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# Olfactory sensitivity in CD-1 mice for the sperm-attractant odorant bourgeonal and some of its structural analogues

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**Sammanfattning**
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**Nyckelord**
Keyword:

Aromatic aldehydes, CD-1 mice, Olfactory detection thresholds

## Content

## 1 Abstract

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Keywords
Aromatic aldehydes, CD-1 mice, Olfactory detection thresholds

## 2 Introduction

The mouse, *Mus musculus*, is a model species in olfactory research. However, only little is known about the olfactory capabilities of mice at the organismal level for odors other than body-borne odors (Beauchamp and Yamazaki, 2003; Schaefer et al, 2002; Wysocki et al, 2004). Olfactory sensitivity in terms of detection thresholds has only been determined for approximately a dozen substances (Passe and Walker, 1985; Laska et al, 2006) and this is surprising given the importance of basic data on olfactory sensitivity for the choice of adequate stimulus concentrations in studies of discrimination performance (Laska et al, 2007b; Laska and Shepherd, 2007a; Laska et al, 2008) and physiological measures. Aromatic aldehydes have recently been shown to be potent ligands for olfactory receptors expressed both in the olfactory epithelium and in mammalian sperm cells (Spehr et al, 2004; Spehr et al., 2003) and to cause directed movement of sperm cells towards the source of this odorant (Fukuda et al., 2004). The aromatic aldehyde bourgeonal has been shown to be a sperm-attractant in humans and lyral to be one in mice. This raises the possibility that bourgeonal or some of its structural analogues may indeed mediate sperm chemotaxis and therefore have broad implications in the fields of fertility and contraception. Assessing the olfactory sensitivity of mice for bourgeonal and six of its structural analogues, including lyral, allows me to address the question of whether small changes in molecular structure may affect detectability of these odorants in a systematic manner.

Olfactory detection threshold values for the same seven aromatic aldehydes have been established in previous studies with humans and spider monkeys. This allows me to compare olfactory sensitivity across species. Additionally, recent genetic studies have demonstrated that mice have 1194 functional genes coding for olfactory receptors whereas humans, for example, have been reported to express only approximately 390 olfactory receptor genes and New World monkeys approximately 900 of such genes (Dryer, 2000; Glusman et al, 2001; Godfrey et al, 2004; Rouquier et al, 2000; Rouquier and Giorgi, 2007; Zhang and Firestein, 2002; Zhang et al, 2007). This allows me to address the question of whether the number of functional olfactory receptor genes has an impact on olfactory sensitivity.

The present study therefore has the following specific aims

1) to determine olfactory detection thresholds for bourgeonal and six of its structural analogues in CD-1 mice,

2) to compare the threshold data obtained to those of other species tested previously on the same set of odorants and to evaluate the impact of the number of functional olfactory receptor genes on olfactory sensitivity, and
3) to assess possible odor structure-activity relationships, that is, correlations between molecular structural properties of the odorants under investigation and their detectability.

## 3 Materials and methods

### 3.1 Animals

Five male CD-1 mice (*Mus musculus*), all 120-150 days old at the beginning of the study, were used for the behavioral testing (Figure 1). The outbred strain CD-1 was used due to its variable genetic background, which is more similar to wild-type mice than that of inbred strains. Furthermore, data on olfactory detection thresholds (Joshi et al., 2006; Laska et al., 2006; Laska et al. 2009) and discrimination capabilities (Laska and Shepherd, 2007a; Laska et al., 2007b; Laska et al. 2008) were obtained in earlier studies using the same mouse strain. The animals were kept on a 12/12h light-dark schedule in individual standard plastic rodent cages, in the animal facility at the University hospital of Linköping. The cages were equipped with nesting material, wood shavings and unlimited access to SDS pellets (CRM (E) rodent). The animals were also given an environmental enrichment composed of one toilet paper roll and a ketchup cup from McDonald´s® which were replaced on a regular basis.

In order to assure high motivation throughout testing, the animals were kept on a water deprivation schedule with no water bottles present in the cage. Instead the animals received water daily, partly as reinforcement during testing and partly by being hand fed using a syringe immediately after the test. The total amount of water per day was 1.5 ml for each individual. All experiments performed comply with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 86-23, revised 1985) and were approved by the Local Ethics Committee.

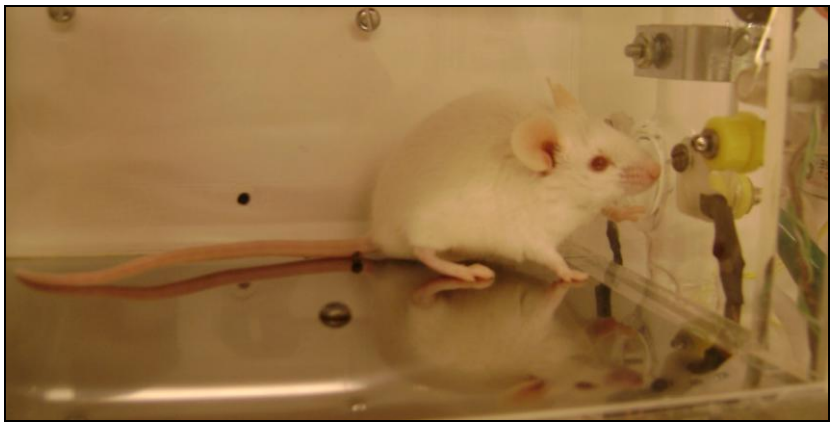

**Figure 1.** CD-1 mouse (*Mus musculus*) at the odor port of the operant chamber.

### 3.2 Odorants

For the critical experiments a set of seven odorants was used: bourgeonal, 3-phenyl propanal (3PPA), canthoxal, cyclamal, helional, lilial and lyral. The odorants all belong to the chemical class of aromatic aldehydes and thus share some molecular features and differ in others allowing me to assess the impact of molecular structure on detectability (Figure 2).

Data on olfactory detection thresholds from humans and spider monkeys for these substances are at hand (Kjeldmand, 2009; Olsson, 2009) allowing me to additionally compare the performance of the mice to that of other species.

Prior to the critical experiments the animals were trained to discriminate between rewarded and unrewarded stimuli using the readily discriminable odorants: amyl acetate, 1.8-cineol, (-)-carvone, (+)-limonene, anethol and eugenol. None of these odorants share any functional groups or other apparent structural similarities with the odorants used in the critical experiments. Amyl acetate was also used as a familiar training odorant in between the critical tests, when needed, in order to prevent the more challenging conditions leading to extinction or loss of motivation.

Bourgeonal and lyral were obtained from Firmenich SA (Geneva, Switzerland) and International Flavors & Fragrances Inc. (New York, NY), respectively. All other substances were obtained from Sigma-Aldrich (St. Louis, MO) and had a nominal purity of at least 99%. They were diluted using near odorless diethyl phthalate (Sigma-Aldrich) as the solvent.

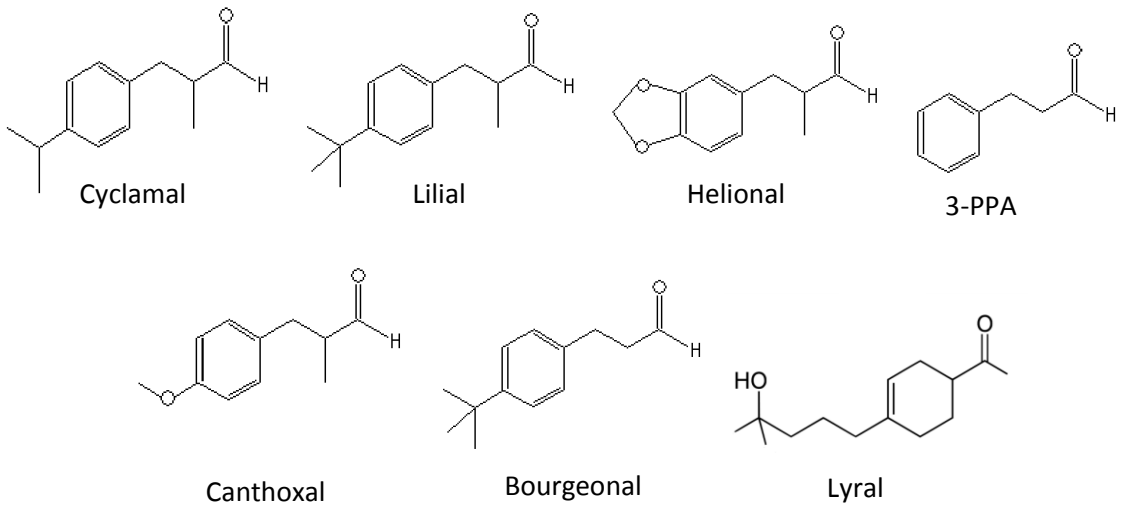


**Figure 2.** Molecular structure of the aromatic aldehydes used in the critical experiments.

### 3.3 Behavioral test

Olfactory detection thresholds were determined using a commercially available automated olfactometer (Knosys, Tampa, FL). Basically, this apparatus consists of a test chamber connected to a computer-controlled odorant delivery device. The mice were trained using standard operant conditioning procedures described by Bodyak and Slotnick (1999) prior to the critical experiments.

#### 3.3.1 Begin program

The begin program was used to train the mice, in two steps, to put their snout into the odor sampling port of the test chamber, to stay in the port when presented with an odorant and then to lick at a waterspout. In the first step the animal was instantly rewarded when licking at the waterspout and in the second step it learned to keep its snout in the odor port and to lick when presented with an odorant in order to be rewarded.

#### 3.3.2 D2 program

Two-odorant discrimination was introduced to the mice using the D2 program in which a rewarded (S+) and an unrewarded (S-) odorant are presented in a pseudorandomized order. Both licking when presented with the S+ and not licking when presented with the S- is

recorded as a correct response whereas not licking in response to the S+ and licking when presented with S- is recorded as an incorrect response.

When entering the odor port the mouse breaks a photo beam that triggers a 2 s presentation of an odorant, which a final valve will bypass the mouse for approximately 0.1 s before presenting. This procedure ensures that the mouse keeps its head in the odor port before making a decision. A correct response to the S+ requires that the mouse licks on the waterspout in at least seven out of ten 0.2 s intervals that constitute a 2 s odorant presentation. A correct response to the S-, in turn, requires that the mouse licks for fewer than seven of the ten 0.2 s intervals (Figure 3). A correct response to a rewarded stimulus will lead to the presentation of 2.5 µl of water as a reward delivered via the waterspout.

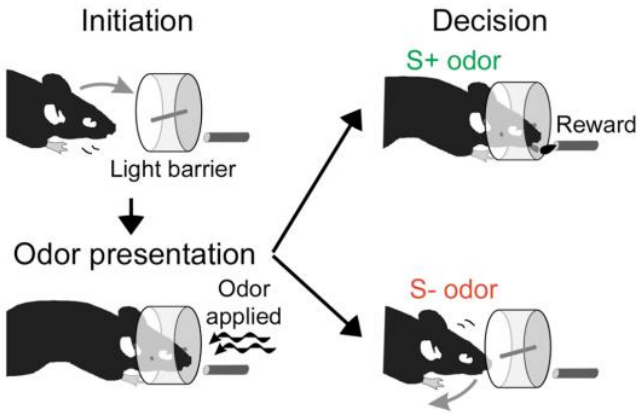


**Figure 3.** Mouse managing odor port in operant chamber.

### 3.4 Experimental procedure

Prior to learning the operant procedure the mice underwent one week of water deprivation receiving 1.5 ml water each day in one serving. Throughout the first step of the begin program the interval between nose poke and reinforcement was successively lengthened. Further on, after learning to stay inside the odor port, the introduction of an S+ odor (amyl acetate) led on to the second step of the begin program. This step included a step-wise increase of the time interval between breaking the photo beam and odor presentation through increased closing time of the final valve. At the same time, the water reinforcement volume is step-wise increased (Table 1). This teaches the mice to lick the waterspout continuously when responding to a rewarded odorant.

As a standard the water reward was kept at 2.5 µl per reinforcement throughout both the begin and the D2 program. However, animals could be granted more reinforcement if regarded necessary.

**Table 1.** Changes in final valve time and reinforcement throughout the second stage of the begin program. 100% equals 2.5 microliters of water**.**

| **Block** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| **Final valve time** | 0 | 0.3 | 0.6 | 0.9 | 1.0 | 1.2 | 1.2 |
| **% Reinforcement** | 80 | 80 | 80 | 100 | 120 | 130 | 140 |

#### 3.4.1Training

Two-odor discrimination was taught to the mice using the D2 program and the odors amyl acetate, (-)-carvone and (+)-limonene were used as rewarded stimuli (S+), while 1,8-cineol, anethol and eugenol were used as unrewarded stimuli (S-) Each of the odor pairs, listed in

Table 2, were presented to the animals for three days before any critical experiments were performed. By subjecting the animals to different S+/S- transfer tasks they learned to rely on different kind of odor stimuli in order to make their decision.

**Table 2.** Rewarded and unrewarded training odors

| S+ | S- | |
|---|---|---|
| amyl acetate | 1.8-cineol | |
| (-)-carvone | 1.8-cineol | First positive transfer |
| (-)-carvone | anethol | First negative transfer |
| (+)-limonene | anethol | Second positive transfer |
| (+)-limonene | eugenol | Second negative transfer |

### 3.4.2 Critical experiments

The D2 program was used throughout the critical experiments presenting each odorant, (bourgeonal, 3-PPA, canthoxal, cyclamal, helional, lilial and lyral) to the animals in decreasing concentrations as rewarded stimulus (S+) and the headspace of the odorless solvent as unrewarded stimulus (S-). First the animals were presented with an easily detectable concentration, 0.1 ppm (parts per million), of the odorant, with the exception of 3-PPA which was presented at a concentration of 1 ppm, for three days in order to robustly learn to discriminate between odorant and solvent. Then the odorant concentration was successively decreased in ten-fold dilution steps as long as the animal reached the criterion of at least 75% correct choices in two consecutive blocks of 20 decisions (trials).

For each concentration the animals were presented with a maximum of five blocks of 20 trials (totaling 50 S+ and 50 S– trials in pseudorandomized order) and the animals performed a maximum of six blocks per day. When an animal failed to significantly discriminate between odorant and solvent an intermediate concentration (0.5 log units between the lowest concentration that was detected above chance and the first concentration that was not) was prepared and tested, providing a more exact threshold value.

### 3.5 Analysis of data

For every dilution step the percentage of correct choices from 40 decisions (two consecutive blocks of 20 trials) per dilution step were calculated for each animal. In order to reach the criterion of 75% correct decisions at least 30 out of 40 decisions need to be correct. This corresponds to an alpha level of 0.05 according to a two-tailed binomial test.

## 4 Results

### 4.1 Olfactory detection thresholds

Figure 4 shows the performance of the mice in discriminating between various dilutions of bourgeonal and the odorless solvent. All five mice significantly distinguished dilutions as low as 1:6,600,000,000 from the solvent (two-tailed binomial test, $p < 0.05$). The mice performed above chance with all higher concentrations of bourgeonal that are not shown in the graph.

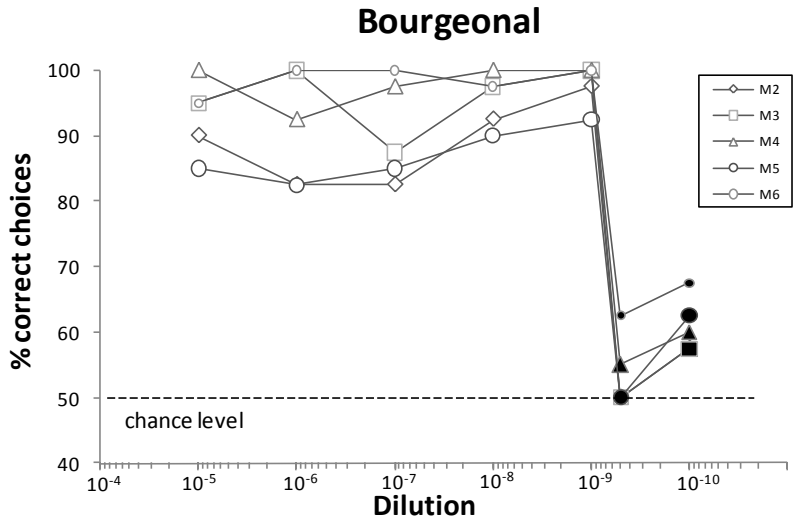


***Figure 4.*** *Performance of the CD-1 mice in discriminating between various dilutions of bourgeonal and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 5 shows the performance of the mice in discriminating between various dilutions of helional and the odorless solvent. Two of the mice (M3 and M5) significantly distinguished dilutions as low as 1:1600 from the solvent and one mouse (M6) significantly distinguished dilutions as low as 1:4800. The last two mice (M2 and M4) significantly distinguished dilutions as low as 1:16,000 from the solvent (two-tailed binomial test, $p < 0.05$).

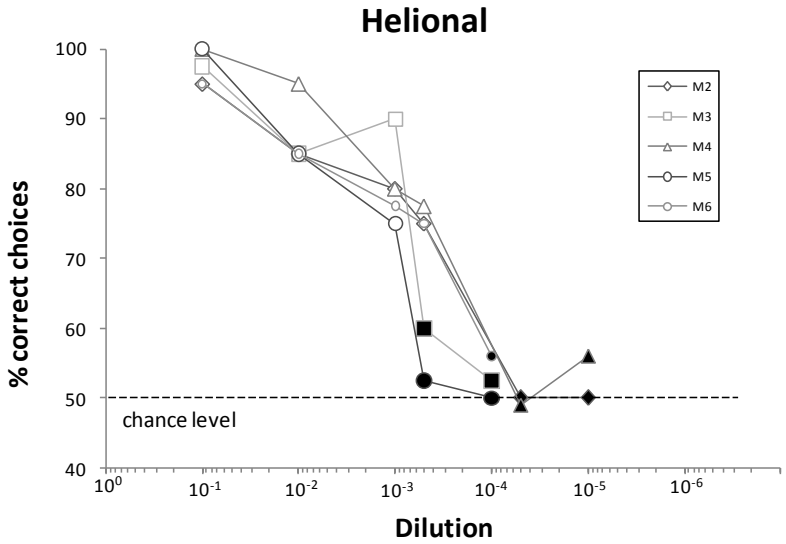


***Figure 5.*** *Performance of the CD-1 mice in discriminating between various dilutions of helional and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 6 shows the performance of the mice in discriminating between various dilutions of 3-phenyl propanal and the odorless solvent. Three of the mice (M2, M3 and M5) significantly distinguished dilutions as low as 1:1400 from the solvent and the other two mice (M4 and M6) significantly distinguished dilutions as low as 1:4200 (two-tailed binomial test, $p < 0.05$).

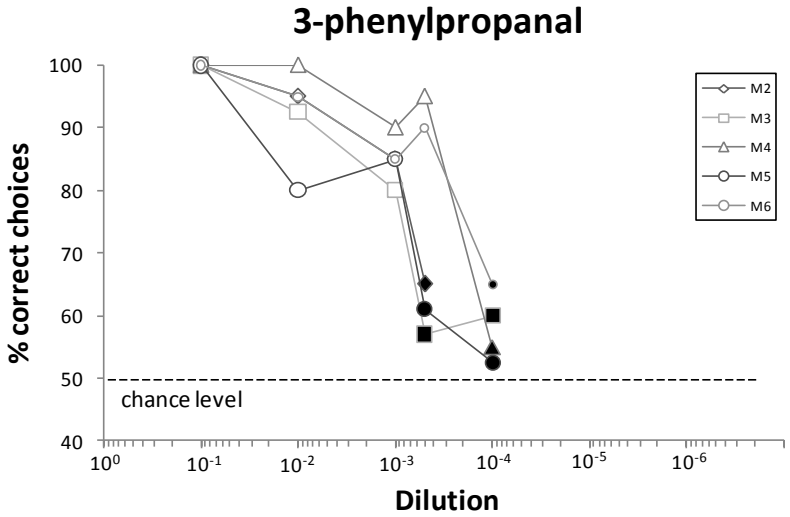


***Figure 6.*** *Performance of the CD-1 mice in discriminating between various dilutions of 3-phenyl propanal and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 7 shows the performance of the mice in discriminating between various dilutions of cyclamal and the odorless solvent. Two of the mice (M5 and M6) significantly distinguished dilutions as low as 1:7800 from the solvent and the other three mice (M2, M3 and M4) significantly distinguished dilutions as low as 1:78,000 (two-tailed binomial test, $p < 0.05$).

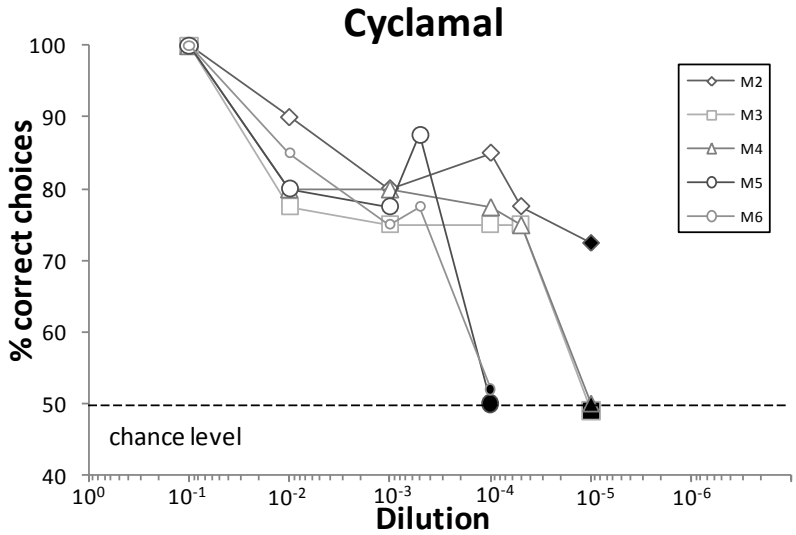


***Figure 7.*** *Performance of the CD-1 mice in discriminating between various dilutions of cyclamal and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 8 shows the performance of the mice in discriminating between various dilutions of canthoxal and the odorless solvent. Two of the mice (M5 and M6) significantly distinguished dilutions as low as 1:1080 from the solvent and the other three mice (M2, M3 and M4) significantly distinguished dilutions as low as 1:3600 (two-tailed binomial test, $p < 0.05$).

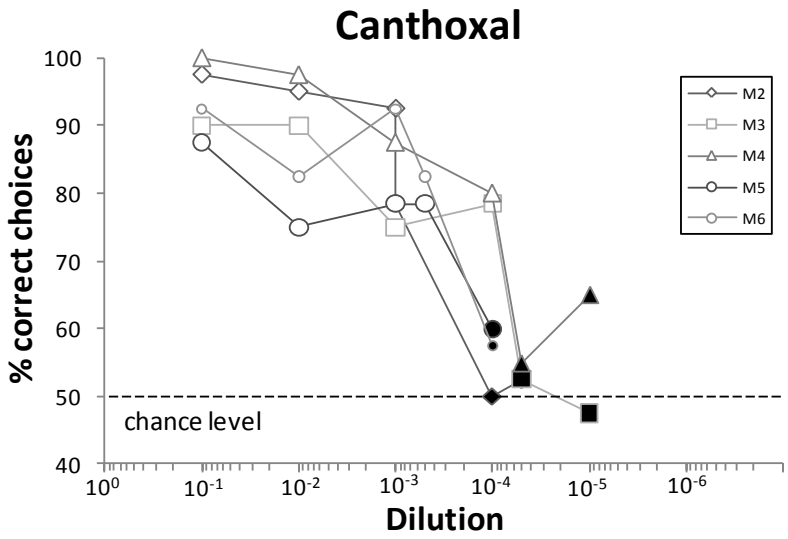


***Figure 8.*** *Performance of the CD-1 mice in discriminating between various dilutions of canthoxal and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 9 shows the performance of the mice in discriminating between various dilutions of lilial and the odorless solvent. One mouse (M5) significantly distinguished dilutions as low as 1:2000 from the solvent and the other four mice (M2, M3, M4 and M6) significantly distinguished dilutions as low as 1:6000 (two-tailed binomial test, $p < 0.05$).

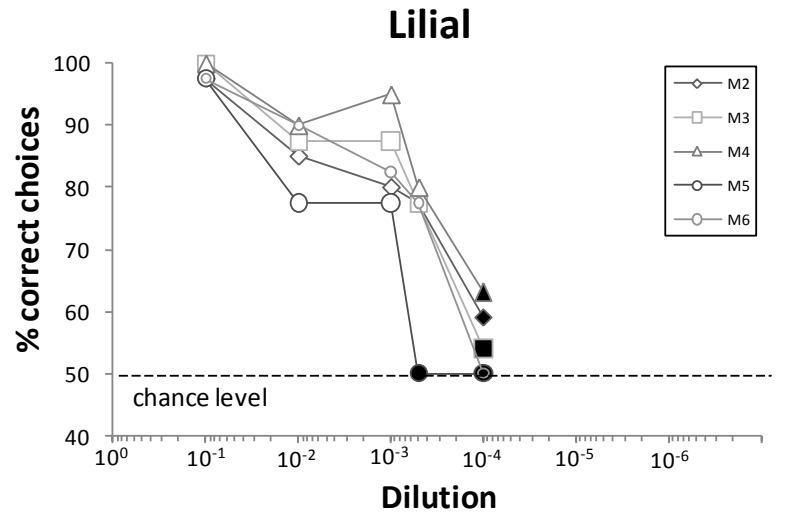


***Figure 9.*** *Performance of the CD-1 mice in discriminating between various dilutions of lilial and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, P < 0.05).*

Figure 10 shows the performance of the mice in discriminating between various dilutions of lyral and the odorless solvent. One mouse (M6) significantly distinguished dilutions as low as 1:2000 from the solvent and three mice (M2, M3 and M5) significantly distinguished dilutions as low as 1:6000. The last mouse (M4) significantly distinguished dilutions as low as 1:20,000 from the solvent (two-tailed binomial test, $p < 0.05$).

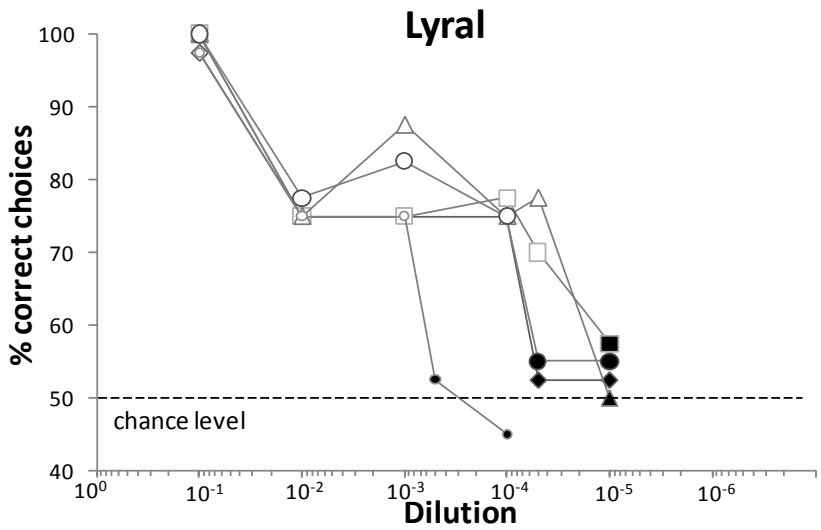


***Figure 10.*** *Performance of the CD-1 mice in discriminating between various dilutions of lyral and the odorless solvent. Each data point represents the percentage of correct choices out of 40 trials. Filled symbols indicate dilutions that the mice did not discriminate significantly above chance level (binomial test, $P < 0.05$).*

## 4.2 Inter- and intraindividual variability

### 4.2.1 Interindividual variability

The individual mice demonstrated very similar threshold values with a given odorant and thus the range between the best and the worst performing mouse was as the most a factor of 33. This was found with lyral. With the odorants helional and cyclamal the threshold values for individual mice differed by a factor of 10 whereas the thresholds for 3-PPA, lilial and canthoxal only differed by a factor of 3 between individuals. With the odorant bourgeonal no difference in threshold was seen among the mice.

### 4.2.2 Intraindividual variability

In order to be able to make proper comparisons between odorants with different vapor pressures a conversion from liquid to vapor phase concentrations is necessary. These data are summarized in Table 3 in which the olfactory detection threshold values determined in this study are shown in various measures of vapor phase concentrations.

A comparison between the animals showed that M4 was among those with the lowest threshold for all seven odorants. M2 and M3 shared the lowest threshold with M4 on cyclamal, canthoxal and lilial for which M6 also reached the lowest threshold value. M2 also reached the lowest threshold with helional whereas M6 reached it with 3-PPA. M5, on the other hand, was among the animals with the highest threshold for lilial, cyclamal, helional, canthoxal and 3-PPA, sharing this position with M6 for cyclamal and canthoxal, M3 with helional and both M3 and M2 with 3-PPA. M6 alone also displayed the highest threshold with lyral.

***Table 3.*** *Olfactory detection threshold values in CD-1 mice for seven aromatic aldehydes, expressed in various measures of vapor phase concentrations.*

| | | liquid | vapor phase concentration | | | | |
|---|---|---|---|---|---|---|---|
| | n | dilution | molec./cm$^3$ | ppm | log ppm | Mol/l | log Mol/l |
| bourgeonal | 5 | 1:6,600,000,000 | $2.5 \cdot 10^{3}$ | 0.0000000001 | -10.00 | $4.5 \cdot 10^{-18}$ | -17.35 |
| helional | 2 | 1:1,600 | $2.5 \cdot 10^{10}$ | 0.001 | -3.00 | $4.5 \cdot 10^{-11}$ | -10.35 |
| | 1 | 1:5,333 | $7.5 \cdot 10^{9}$ | 0.0003 | -3.52 | $1.3 \cdot 10^{-11}$ | -10.87 |
| | 2 | 1:16,000 | $2.5 \cdot 10^{9}$ | 0.0001 | -4.00 | $4.5 \cdot 10^{-12}$ | -11.35 |
| cyclamal | 2 | 1:8,667 | $7.5 \cdot 10^{9}$ | 0.0003 | -3.52 | $1.3 \cdot 10^{-11}$ | -10.87 |
| | 3 | 1:86,667 | $7.5 \cdot 10^{8}$ | 0.00003 | -4.52 | $1.3 \cdot 10^{-12}$ | -11.87 |
| 3-PPA | 3 | 1.1,400 | $2.5 \cdot 10^{11}$ | 0.01 | -2.00 | $4.5 \cdot 10^{-10}$ | -9.35 |
| | 2 | 1:4,667 | $7.5 \cdot 10^{10}$ | 0.003 | -2.52 | $1.3 \cdot 10^{-10}$ | -9.87 |
| canthoxal | 2 | 1:1,200 | $7.5 \cdot 10^{10}$ | 0.003 | -2.52 | $1.3 \cdot 10^{-10}$ | -9.87 |
| | 3 | 1:3,600 | $2.5 \cdot 10^{10}$ | 0.001 | -3.00 | $4.5 \cdot 10^{-11}$ | -10.35 |
| lilial | 1 | 1:2,000 | $2.5 \cdot 10^{10}$ | 0.001 | -3.00 | $4.5 \cdot 10^{-11}$ | -10.35 |
| | 4 | 1:6,667 | $7.5 \cdot 10^{9}$ | 0.0003 | -3.52 | $1.3 \cdot 10^{-11}$ | -10.87 |
| lyral | 1 | 1:2,000 | $2.5 \cdot 10^{10}$ | 0.001 | -3.00 | $4.5 \cdot 10^{-11}$ | -10.35 |
| | 3 | 1:20,000 | $2.5 \cdot 10^{9}$ | 0.0001 | -4.00 | $4.5 \cdot 10^{-12}$ | -11.35 |
| | 1 | 1:66,667 | $7.5 \cdot 10^{8}$ | 0.00003 | -4.52 | $1.3 \cdot 10^{-12}$ | -11.87 |

*N indicates the number of individuals who reached a given threshold.*

Overall, bourgeonal yielded by far the lowest threshold values among these odorants while 3-PPA yielded the highest threshold values.

## 5 Discussion

### 5.1 Comparison among odorants

The results of the present study show that the ability of the mice to detect aromatic aldehydes varies with the structure of the stimuli. Even a small change in the molecular structure can lead to a marked change in detectability. Among the odorants tested bourgeonal yielded the lowest threshold value by far. Cyclamal yielded the second lowest threshold value, five log units higher than bourgeonal. Close behind cyclamal comes lyral followed by helional and lilial, while 3-PPA and canthoxal yielded the highest threshold values. With these results a possible correlation between structure and detectability of these odorants can be assessed.

Bourgeonal was detected by the mice when only $2.5 \cdot 10^{3}$ molecules were present in one cubic centimeter of air (molecules/cm$^3$) and cyclamal needed between $7.5 \cdot 10^{8}$ and $7.5 \cdot 10^{9}$ molecules in order to be detected. Bourgeonal and cyclamal, with their synthetic lily of the valley scents, are structurally similar to each other. Cyclamal possesses an isopropyl group attached to the benzene ring and at that position bourgeonal possesses a tertiary butyl group

(figure 11). The only other difference between these two odorants is a methyl group next to the aldehyde group which cyclamal possesses and bourgeonal is lacking. This lack of a methyl group next to the aldehyde group is only shared between bourgeonal and 3-PPA (figure 12), which has the highest threshold value of the seven odorants tested. Thus, the presence of a tertiary butyl group together with the lack of a methyl group next to the aldehyde group might be the reason why bourgeonal was detected by the mice at such low concentrations. However, lilial, which shares the tertiary butyl functional group with bourgeonal, possesses a methyl group next to the aldehyde group like cyclamal and has a much higher threshold. Thus, you can draw the conclusion that the tertiary butyl group alone cannot be responsible for the extraordinarily low detection threshold value found with bourgeonal.

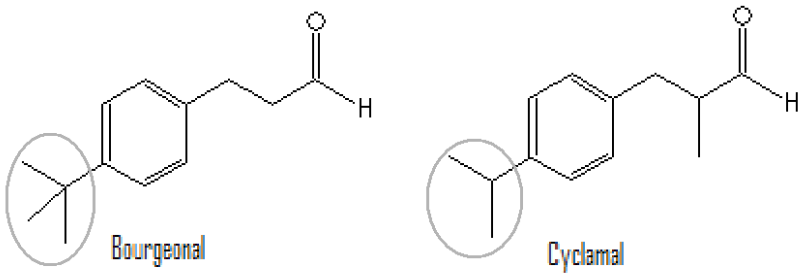


***Figure 11.*** *Molecular structure of bourgeonal and cyclamal. Functional groups encircled consist of a tertiary butyl group and an isopropyl group respectively.*

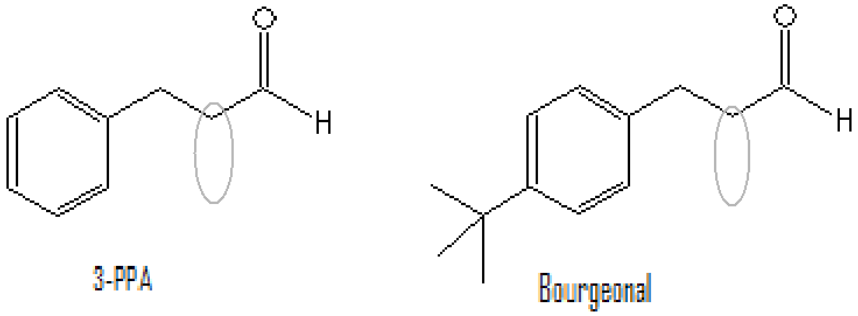


***Figure 12.*** *Molecular structure of 3-PPA and bourgeonal, both lack a methyl group next to the aldehyde group.*

Lyral, helional and lilial were very similar in their respective thresholds. However, these three odorants differ in structure by the functional groups attached to the benzene ring. Lyral possesses a hydroxyl group at the opposite side of the aldehyde group. This oxygen containing feature puts it in the same category as helional and canthoxal which possess a dioxo- and methoxy group respectively (figure 13). A study performed by Laska et al (2000) about olfactory discrimination ability as a function of oxygen moiety found that odors such as ketones and carboxylic acid were easier to discriminate compared to alcohols and aldehydes of the same carbon chain length. This indicates that the type of functional group is important with regard to discrimination ability. Results in the present study show that an extra oxygen feature in addition to the aldehyde group shared by all seven odorants led to a higher detection threshold value for aromatic aldehydes. Lilial, on the other hand, does not possess an extra oxygen containing functional group and shares the feature of a tertiary butyl group attached to the benzene ring with bourgeonal (figure 14). The methyl group located next to the aldehyde

group, the only feature differing between lilial and bourgeonal, is therefore likely to be the reason for the higher detection threshold value obtained for lilial.

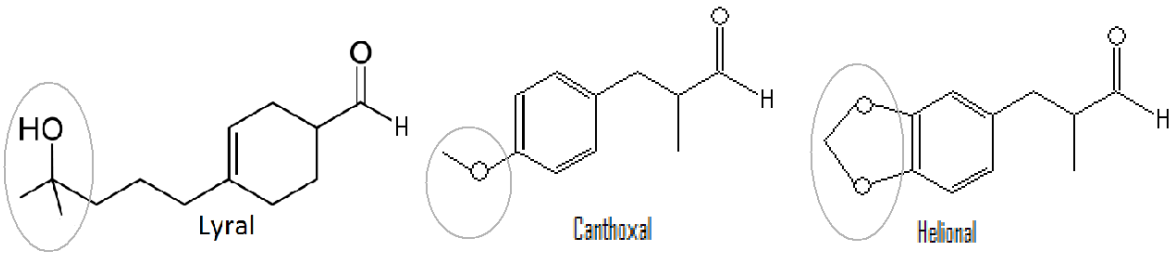


***Figure 13.*** *Molecular structure of the oxygen containing odorants, lyral, canthoxal and helional. Functional groups encircled consist of a hydroxyl, methoxy and dioxo group respectively.*

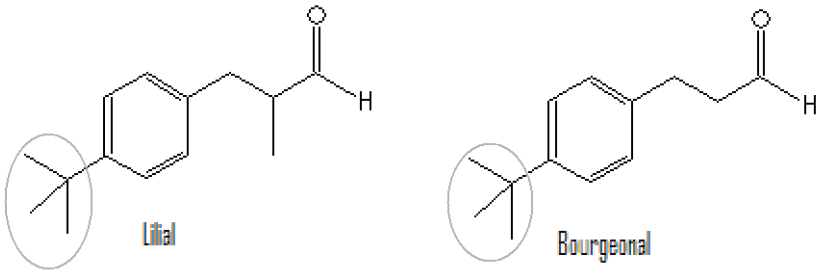


***Figure 14.*** *Molecular structure of lilial and bourgeonal sharing the functional tertiary butyl group.*

The highest threshold values were found with 3-PPA and canthoxal. The structure of 3-PPA is more similar to bourgeonal (figure 12) than to canthoxal which contains both a methoxy group and the additional methyl group, shared by several of the odorants, close to the aldehyde group. 3-PPA is also the only one of these seven odorants lacking both a functional group attached to the benzene ring opposite to the aldehyde group and a methyl group next to the aldehyde group. This might be the reason for its high detection threshold. However, it is surprising to find 3-PPA among the highest threshold values when considering the finding by Spehr et al (2003) who identified 3-PPA as a lead structure for the human testicular olfactory receptor, hOR17-4, involved in human sperm chemotaxis. Bourgeonal, which has the lowest threshold values in the present study, was also found to elicit chemotaxis in human sperm.
As both the presence or absence and the type of oxygen moiety may affect the detection threshold values as shown by Laska et al (2000), more studies have to be performed in order to fully understand why oxygen containing functional groups can affect the threshold in either way. For lyral the higher detection threshold might be due to the longer carbon chain leading to the functional alcohol group and for helional and canthoxal it might be the methyl group next to the aldehyde, in addition to the oxygen moiety, that affect the detection threshold value.

### 5.2 Comparison with other classes of odorants tested with CD-1 mice

Figure 15 compares the olfactory detection threshold values of CD-1 mice found in the present study to those found in earlier studies for other classes of odorants. With the notable exception of bourgeonal, the detection threshold values found here for aromatic aldehydes are in the same range as those found in previous studies, for aliphatic aldehydes (Laska et al, 2006a), alkylpyrazines (Laska et al, 2009) and terpenoids (Joshi et al, 2006).

Laska et al (2006a) tested CD-1 mice with six aliphatic aldehydes (n-butanal, n-pentanal, n-hexanal, n-heptanal, n-octanal and n-nonanal) in order to assess their individual detection threshold value and to see if detectability correlated with carbon chain length. The authors

found detection threshold values ranging from $1.0 \cdot 10^9$ to $1.0 \cdot 10^{11}$ molecules/cm$^3$ and thus in the same range as found here for six of the seven aromatic aldehydes. The authors also found no correlation between detection threshold value and carbon chain length when investigating aliphatic aldehydes in mice.

Laska et al (2009) determined olfactory detection thresholds for alkylpyrazines in CD-1 mice and they concluded that the detection threshold value for this class of odorants correlated positively with the number of functional groups attached to the pyrazine ring. The authors used pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine and 2,3,5,6-tetramethylpyrazine. For these six compounds they obtained detection threshold values ranging between $2.5 \cdot 10^7$ and $7.5 \cdot 10^{11}$, molecules/cm$^3$. A comparison shows that some of the alkylpyrazines were perceived at lower concentrations than the aromatic aldehydes, with the exception of bourgeonal, and others were detected only at higher concentrations.

Joshi et al (2006) determined olfactory detection thresholds for two enantiomeric odor pairs belonging to the chemical class of terpenoids in CD-1 mice and they concluded that the effect of chirality was substance specific in terms of detectability of the enantiomers. Obtained in this study were threshold values of $7.5 \cdot 10^8$ and $2.5 \cdot 10^9$ molecules/cm$^3$ respectively for (+)- and (-)-carvone and threshold values of $7.5 \cdot 10^{10}$ and $2.5 \cdot 10^8$ molec/cm$^3$ respectively for (+)-and (-)-limonene. These detection threshold values are in the same range as those found with the aromatic aldehydes in the present study, with the exception of bourgeonal.

Olfactory detection threshold values for the L- and D-forms of three different amino acids were determined by Wallén (2010) and no clear correlation between structure and detectability was found. Detection threshold values of $3.6 \cdot 10^8$ and $1.2 \cdot 10^8$ were found for the L- and D-forms of cysteine, respectively, and for the enantiomers of methionine and proline the threshold values were $2.9 \cdot 10^9$, $9.8 \cdot 10^8$ and $1.8 \cdot 10^{12}$, $6.1 \cdot 10^{11}$ molec/cm$^3$ of air, respectively. Compared to the aromatic aldehydes in the present study one can conclude that some of the amino acids were perceived at lower concentrations, with the exception of bourgeonal, and others were detected only at higher concentrations.

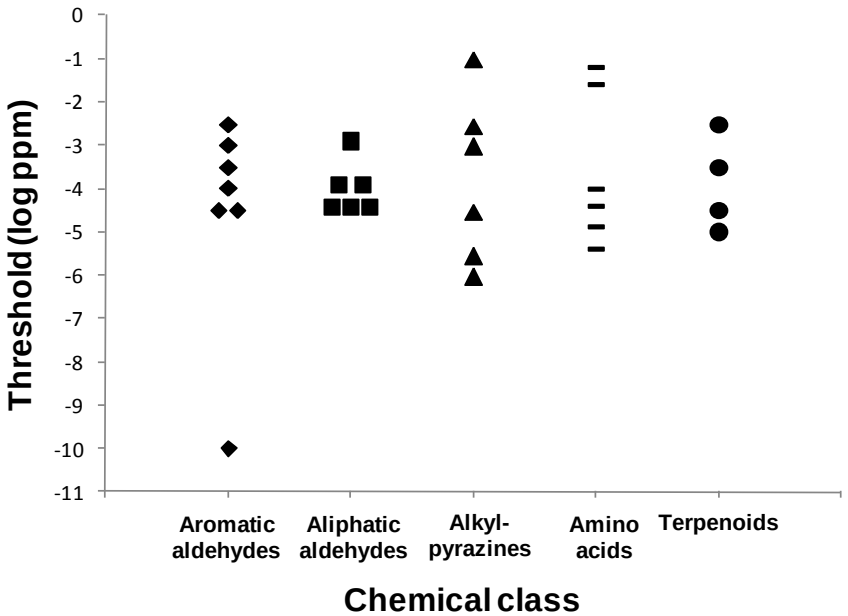


**Figure 15.** Comparison of olfactory detection threshold values for different odorant groups studied in mice, expressed as vapor phase concentrations. Each symbol represents the lowest threshold value in log ppm for each odorant tested in each class of odorants. (Laska et al, 2006; Laska et al, 2009; Joshi et al, 2006; Wallén, 2010)

### 5.3 Comparison with other species

Figure 16 compares the olfactory detection threshold values of CD-1 mice found in the present study to those found in earlier studies with humans (Olsson, 2009; Laska unpublished data) and spider monkeys (Kjeldmand, 2009). With the notable exception of bourgeonal, the detection threshold values found here for the aromatic aldehydes with CD-1 mice are two to three log units lower than the ones obtained in spider monkeys. Similarly, the threshold values found in humans are higher than those found in mice.

Olsson (2009) determined olfactory detection threshold values for bourgeonal and helional in a total of 500 human subjects. The threshold values were found to be $4.7 \cdot 10^{11}$ and $1.4 \cdot 10^{13}$ molecules/cm$^3$, respectively, for bourgeonal and helional. The olfactory sensitivity for both bourgeonal and helional together with the other five aromatic aldehydes used in the present study was also determined in an additional 20 human subjects (Laska, unpublished data). The threshold value for bourgeonal, 3-PPA, lilial and lyral were found to be $1.1 \cdot 10^{11}$, $7.9 \cdot 10^{11}$, $5.6 \cdot 10^{11}$ and $7.5 \cdot 10^{11}$ molecules/cm$^3$ respectively and helional, cyclamal and canthoxal were determined to have threshold values of $2.1 \cdot 10^{12}$, $3.5 \cdot 10^{12}$ and $1.8 \cdot 10^{13}$ molecules/cm$^3$.

Comparing these results for humans with the ones from the present study for CD-1 mice reveals that the detection threshold values for bourgeonal differ eight log units between species. The detection threshold for helional ranges from $2.1 \cdot 10^{12}$ to$1.4 \cdot 10^{13}$ molecules/cm$^3$ in humans and is therefore two to three log units higher than the highest threshold value of the mice with $2.5 \cdot 10^{10}$ molecules/cm$^3$. The human detection threshold values for lilial and lyral are only one log unit higher than the highest threshold values with mice and 3-PPA was found to have similar threshold values in both species. For cyclamal and canthoxal the threshold values for mice were three log units lower than those for humans.

The olfactory detection threshold values for bourgeonal differ between mice and spider monkeys, with detection threshold values for the mice being five to six log units lower than the values obtained in spider monkeys. 3-PPA gave the same detection threshold values in mice and spider monkeys. However, whereas 3-PPA scored the highest detection threshold value in mice, among the seven aromatic aldehydes, this odorant is found among the lowest threshold values in spider monkeys. Overall, for aromatic aldehydes as a chemical class excluding bourgeonal, mice have detection threshold values two to three log units lower than those found in spider monkeys.

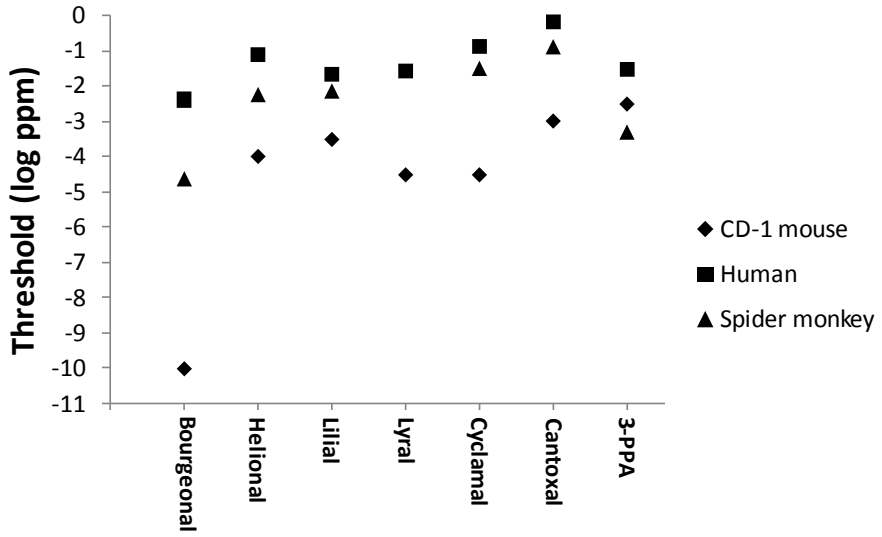


**Figure 16.** Comparison of olfactory detection threshold values of mice, humans and spider monkeys for the seven aromatic aldehydes, expressed as vapor phase concentrations. Each symbol represents the lowest threshold value in log ppm for each odorant tested. (Laska unpublished data; Olsson 2009; Kjeldmand 2009)

### 5.4 Odor structure-activity relationship

Even a small change in the molecular structure of an odorant can change its quality and affect its detectability (Joshi et al, 2006; Laska and Teubner, 1999a; Laska et al, 1999b; Laska et al, 2000; Laska et al, 2006; Laska et al, 2007). It has been shown that carbon chain length is an important molecular feature (Hommen, 2007) when it comes to the interaction between stimulus and receptor and a change of functional groups can lead to the inactivation of an odorant.

Laska et al (2008) showed that aliphatic odorants such as alcohols, aldehydes, ketones, acetic esters and carboxylic acids all were easily discriminated by mice at a vapor phase concentration of 1 ppm. The authors also found that the correlation between discrimination performance and differences in carbon chain length was odorant class-specific, and that compounds differing in their functional groups were easier to discriminate that those differing in carbon chain length. Therefore, the authors suggested that differences in both carbon chain length and functional group, such as oxygen features, allow CD-1 mice to discriminate between aliphatic odorants.

Similarly, the addition of methyl groups has been shown to lower the detection threshold value for alkylpyrazines (Laska et al, 2009) and oxygen moieties affect the detection of an odorant in either a positive or negative way depending on odor-class (Laska et al, 2000).
Spehr et al (2003) showed that the most effective ligand for the testicular olfactory receptor, hOR 17-4, had an aldehyde group connected to an aromatic ring via a carbon chain of 2-4 carbons. Also additional methyl groups in the side chain are tolerable, and a substitution of isopropyl or tertbutyl at the para position amplified the effect of the ligand.

In the present study functional group and oxygen moiety were found to influence the detectability of aromatic aldehydes. However, no generalizable rules with regard to odor structure-activity relationships can be drawn from the set of odorants used in this study.

### 5.5 Possible factors affecting olfactory sensitivity

The largest gene superfamily in the vertebrate genome is coding for olfactory receptors, which, in turn, comprise the largest and most diverse family of G-protein-coupled receptors. In humans the olfactory receptor genes are located on all chromosomes except 20 and Y.

Humans have approximately 390 functional olfactory receptor genes, mice possess approximately 1194 functional genes coding for olfactory receptors and New World monkeys approximately 900 of such genes (Dryer, 2000; Glusman et al, 2001; Godfrey et al, 2004; Rouquier et al, 2000; Rouquier and Giorgi, 2007; Zhang and Firestein, 2002; Zhang et al, 2007). This raises the question whether the size of the olfactory receptor repertoire affects olfactory sensitivity. Also, the difference in relative size of olfactory brain structure might be a reason for differences in detection threshold. However, several studies refute this idea and show that there are no clear correlations between the relative size of olfaction brain structures or the numbers of functional olfactory receptor genes and a species´ olfactory sensitivity (Laska et al, 1999b; Laska et al, 2005). The present study as well, found no clear correlation between olfactory sensitivity and the size of the olfactory receptor repertoire.

### 5.6 Future implications

The fact that bourgeonal and lyral mediate sperm chemotaxis in human and mice, respectively, might in the future have broad implications in the fields of fertility and contraception. By further studying the possibilities that the woman's egg secretes an analogue to bourgeonal that help the sperm cell to locate it and the exact role of secretion many women that today have problems to conceive might get helped.

### 5.7 Conclusion

The olfactory detection threshold value for bourgeonal is the lowest ever reported for any odorant in mice. The differences in detection threshold values between aromatic aldehydes are likely to be due to small differences in molecular structure such as presence or absence and type of functional groups.

## 6 Acknowledgements

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