioral relevance of odorants may be responsible for the observed differences in olfactory sensitivity between species. Frugivorous and carnivorous species, for example, have been shown to clearly outperform each other with regard to sensitivity for odorants that are typical of their respective dietary habits, which makes sense in terms of an evolutionary adaptation to optimal foraging (Hernandez Salazar et al. 2003; Laska et al. 2004). Similarly, prey species have been found to be more sensitive to odorants of their natural predators compared with nonprey species (Apfelbach et al. 2005; Laska et al. 2005).

Concerning the possible biological function of gender differences in olfactory sensitivity within a given species, sex pheromones comprise a group of odorants offering a plausible explanation as they typically act on either males or females, and thus, a superior sensitivity of the receiving gender should be adaptive. Not surprisingly, such gender differences for odorants known or at least presumed to serve as a means of intraspecific social communication have been found both in humans (Labows and Wysocki 1984; Lundström et al. 2003) and nonhuman species (e.g., Dorries et al. 1994; Laska et al. 2006).

The recent discovery that ectopically expressed olfactory receptors play a critical role in human sperm chemotaxis (Spehr et al. 2003; Spehr, Schwane, Riffell, et al. 2004; Gakamsky et al. 2009) suggests that these receptors should be subject to sexual selection favoring males over females. Our finding that male subjects detected bourgeonal at significantly lower concentrations compared with female subjects is consistent with this idea as this aromatic aldehyde has been demonstrated to activate the human olfactory receptor OR1D2, which is expressed both in human sperm cells and in the human nasal epithelium. The idea of sexual selection acting on olfactory receptors that are expressed in sperm cells is supported by findings from comparative genetic analyses, which found that the subset of orthologous olfactory receptor genes with conserved ectopic expression evolved under stronger evolutionary constraint than olfactory receptor genes expressed exclusively in the olfactory epithelium. This suggests that ectopic olfactory receptors may indeed carry out additional functions in nonolfactory tissues (De la Cruz et al. 2009).

In conclusion, our finding of a human male superiority in sensitivity for the sperm attractant odorant bourgeonal might be due to differences in its behavioral relevance for males and females. Genetic mechanisms such as SNPs and CNVs might underlie this first example of a gender-specific phenotypic difference in olfactory sensitivity in favor of males.

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References


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