Master Thesis

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

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Abstract:

The present study aimed at assessing possible gender differences in human olfactory detection thresholds for three odorants; bourgeonal, an aromatic aldehyde, helional, a structural analogue of bourgeonal, and n-pentyl acetate, an aliphatic ester. A total of 500 subjects, 250 males and 250 females between 18 and 40 years of age, were tested using a triangular forced choice method with an ascending staircase procedure. The subjects were asked to sniff the contents of three bottles and to identify the one containing the stimulus presented at a given concentration. Males were found to have a significantly lower olfactory detection threshold for bourgeonal compared to females (3.4·10^{11} molecules·cm^{-3} air and 5.9·10^{11} molecules·cm^{-3} air, respectively) whereas no significant gender difference in the olfactory detection thresholds for helional (1.4·10^{13} molecules·cm^{-3} air and 1.3·10^{13} molecules·cm^{-3} air, respectively) or n-pentyl acetate (1.4·10^{14} molecules·cm^{-3} air and 1.1·10^{14} molecules·cm^{-3} air, respectively) were found. This is the first study ever to report a human male superiority in olfactory sensitivity for a monomolecular odorant. One possible explanation for this finding is that bourgeonal may differ in its biological significance for males and females. It has recently been demonstrated that bourgeonal elicits chemotaxis in human sperm cells and olfactory receptors activated by bourgeonal might thus be subject to sexual selection.

Keyword:

Olfactory detection threshold, gender difference, bourgeonal, helional, n-pentyl acetate
Abstract

The present study aimed at assessing possible gender differences in human olfactory detection thresholds for three odorants; bourgeonal, an aromatic aldehyde, helional, a structural analogue of bourgeonal, and n-pentyl acetate, an aliphatic ester. A total of 500 subjects, 250 males and 250 females between 18 and 40 years of age, were tested using a triangular forced choice method with an ascending staircase procedure. The subjects were asked to sniff the contents of three bottles and to identify the one containing the stimulus presented at a given concentration. Males were found to have a significantly lower olfactory detection threshold for bourgeonal compared to females (3.4·10^{11} molecules·cm^{-3} air and 5.9·10^{11} molecules·cm^{-3} air, respectively) whereas no significant gender difference in the olfactory detection thresholds for helional (1.4·10^{13} molecules cm^{-3} air and 1.3·10^{13} molecules cm^{-3} air, respectively) or n-pentyl acetate (1.4·10^{14} molecules·cm^{-3} air and 1.1·10^{14} molecules·cm^{-3} air, respectively) were found. This is the first study ever to report a human male superiority in olfactory sensitivity for a monomolecular odorant. One possible explanation for this finding is that bourgeonal may differ in its biological significance for males and females. It has recently been demonstrated that bourgeonal elicits chemotaxis in human sperm cells and olfactory receptors activated by bourgeonal might thus be subject to sexual selection.

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1 Introduction

There is a general scientific interest in gender differences in both sensory and cognitive performance (Lippa 2005; Doty and Cameron 2009). Recent advances in the field of genetics have yielded new tools which now allow us to assess possible correlations between gender-specific genotypical differences and gender-specific phenotypical differences in sensory and cognitive performance, such as the human genome project (International human genome sequencing consortium 2001). With regard to sensory performance three different aspects are usually considered: sensitivity, discrimination performance and identification ability. In olfaction, sensitivity refers to the lowest concentration of an odorant which can be perceived at all or discriminated from a blank, odorless, stimulus. Discrimination performance refers to the ability to distinguish a perceptible odorant from one or several other perceptible odorants of equal intensity. Identification ability refers to the ability to correctly name or label a given odorant. In human subjects gender differences in favor of females have been found consistently with odor identification (Doty et al. 1985; Velle 1987; Ship and Weiffenbach 1993; Brand and Millot 2001; Doty and Kerr 2005; Fusari and Ballesteros 2008), whereas they have hardly ever been found with odor discrimination. Gender differences in human olfactory sensitivity have often been studied but rarely found. However, all studies that did report gender differences
in human olfactory sensitivity found, without an exception, a female superiority (Koelega 1970; Jacob et al. 2003; Menashe 2007; Chopra et al. 2008).

One possible explanation for the observed gender differences in odor identification is a superior verbal ability observed in females compared to males (Larsson et al. 2003). This is intuitive considering that odor identification has a strong verbal component as it requires naming or labeling of odor stimuli. Experiments on olfactory sensitivity, in contrast, usually do not require a verbal component, as the subject can indicate his or her response by pointing or other non-verbal means. A possible explanation for the observed female superiority in olfactory sensitivity for some odorants could be a possible difference in the biological relevance of these odorants between males and females, as is the case with pheromones. Pheromones are chemicals that are released by one individual of a species and when received by another individual of the same species affect the behavior or hormonal status of the recipient. In pigs, for example, the volatile testosterone-derivate androstenone is secreted by male pigs and elicits an immediate behavioral response, the mating stance, in female pigs (Melrose et al. 1971; Reed et al. 1974). Dorries et al. (1994) reported that adult female pigs are clearly more sensitive to androstenone compared to male pigs which makes sense as this sex pheromone acts on female pigs.

It has been reported that some olfactory receptors are not only expressed in the olfactory epithelium but also in the sperm cells in several mammalian species (Parmentier et al. 1992; Vanderhaeghen et al. 1997; De la Cruz et al. 2009). From these studies it was hypothesized that the chemicals that activate olfactory receptors present in sperm cells might mediate chemotaxis aiding fertilization. If the olfactory receptors on sperm cells are involved in fertilization it is possible that they are subjected to sexual selection. Spehr et al. (2003) did indeed find that the activation of hOR17-4, one of the olfactory receptors expressed in human sperm cells, by bourgeonal mediated chemotaxis. However, the receptor hOR17-4 is also expressed in the human nasal epithelium, raising the possibility of a gender difference in the olfactory sensitivity to this odorant.

In the present study I therefore aimed to establish the olfactory detection threshold of 250 human female subjects and 250 human male subjects 18 to 40 years of age for three odorants: the sperm attractant odorant bourgeonal, its structural analogue helional which is also an aromatic aldehyde, and n-pentyl acetate which is an aliphatic ester. N-pentyl acetate is used as a control odorant as previous studies on human olfactory sensitivity failed to find a gender difference in sensitivity for this odorant.

2 Materials and Methods

2.1 Subjects

A total of 500 subjects, 250 males and 250 females between 18 and 40 years of age, participated in the study. The subjects were approached and asked to participate on the Linköping University Campus. The vast majority of subjects were students at
Linköping University and most were Swedish. However, ethnicity was not a variable in this study and students from other countries were included in the experiment. Female subjects were asked if they were menstruating or not to assess possible effects of cycle phase on olfactory detection thresholds. None of the subjects had any history of olfactory dysfunction or suffered from an acute upper respiratory tract infection. All subjects were informed as to the aims of the study and provided a written consent. The study was performed in accordance with the declaration of Helsinki/Hong Kong.

2.2 Odorants

In this study three odorants were used, bourgeonal, helional and n-pentyl acetate. Figure 1 shows the molecular structure of these odorants. Bourgeonal is an aromatic aldehyde and has been described as smelling like Lily of the valley (Convallaria majalis). Helional belongs to the same chemical class and is a structural analogue to bourgeonal. The smell of helional resembles that of melon. The molecular structure of n-pentyl acetate is different from that of bourgeonal and helional, it does not have the same functional groups and it is aliphatic rather than aromatic. The smell of n-pentyl acetate resembles that of pears or bananas. n-pentyl acetate was chosen because previous studies failed to find gender differences in sensitivity with this odorant.

Figure 1. Molecular structure of the three odorants

For each odorant a dilution series in twelve steps was prepared. For helional and bourgeonal a stem solution of 1:10 was prepared by mixing 2 ml odorant and 18 ml of solvent and for n-pentyl acetate a stem solution of 1:100 was prepared by mixing 200 µl and 19.8 ml of solvent. Each odorant was then dissolved further from the stem solution by a dilution factor of three until a total of twelve steps had been created for each odorant. Table 1 summarizes the actual liquid dilutions used and the numbers assigned to each dilution step. Fresh dilutions were prepared on a regular basis following the initial preparations. Odorless diethyl phthalate was used as the solvent for all three odorants. All substances where of the highest available purity and were obtained from Givaudan (bourgeonal), and Sigma-Aldrich (helional, n-pentyl acetate and diethyl phthalate).
Table 1. Liquid dilutions used with the three odorants.

<table>
<thead>
<tr>
<th>dilution step</th>
<th>bourgeonal</th>
<th>helional</th>
<th>n-pentyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:10</td>
<td>1:10</td>
<td>1:100</td>
</tr>
<tr>
<td>2</td>
<td>1:30</td>
<td>1:30</td>
<td>1:300</td>
</tr>
<tr>
<td>3</td>
<td>1:90</td>
<td>1:90</td>
<td>1:900</td>
</tr>
<tr>
<td>4</td>
<td>1:270</td>
<td>1:270</td>
<td>1:2700</td>
</tr>
<tr>
<td>5</td>
<td>1:810</td>
<td>1:810</td>
<td>1:8100</td>
</tr>
<tr>
<td>6</td>
<td>1:2430</td>
<td>1:2430</td>
<td>1:24300</td>
</tr>
<tr>
<td>7</td>
<td>1:7290</td>
<td>1:7290</td>
<td>1:727900</td>
</tr>
<tr>
<td>8</td>
<td>1:21870</td>
<td>1:21870</td>
<td>1:218700</td>
</tr>
<tr>
<td>9</td>
<td>1:65610</td>
<td>1:65610</td>
<td>1:656100</td>
</tr>
<tr>
<td>10</td>
<td>1:196830</td>
<td>1:196830</td>
<td>1:1968300</td>
</tr>
<tr>
<td>11</td>
<td>1:590490</td>
<td>1:590490</td>
<td>1:5904900</td>
</tr>
<tr>
<td>12</td>
<td>1:1771470</td>
<td>1:1771470</td>
<td>1:17714700</td>
</tr>
</tbody>
</table>

2.3 Experimental procedure

Olfactory detection thresholds were determined using a triangular forced choice procedure. The subject was presented with three 250 ml volume polyethylene bottles with flip-up spouts, two of which contained the solvent (20 ml) and the third bottle contained a given concentration of the odorant (20 ml). The subject’s task was to sniff the contents of all three bottles and to identify the bottle that contained the odorant. The subjects were restricted to sampling each bottle twice for one inhalation cycle each, this restriction was imposed to minimize the risk of adaptation effects. Each dilution step was presented twice and in order to pass one dilution step the subject had to correctly identify the bottle that contained the odorant on both presentations. Prior to the main study a small pilot study was performed. I used the mean detection thresholds from the pilot study to determine from which dilution step I should start testing. With bourgeonal and n-pentyl acetate testing started with presentation of dilution step six, and with helional it started with dilution step four. If the subject failed with at least one of the two presentations an ascending staircase procedure was adopted and increasing concentrations of the odorant were presented until the subject made two correct choices. If this dilution step was preceded by two incorrect choices it was accepted as the detection threshold. However, if it was preceded by one incorrect choice and one correct choice the previous dilution step (with a lower concentration of the odorant) was presented twice again and only accepted as the detection threshold when the subject now made two correct choices. In the cases when a subject correctly identified the first dilution step presented a descending staircase procedure was adopted. Here, decreasing concentrations of the
odorant were presented until the subject made two incorrect choices or one incorrect and one correct choice. In the case of two incorrect choices the next higher concentration was taken as the threshold. In the case of one correct and one incorrect choice the subject got a second chance to pass that dilution step. If the subject had one or two incorrect choices on the second chance the subject failed that dilution step and the previously presented, less diluted, step was taken as the threshold. In the case when the subject passed the second chance this dilution step was taken as the threshold and testing was concluded for that odorant.

Presentations of two different dilution steps were separated by a one minute interval during which the subject’s decision was recorded and new bottles were selected. Within one dilution step the two presentations were separated by a 30 second interval.

2.4 Statistical Analysis

The Mann-Whitney U-test was used to assess possible differences in sensitivity between male and female subjects as well as between menstruating and non-menstruating females. The Spearman rank-correlation test was used to assess possible correlations between age and sensitivity. If not otherwise mentioned, data are reported as means ± SDs.

3 Results

3.1 Olfactory detection thresholds

Figure 2 shows the mean olfactory detection thresholds for the three odorants tested, subdivided by male and female subjects. A statistically significant difference between the genders in their mean olfactory detection threshold for bourgeonal was found (Mann-Whitney U-test 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 two other odorants. For helional the male and female detection thresholds were at dilution step 3.26±1.75 and 3.22±1.69, respectively (Mann-Whitney U-test p>0.05) and for n-pentyl acetate the male and female detection thresholds were at dilution step 5.82±1.56 and 5.58±1.67, respectively (Mann-Whitney U-test p>0.05).
3.2 Frequency distribution of olfactory detection threshold values

Figures 3 to 5 illustrate the frequency distribution of the olfactory detection threshold values for each of the three odorants. All distributions are monomodal for both genders. The highest incidence of failure to detect the highest concentration (that is: dilution step 1) of a given odorant occurred with helional. With this odorant ten (4% of 250) males and eleven (4.4% of 250) females failed to detect the highest concentration. With bourgeonal four (1.6% of 250) males and four (1.6% of 250) females failed to detect the highest concentration. With n-pentyl acetate only one female (0.4% of 250) failed to detect the highest concentration.
Figure 3. Frequency distribution of the olfactory detection thresholds for bourgeonal obtained from male (n=250) and female (n=250) subjects. Dilution step 1 refers to the highest concentration tested, dilution step 12 refers to the lowest concentration tested, and 0 refers to a lack of detection of dilution step 1.

Figure 4. Frequency distribution of the olfactory detection thresholds for helional obtained from male (n=250) and female (n=250) subjects. Dilution step 1 refers to the highest concentration tested, dilution step 12 refers to the lowest concentration tested, and 0 refers to a lack of detection of dilution step 1.
Figure 5. Frequency distribution of the olfactory detection thresholds for n-pentyl acetate obtained from male (n=250) and female (n=250) subjects. Dilution step 1 refers to the highest concentration tested, dilution step 12 refers to the lowest concentration tested, and 0 refers to a lack of detection of dilution step 1.

3.3 Threshold difference between menstruating and non menstruating women

227 of the 250 females provided information as to their cycle phase at the day of testing. 193 women were not menstruating and 34 women were menstruating. Table 2 shows that there was no significant difference between these two groups in their detection threshold for any of the three odorants.

Table 2. Mean olfactory detection threshold (±SD) for the three odorants tested for non-menstruating females (n=194) and menstruating females (n=34).

<table>
<thead>
<tr>
<th></th>
<th>Non-menstruating females (N=193)</th>
<th>Menstruating females (N=34)</th>
<th>Mann-Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>bourgeonal</td>
<td>5.42±1.74</td>
<td>5.24±2.27</td>
<td>n.s.</td>
</tr>
<tr>
<td>helional</td>
<td>3.16±1.60</td>
<td>3.44±1.83</td>
<td>n.s.</td>
</tr>
<tr>
<td>n-pentyl acetate</td>
<td>5.59±1.57</td>
<td>6.06±2.21</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

3.4 Age dependence of threshold values

Table 3 summarizes the correlation between age and detection threshold for the three odorants tested, subdivided by males and females. There was no statistically significant correlation for any of the three odorants for any gender except for a weak correlation with n-pentyl acetate for females (Spearman rank test p<0.05).
Table 3. The correlation between olfactory detection threshold and age for male (n=250) and female (n=250) subjects.

<table>
<thead>
<tr>
<th></th>
<th>Correlation between threshold and age (Spearman rank test)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>males</td>
</tr>
<tr>
<td></td>
<td>Spearman r&lt;sub&gt;s&lt;/sub&gt;</td>
</tr>
<tr>
<td>bourgeonal</td>
<td>-0.03</td>
</tr>
<tr>
<td>helional</td>
<td>-0.06</td>
</tr>
<tr>
<td>n-pentyl acetate</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

3.5 Gas phase concentrations

Since odorants may differ in their vapor pressure two odorants presented at the same liquid dilutions may also differ in the concentration of their gas phase. Furthermore, the molecules in the gas phase rather than the liquid dilutions are ultimately perceived by the subjects. To be able to compare the detection thresholds for the three odorants tested in this study the gas phase concentrations for the average detection threshold were therefore calculated for each odorant and gender and are summarized below.

Table 4 shows that bourgeonal was detected at a lower gas phase concentration than the other two odorants and that helional was detected at a lower gas phase concentration than n-pentyl acetate. This was true for both males and females.

Table 4. Liquid dilution and gas phase concentrations of the mean detection thresholds for the three odorants tested, subdivided by males and females.

<table>
<thead>
<tr>
<th></th>
<th>liquid gas phase concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dilution molec./cm&lt;sup&gt;3&lt;/sup&gt; ppm log ppm M log M</td>
</tr>
<tr>
<td>males</td>
<td></td>
</tr>
<tr>
<td>bourgeonal</td>
<td>1:1950 3.4·10&lt;sup&gt;11&lt;/sup&gt; 0.01 -1.90 5.6·10&lt;sup&gt;-10&lt;/sup&gt; -9.25</td>
</tr>
<tr>
<td>helional</td>
<td>1:112 1.4·10&lt;sup&gt;13&lt;/sup&gt; 0.52 -0.29 2.3·10&lt;sup&gt;8&lt;/sup&gt; -7.63</td>
</tr>
<tr>
<td>n-pentyl acetate</td>
<td>1:1566 1.4·10&lt;sup&gt;14&lt;/sup&gt; 5.19 0.71 2.3·10&lt;sup&gt;-7&lt;/sup&gt; -6.63</td>
</tr>
<tr>
<td>females</td>
<td></td>
</tr>
<tr>
<td>bourgeonal</td>
<td>1:1126 5.9·10&lt;sup&gt;11&lt;/sup&gt; 0.02 -1.66 9.8·10&lt;sup&gt;-10&lt;/sup&gt; -9.01</td>
</tr>
<tr>
<td>helional</td>
<td>1:125 1.3·10&lt;sup&gt;13&lt;/sup&gt; 0.48 -0.32 2.2·10&lt;sup&gt;8&lt;/sup&gt; -7.67</td>
</tr>
<tr>
<td>n-pentyl acetate</td>
<td>1:1951 1.1·10&lt;sup&gt;14&lt;/sup&gt; 4.07 0.61 1.8·10&lt;sup&gt;7&lt;/sup&gt; -6.74</td>
</tr>
</tbody>
</table>
4 Discussion

The main finding of the present study is that bourgeonal was detected at significantly lower concentrations by male compared to female subjects, whereas no such gender difference in olfactory sensitivity was found with helional and n-pentyl acetate. This is the first study ever to find a male superiority in olfactory performance. I argue that the gender difference observed in this study is due to a difference in the biological relevance of bourgeonal between males and females.

Experimental studies on gender differences in olfactory performance date back till the 19th century, (Tolouse and Vaschide 1899). Despite this long history, to the best of my knowledge, no study ever reported males being more sensitive to a given odorant than females. Among the few studies that reported gender differences in olfactory sensitivity, females always displayed lower detection thresholds compared to males, for example with 2-methyl-3-mercapto-butanol (Chopra et al 2008) skatol and valeric acid (Jacob et al. 2003), exaltolide (Koelega 1970), isoamyl acetate, isovaleric acid, L-carvone and cineole (Menashe 2007). Whereas gender differences in olfactory sensitivity are rather the exception than the rule, with the majority of studies failing to find significant differences between males and females in olfactory detection thresholds, studies on odor identification have consistently found a female superiority (Doty et al. 1985; Doty and Kerr 2005; Fusari and Ballesteros 2008; Ship and Weiffenbach 1993; Velle 1987). Odor identification refers to the ability to correctly name an odor. The female superiority in odor identification is usually small but consistent and includes the ability to recognize one’s own body odor (Platek et al. 2001). It has also been found that women are better than men at odor memory tasks (Choudry et al. 2003; Larsson et al. 2003) such as long term olfactory memory (Lehrner 1993) and retention of new odor names (Dempsey and Stevenson 2002). Some argue that the female superiority in odor identification, which is regarded as a verbal task, can be explained by a female superiority in verbal recognition tasks (Larsson et al. 2003).

Brand and Millot (2001) discuss two hypotheses to explain the observed differences between the genders in several aspects of olfactory performance. The first hypothesis states that learning and environmental factors may underlie the gender difference. Several studies reported that repeated exposure to an odorant can increase olfactory sensitivity for this, but not for other, odorants (Dalton et al. 2002; Boulkroune et al. 2007). Similarly, specific anosmia, that is: an inability to perceive a given odorant, can be reversed by repeated exposure to this odorant (Wysocki et al. 1989). This hypothesis should affect males and females and does not properly explain why females are consistently better at identifying odors and are more sensitive to some odorants unless a female superiority in learning or a higher proneness to respond to environmental factors in females compared to males is postulated. A prediction from this hypothesis is that children should not show any gender difference. However, some studies show that female children are more sensitive to certain odorants than
male children (Koelega and Köster 1974). The second hypothesis states that there is an evolutionary biological difference between the genders that may be related to workload differences among males and females in prehistoric times, specifically that females gathered food from plant material. This could lead to a selection pressure upon females to increase olfactory sensitivity and odor identification ability, as females with higher olfactory sensitivity and better odor identification abilities are likely to be more successful in gathering food for their offspring. Some studies also implicate the odor of a potential partner to be a more important factor in mate selection for females than for males (Herz and Inzlicht 2002). As with all evolutionary explanations, it is difficult to test this hypothesis experimentally.

4.1 Hormonal influences on olfactory performance

The second hypothesis by Brand and Millot implicates that hormones could play an important role in olfactory capabilities. Some studies lend support to this idea. It has been shown that the menstrual cycle affects, however only slightly, the olfactory sensitivity of women (Doty 1986). The effect of the menstrual cycle on sensitivity seems to be unrelated to levels of luteinizing hormone (LH), estrogen, progesterone and follicle stimulating hormone (FSH), whereas secretion from the adrenals seems to be responsible for the fluctuation in olfactory performance in women. Pregnant women have been found to rate mercaptans and isoamyl acetate as more intense compared to non pregnant females and androstenone and galaxolide as less intense compared to non pregnant controls (rating the quality of an odor is referred to as hedonic rating) (Gilbert and Wysocki 1991). It has also been shown that olfactory sensitivity for the odorant n-butanol increases during pregnancy, whereas changes in the hedonic evaluation of odorants are restricted to food-associated odorants during the first trimester of pregnancy (Laska et al 1996). Effects of hormonal status on the sense of smell have been studied in other mammalian species as well. Cardwell et al. (1994) showed that treatment with androgen increased the olfactory receptor response to a putative pheromone in two species of fish. Dorries et al. (1994) found that female pigs have a detection threshold for androstenone five times lower than male pigs but not significantly different from castrated male pigs indicating that testosterone might be responsible for the lower sensitivity observed in intact male pigs. Sorwell et al. (2008) found no difference in detection threshold for male mouse urinary odor between females and gonadectomized males but treatment with estradiol lowered female detection threshold and did not affect the gonadectomized males. However some reports do find that olfactory performance can vary with circulating hormone levels, few have examined cause and effect relationships (Doty and Cameron 2009). It is unlikely, though, that hormonal differences between the genders should explain the observed results in the present study as there is no difference between the genders in sensitivity to helional and n-pentyl acetate.
4.2 Genotypical influences on olfactory performance

Recent studies have found that gender differences in sensitivity for and hedonic evaluation of odorants can be correlated with certain genotypes. Keller et al. (2007) tested the olfactory sensitivity and hedonic ratings for androstenone and androstanediene in a large group of over 300 individuals. They genotyped all subjects for a specific gene coding for a human olfactory receptor, OR7D4, which responds to androstenone and androstadienone. It was found that two single nucleotide polymorphisms (SNPs) affected the receptor resulting in two homozygous genotypes (RT/RT and WM/WM) and one heterozygous genotype (RT/WM) and further that subjects with the RT/RT homozygous genotype were more sensitive and rated androstenone and androstadienone as more pleasant compared to subjects with the RT/WM and WM/WM genotypes. Menashe et al. (2007) reported that damaging SNPs that affected the gene for the human olfactory receptor OR11H7P yielding an intact allele not affected by the SNP and one affected allele which yields a product less sensitive compared to the gene product of the intact allele. They further found that the group of subjects that were hyperosmic to isovaleric acid contained a very low frequency of homozygotes for the affected allele of OR11H7P. Genotypic differences in olfactory performance as reported by Keller et al. (2007) and Menashe et al. (2007) should be gender neutral as they seem to spread throughout the genome neutrally and thus are unlikely to explain the difference between males and females in sensitivity for bourgeonal observed in the present study.

A recent study performed by Knape et al. (2008) focused on a single human olfactory receptor, hOR17-40, which is responsive to bourgeonal and helional. The study looked at Single Nucleotide Polymorphisms (SNPs) that affect the gene of this specific olfactory receptor. Three SNPs, one which causes a non functional variant of the gene, are located in the coding region of the gene and can affect the functionality of the receptor. Another six SNPs are located in the non coding region of the gene. Ninety subjects were genotyped for this olfactory receptor and their ethnicity and gender were noted. Interestingly, it was found that the distribution of the SNPs between the ethnic groups and, in some cases, between the genders, was different. One of the three SNPs found in the coding region, for example, was nine times more prevalent in Sub-Saharan African males than in females and the same SNP was not even found among African American males but in 16% of the African American females. Similarly uneven distributions for the frequencies of other SNPs were found for other ethnic groups. The subject population used in the current study consisted almost exclusively of European subjects and it is possible that there is an uneven distribution of SNPs among the genders that may affect the olfactory sensitivity to bourgeonal and explain the observed pattern in olfactory sensitivity.

An interesting idea to explain individual variations in the olfactory sensitivity and odor identification capabilities is that of copy-number variations (CNV). CNV refers to the fact that some genes can be found in one or several copies throughout the
genome. If a gene is present in several functional copies it is likely that the product of that gene is expressed in the organism in an amount corresponding to the number of copies. Nozawa et al. (2007) and Young et al. (2008) found copy-number variations for functional olfactory receptor genes in the human genome. Nozawa et al. (2007) reported an average difference of 11 functional copies of olfactory receptor genes between individuals in the entire genome. The amount of copy-number variation is discussed by both studies to have neutral selection pressure and that genomic drift is the mechanism that most likely affects the amount of copy-number variation. It is likely 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, which could lead to a higher sensitivity and a better ability to correctly identify an odorant at lower concentrations. In humans, the majority of olfactory receptor genes (73%) are located on chromosomes 1, 6, 9, 11, 14 and 19, the remaining receptor genes are spread on all other chromosomes except chromosome 20 and the Y chromosome (Glusman et al. 2001). CNV could therefore be an important mechanism underlying differences in sensitivity between males and females.

4.3 Possible gender differences in biological relevance of Bourgeonal

The findings of the present study that males were more sensitive to bourgeonal than females whereas no gender difference in sensitivity was found with helional and n-pentyl acetate raise the question whether bourgeonal might have a higher behavioral relevance for males than for females. Recent studies have found that some of the 390 olfactory receptor types found in the human olfactory epithelium are also expressed in human sperm cells, and at least 83 olfactory receptor types are expressed in human testis (De la Cruz et al. 2009). De la Cruz et al (2009) investigated the rate of evolution of orthologous olfactory receptor genes expressed in non olfactory tissue in humans and chimpanzees and found that these olfactory receptor genes evolve slower compared to olfactory receptor genes expressed only in the olfactory epithelium. De la Cruz et al. (2009) suggests that the lower rate of evolution is due to an evolutionary constraint imposed by a given function of these receptors that are expressed outside the olfactory epithelium. Spehr et al. (2003) found that one of the olfactory receptors, hOR17-4, expressed in human sperm cells is activated by bourgeonal. A later study confirmed the position of hOR17-4 to the surface of the sperm cell (Spehr et al. 2004). The fact that olfactory receptors are expressed in the sperm cells is not uncommon and not unique to humans, it has been found in rats, mice and dogs (Parmentier et al. 1992; Vanderhaeghen et al. 1997) and in the testis of chimpanzees (De la Cruz et al. 2009). The possible function of olfactory receptors in the sperm cells is most likely chemotaxis to help the sperm cells navigate towards the egg cell and thus aid fertilization. Spehr et al. (2003) did indeed show that the swimming pattern of the sperm cells was affected by stimulation with bourgeonal consistent with the idea of positive chemotaxis. Interestingly, if one were to expand the concept of pheromones to incorporate sperm cells which in many ways fulfill the criteria of a living organism bourgeonal could earn the status of a pheromone. If the
receptor hOR17-4 is involved in aiding fertilization then the expression of this receptor is likely to be affected by sexual selection. Males whose sperm express more of the olfactory receptor might be more successful at fertilizing, especially if sperm competition is the case. It is also reasonable to assume that the expression rate of hOR17-4 should be different between males and females as males express the receptor in the olfactory epithelium and in sperm cells, whereas females should express the receptor only in the olfactory epithelium. This putative heightened expression rate in males could affect the number of hOR17-4 receptors in the nasal olfactory epithelium and could explain the observed difference in sensitivity between males and females to bourgeonal in this study.

I argue that males are more sensitive to bourgeonal compared to females due to a relative increase in the number of receptors that are responsive to bourgeonal present in the olfactory nasal epithelium. This could be examined by taking samples from the olfactory nasal epithelium from both males and females and apply bourgeonal linked with marked antibodies, from which it would be possible to find the number and location of active receptors to bourgeonal. The gene for hOR17-4 is located on chromosome 17, therefore CNV at this gene alone should be gender neutral. The expression of hOR17-4 could be functionally linked to a gene on the Y chromosome rendering CNV at this locus an important mechanism possibly underlying gender differences in the sensitivity to bourgeonal. It is advised that further studies should genotype the subjects and thereby be able to verify the possibility that there is an uneven distribution of damaging SNPs between the genders that may affect the pattern of olfactory sensitivity to the odorant tested.

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References


Boulkroune, N., Wang, L., March, A., Walker, N. and Jacob, T.J.C. 2007. Repetitive olfactory exposure to the biologically significant steroid androstadienone causes a
hedonic shift and gender dimorphic changes in olfactory evoked potentials. Neuropsychopharmacology 32:1822-1829.


De la Cruz, O., Blekham, R., Zhang, X., Nicolae, D., Firestein, S. And Gilad, Y. A signature of evolutionary constraint on a subset of ectopically expressed olfactory receptor genes. Molecular Biology of Evolution 26:491-494


Shusterman, D., Murphy, M.A and Balmes, J. 2003. Differences in nasal irritant sensitivity by age, gender and allergic rhinitis status. International Archives of Occupational and Environmental Health 76: 577-583.


