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Olfactory Sensitivity for Enantiomers and Their Racemic Mixtures—A Comparative Study in CD-1 Mice and Spider Monkeys

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Abstract

Using a conditioning paradigm, the olfactory sensitivity of six CD-1 mice for the enantiomers of carvone and of limonene as well as for their racemic mixtures was investigated. With all six stimuli, the animals significantly discriminated concentrations ≤0.1 ppm (parts per million) from the odorless solvent, and with five of the six stimuli, the best-scoring animals were even able to detect concentrations ≤1 ppb (parts per billion). Five spider monkeys tested in parallel were found to detect the same stimuli at concentrations <1 ppm, and with two of the stimuli, they were also able to discriminate concentrations <1 ppb from the solvent. The results showed 1) both CD-1 mice and spider monkeys to have a well-developed olfactory sensitivity for the stimuli tested, with no systematic difference in performance between species; 2) the effect of chirality on detectability of the enantiomers to be substance specific; 3) no systematic effect of the presence (carvone) or absence (limonene) of a functional carbonyl group on detectability of the enantiomers; and 4) that spider monkeys detected the racemic mixtures of both carvone and limonene at lower concentrations compared to the unmixed compounds, whereas the mice failed to do so. These findings lend support to the growing body of evidence suggesting that between-species comparisons of the relative size of olfactory brain structures do not allow us to reliably predict olfactory sensitivity. As mice and spider monkeys are thought to share a similar number of functional olfactory receptor genes, the findings further suggest that differences in the relative abundance of chiral-specific olfactory receptor types might account for the observed difference in mixture additivity at threshold level between the two species. These threshold data may provide useful information for the choice of adequate stimulus concentrations in electrophysiological or imaging studies of the olfactory system or investigations of the discriminative abilities of mice and spider monkeys.

Key words: CD-1 mice, detection thresholds, enantiomers, olfactory sensitivity, racemic mixtures, spider monkeys

Introduction

A great deal of the current knowledge about the anatomy (Zou *et al.*, 2004; T. Kosaka and K. Kosaka, 2005), physiology (Xu *et al.*, 2003; Wachowiak *et al.*, 2005; Yamaguchi and Mori, 2005), development (Burd and Tolbert, 2000; Mombaerts, 2001), and genetics of olfaction (Young *et al.*, 2001; Zhang and Firestein, 2002; Godfrey *et al.*, 2004) as well as the mechanisms underlying the neural coding of olfactory information (Wachowiak and Cohen, 2003; Katada *et al.*, 2005; Zou *et al.*, 2005) has been obtained using the mouse as a model species. Surprisingly, few studies, however, have so far assessed olfactory performance in the mouse at the organismal level. With regard to olfactory sensitivity of *Mus musculus*, for example, detection thresholds for little more than a dozen substances have been reported (Passe and Walker, 1985; Walker and Jennings, 1991; Laska *et al.*, 2006a) and, to the best of our knowledge, none for any chiral odorants or binary mixtures. This is all the more surprising given the importance of basic data on olfactory sensitivity for the choice of adequate stimulus concentrations in electrophysiological or imaging studies and the frequent use of structurally related odorants for elucidating possible correlations between molecular structural features and measures of physiological activity (Xu *et al.*, 2003; Johnson *et al.*, 2004) or discrimination performance (Laska *et al.*, 1999a,b, 2005; Laska and Teubner, 1999).

Enantiomers appear to be particularly useful for assessing how molecular structure is encoded by the olfactory system, finally leading to detectable and discriminable odor qualities. Whereas perceptual differences between nonenantiomeric odorants can be, at least partially, due to properties such

as differing diffusion rates in the mucus covering the olfactory sensory epithelium or differing air–mucus partition coefficients (Hahn *et al.*, 1994; Abraham *et al.*, 2001, 2002), enantiomers exhibit identical chemical and physical properties (except for their optical activity, i.e., rotation of polarized electromagnetic waves), and thus, any difference in odor perception must originate from chiral selectivity at the peripheral level (Rossiter, 1996). Therefore, the systematic assessment of both the sensitivity for and discriminability of enantiomeric odor pairs may contribute to our understanding of odor perception and coding.

In order to begin to provide the needed data, it was the aim of the present study to determine olfactory detection thresholds in CD-1 mice for two pairs of enantiomers, allowing us to assess the impact of chirality on detectability. We have chosen the optical isomers of carvone and limonene as stimuli because previous studies have shown both enantiomeric odor pairs to be discriminable for a number of species, including human subjects (Laska and Teubner, 1999), rats (Linster *et al.*, 2002), squirrel monkeys (Laska *et al.*, 1999a), pigtail macaques (Laska *et al.*, 2005), honeybees (Laska and Galizia, 2001), and also for CD-1 mice and spider monkeys (M. Laska, unpublished data). This suggests that the (+)- and (−)-forms of these substances should interact with at least partially different subsets of olfactory receptors (Hamana *et al.*, 2003; Kirner *et al.*, 2003). This, in turn, might affect olfactory sensitivity for both the single compounds and their racemic (i.e., 50:50) mixtures that were therefore also used as stimuli. Furthermore, carvone and limonene only differ from each other in the presence versus absence of a functional carbonyl group, allowing us to assess the impact of this structural feature on detectability.

The opportunity to test five spider monkeys, a mammalian species thought to have a similar number of functional olfactory receptor genes as the mouse (Gilad *et al.*, 2004; Godfrey *et al.*, 2004) but a considerably smaller relative size of olfactory brain structures (Stephan *et al.*, 1988), in parallel allowed us to additionally assess the impact of this neuroanatomical feature on olfactory sensitivity.

Materials and methods

Animals

Testing was carried out using six male CD-1 mice (*M. musculus*) and five adult female spider monkeys (*Ateles geoffroyi*). The rationale for choosing this outbred strain of mice was to use animals with a variable genetic background that is more similar to wild-type mice than that of inbred strains. Furthermore, data on olfactory detection thresholds for a homologous series of aliphatic aldehydes were obtained in an earlier study using the same mouse strain (Laska *et al.*, 2006a). The rationale for choosing spider monkeys was to use a mammal species presumed to have a number of olfactory receptor genes (Gilad *et al.*, 2004) similar to those reported in mice (Zhang and Firestein, 2002) but a considerably smaller relative size of olfactory brain structures (Stephan *et al.*, 1988), allowing us to assess the impact of this neuroanatomical feature on olfactory sensitivity. Furthermore, data on olfactory detection thresholds for homologous series of aliphatic esters (Hernandez Salazar *et al.*, 2003), carboxylic acids (Laska *et al.*, 2004), alcohols, and aldehydes (Laska *et al.*, 2006b) were obtained in earlier studies using the same animals. Maintenance of both species has been described in detail elsewhere (mice: Laska *et al.*, 2006a; spider monkeys: Laska *et al.*, 2003). One of the spider monkeys only participated in the tests with the limonenes but not in the tests with the carvones.

The experiments reported here comply with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication no. 86-23, revised 1985) and were performed according to a protocol approved by the Yale University Institutional Animal Care and Use Committee.

Stimuli

A set of six stimuli was used: (+)-carvone, (−)-carvone, (+)-limonene, (−)-limonene, and the racemic (i.e., 50:50) mixtures of the (+)- and (−)-forms of carvone and of limonene. The rationale for choosing these stimuli was to assess the sensitivity of both species for two pairs of enantiomers, that is, substances that are identical in their physical and chemical properties except for chirality, allowing us to assess the impact of this structural feature on detectability. Furthermore, carvone and limonene only differ from each other in the presence versus absence of a functional carbonyl group, allowing us to assess the impact of this structural feature on detectability (Figure 1). All substances were obtained from Sigma–Aldrich (St Louis, MO) and had a nominal purity of at least 99%. They were diluted using odorless diethyl phthalate (Sigma–Aldrich) as the solvent.

Behavioral tests

Olfactory sensitivity of the mice was assessed using an automated liquid-dilution olfactometer (Knosys, Tampa, FL). Animals were trained using standard operant conditioning procedures (Bodyak and Slotnick, 1999) to insert their snout into the odor sampling port of a test chamber. This triggered the 2-s presentation of either an odorant used as the

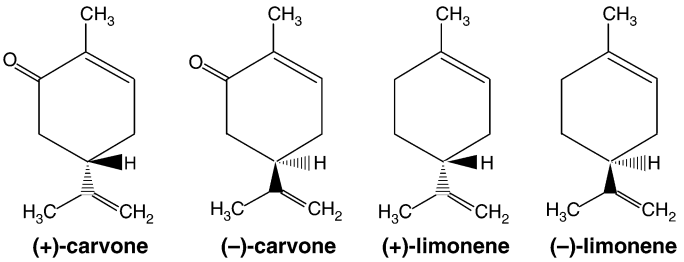

Figure 1 Chemical structure of the enantiomers used.

rewarded stimulus (S+) or a blank (headspace of the solvent) used as the unrewarded stimulus (S−). Licking at a steel tube providing 2.5 μl of water reinforcement in response to presentation of the S+ served as the operant response. A total of 100 such trials (50 S+ and 50 S− trials in pseudorandomized order) using the same concentration of a given S+ were conducted per animal and condition.

The spider monkeys were tested using a food-rewarded instrumental conditioning paradigm, which has been described in detail elsewhere (Laska *et al.*, 2003). Briefly, the animals were trained to sniff at manipulation objects equipped with absorbent paper strips that were impregnated with 10 μl of an odorant or the odorless solvent signaling either that they contained a food reward (S+) or that they did not (S−). Opening of one of the options served as the operant response. A total of 30 such trials (15 S+ and 15 S− trials in pseudorandomized order) using the same concentration of a given S+ were conducted per animal and condition.

With both species, olfactory detection thresholds were determined by testing the animals' ability to discriminate between increasing dilutions of an odorant used as S+ and the odorless solvent alone used as S−. Starting with a gas-phase concentration of 1 ppm (parts per million) (in the case of the mice) and a 100-fold liquid dilution (in the case of the spider monkeys), each stimulus was successively presented in 10-fold dilution steps until an animal failed to significantly discriminate the odorant from the solvent. Subsequently, an intermediate concentration (0.5 log units between the lowest concentration that was detected above chance and the first concentration that was not) was tested in order to determine the threshold value more exactly.

Data analysis

For each individual animal, the percentage of correct choices from 100 (mice) and 30 (spider monkeys) decisions per dilution step was calculated. With the mice, correct choices consisted of both licking in response to presentation of the S+ and not licking in response to the S−, and errors consisted of animals showing the reverse pattern of operant responses. With the spider monkeys, correct choices consisted both of animals rejecting negative manipulation objects by failing to open them and of animals identifying positive manipulation objects by opening them to obtain the food reward. Conversely, errors consisted of animals opening negative manipulation objects or failing to open positive manipulation objects.

Significance levels were determined by calculating binomial z-scores corrected for continuity from the number of correct and false responses for each individual and condition. All tests were two tailed, and the alpha level was set at 0.05.

Results

Figure 2 shows the performance of the mice in discriminating between various dilutions of a given stimulus and the odorless solvent. All six animals significantly distinguished dilutions as low as 1:13,200 (+)-carvone and (−)-carvone, 1:4400 (±)-carvone, 1:34,000 (+)-limonene, 1:3.4 million (−)-limonene, and 1:3.4 million (±)-limonene from the solvent (binomial test, $P < 0.01$), with single individuals even scoring better.

The individual mice generally demonstrated similar threshold values with a given stimulus, and with two of the six stimuli, (+)-limonene and (±)-limonene, they differed only by a dilution factor of 30 between the highest and the lowest scoring animal. In the case of (−)-carvone, (±)-carvone, and (−)-limonene, the individual threshold values differed by a factor of 100. The largest difference in sensitivity for a given stimulus between individuals was a dilution factor of 1000 and was found with (+)-carvone.

Table 1 summarizes the threshold dilutions of the mice and shows various measures of corresponding vapor-phase concentrations (Weast, 1987), allowing readers to easily compare the data obtained in the present study to those reported by other authors using one of these convertible measures. In all cases, threshold dilutions correspond to vapor-phase concentrations ≤0.1 ppm, and with five of the six stimuli, the best-scoring animal was even able to detect a concentration ≤1 ppb (parts per billion).

Figure 3 compares the threshold values of the six mice for the six stimuli tested. The sensitivities of *M. musculus* for the three carvone stimuli were generally similar and did not differ significantly from each other (Wilcoxon, $P > 0.05$). Four of the six animals displayed identical threshold values for (+)- and (−)-carvone. It is interesting to note, however, that none of the animals were more sensitive for the racemic mixture of (+)- and (−)-carvone compared to its constituents. Rather, four of the six animals displayed higher thresholds for the mixture than for the unmixed compounds. With limonene, all six mice were more sensitive for the (−)-form and for the racemic mixture compared to the (+)-form (Wilcoxon, $P < 0.05$). The sensitivity of the mice for (−)-limonene and the racemic mixture of the two optical isomers of limonene did not differ significantly (Wilcoxon, $P > 0.05$). No clear difference was found in sensitivity of the mice for (+)-carvone and (+)-limonene (Wilcoxon, $P > 0.05$), whereas five of the six animals displayed lower thresholds with (−)-limonene compared to (−)-carvone (Wilcoxon, $P < 0.05$). The racemic mixture of (+)- and (−)-limonene was perceived at lower concentrations than the racemic mixture of (+)- and (−)-carvone by all six mice (Wilcoxon, $P < 0.05$).

Figure 4 shows the performance of the spider monkeys in discriminating between various dilutions of a given stimulus and the odorless solvent. All animals significantly distinguished dilutions as low as 1:30,000 (+)-carvone and (−)-carvone, 1:1 million (±)-carvone, 1:100,000 (+)-limonene, 1:3000 (−)-limonene, and 1:3 million (±)-limonene from the solvent (binomial test, $P < 0.01$), with single individuals even scoring better.

The individual spider monkeys demonstrated similar threshold values with a given stimulus, and with two of the

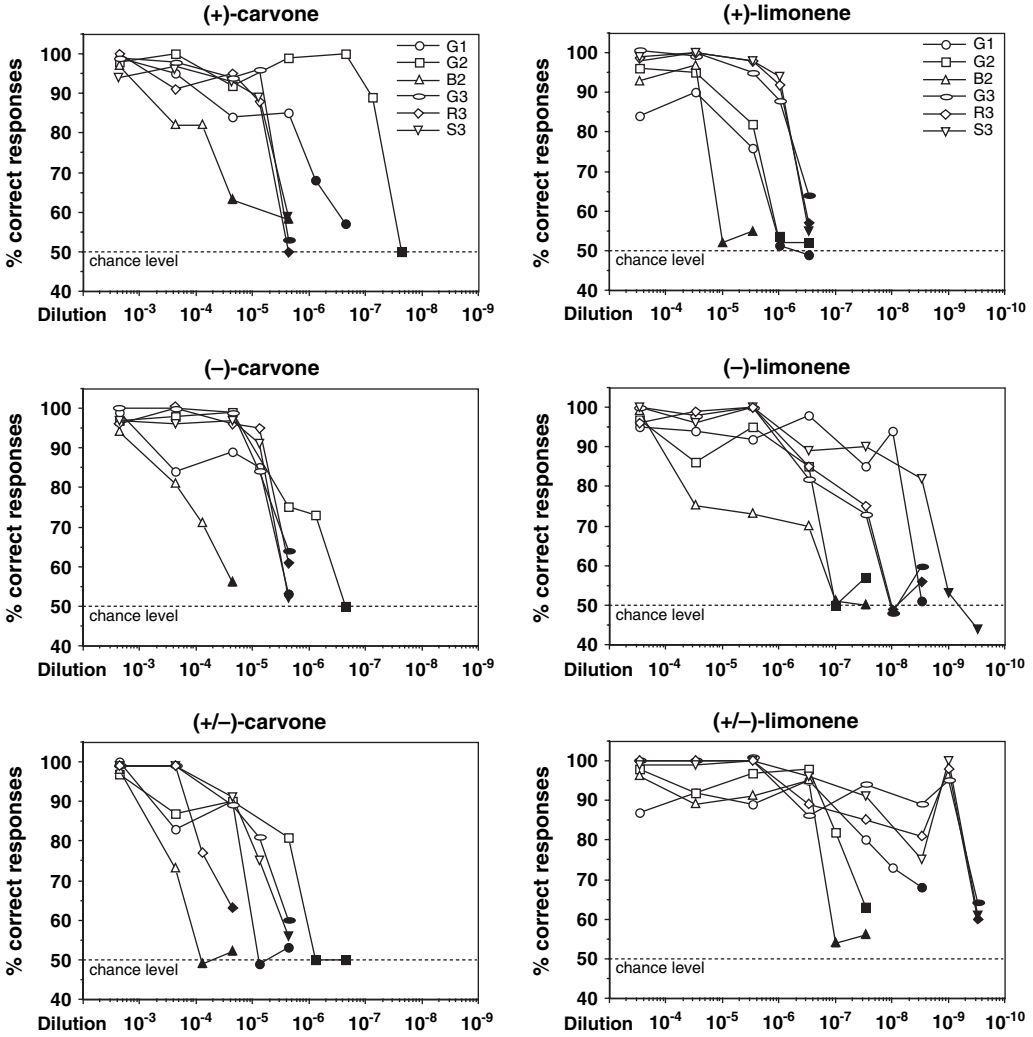

Figure 2 Performance of CD-1 mice in discriminating between various dilutions of an enantiomer or its racemic mixture and the odorless solvent diethyl phthalate. Each data point represents the percentage of correct choices from a total of 100 decisions per individual animal. The six different symbols represent data from each of the six individual animals tested per stimulus. Filled symbols indicate dilutions that were not discriminated significantly above chance level (binomial test, $P > 0.05$).

six stimuli, (+)-carvone and (+)-limonene, they differed only by a dilution factor of 10 between the highest and the lowest scoring animal. In the case of (±)-carvone, the individual threshold values differed by a factor of 30, and with (−)-carvone, the animals even demonstrated identical threshold values. The largest difference in sensitivity for a given stimulus between individuals was a dilution factor of 100 and was found with (−)-limonene and (±)-limonene.

Table 2 summarizes the threshold dilutions of the spider monkeys and shows various measures of corresponding vapor-phase concentrations. In all cases, threshold dilutions correspond to vapor-phase concentrations <1 ppm, and with two of the six stimuli, all animals were even able to detect a concentration <1 ppb.

Figure 5 compares the threshold values of the spider monkeys for the six stimuli tested. The sensitivities of *A. geoffroyi* for (+)- and (−)-carvone were generally similar. However, all animals were more sensitive for the racemic mixture of (+)- and (−)-carvone compared to its constituents. With limonene, all five spider monkeys were more sensitive for the (+)-form compared to the (−)-form (Wilcoxon, $P < 0.05$). Here, too, all animals were more sensitive to the racemic mixture of (+)- and (−)-limonene than to its constituents (Wilcoxon, $P < 0.05$). No clear difference was found in sensitivity of the spider monkeys for (+)-carvone and (+)-limonene, whereas all but one animal were more sensitive for (−)-carvone than for (−)-limonene. A comparison between the spider monkeys' thresholds for the racemic mixtures of carvone and of limonene showed no clear difference.

Discussion

The results of this study demonstrate 1) both CD-1 mice and spider monkeys to have a well-developed olfactory

Table 1 Olfactory detection threshold values for the enantiomers of carvone and limonene plus their racemic mixtures in CD-1 mice, expressed in various measures of vapor-phase concentrations

	n	Liquid dilution	Vapor-phase concentrations				
			Molecules/cm^3	ppm	Log ppm	mol/l	Log mol/l
(+)-Carvone	1	1:13,200	7.5×10^{11}	0.03	−1.52	1.3×10^{-9}	−8.87
	3	1:132,000	7.5×10^{10}	0.003	−2.52	1.3×10^{-10}	−9.87
	1	1:440,000	2.5×10^{10}	0.001	−3.00	4.5×10^{-11}	−10.35
	1	1:13.2 million	7.5×10^{8}	0.00003	−4.52	1.3×10^{-12}	−11.87
(−)-Carvone	1	1:13,200	7.5×10^{11}	0.03	−1.52	1.3×10^{-9}	−8.87
	4	1:132,000	7.5×10^{10}	0.003	−2.52	1.3×10^{-10}	−9.87
	1	1:1.32 million	7.5×10^{9}	0.0003	−3.52	1.3×10^{-11}	−10.87
(±)-Carvone	1	1:4400	2.5×10^{12}	0.1	−1.00	4.5×10^{-9}	−8.35
	1	1:13,200	7.5×10^{11}	0.03	−1.52	1.3×10^{-9}	−8.87
	1	1:44,000	2.5×10^{11}	0.01	−2.00	4.5×10^{-10}	−9.35
	2	1:132,000	7.5×10^{10}	0.003	−2.52	1.3×10^{-10}	−9.87
	1	1:440,000	2.5×10^{10}	0.001	−3.00	4.5×10^{-11}	−10.35
(+)-Limonene	1	1:34,000	2.5×10^{12}	0.1	−1.00	4.5×10^{-9}	−8.35
	2	1:340,000	2.5×10^{11}	0.01	−2.00	4.5×10^{-10}	−9.35
	3	1:1.02 million	7.5×10^{10}	0.003	−2.52	1.3×10^{-10}	−9.87
(−)-Limonene	2	1:3.4 million	2.5×10^{10}	0.001	−3.00	4.5×10^{-11}	−10.35
	2	1:34 million	2.5×10^{9}	0.0001	−4.00	4.5×10^{-12}	−11.35
	1	1:102 million	7.5×10^{8}	0.00003	−4.52	1.3×10^{-12}	−11.87
	1	1:340 million	2.5×10^{8}	0.00001	−5.00	4.5×10^{-13}	−12.35
(±)-Limonene	1	1:3.4 million	2.5×10^{10}	0.001	−3.00	4.5×10^{-11}	−10.35
	1	1:10.2 million	7.5×10^{9}	0.0003	−3.52	1.3×10^{-11}	−10.87
	1	1:102 million	7.5×10^{8}	0.00003	−4.52	1.3×10^{-12}	−11.87
	3	1:1.02 billion	7.5×10^{7}	0.000003	−5.52	1.3×10^{-13}	−12.87

n, number of animals.

sensitivity for the stimuli tested, with no systematic difference in performance between species; 2) the effect of chirality on detectability of the enantiomers to be substance specific; 3) no systematic effect of the presence (carvone) or absence (limonene) of a functional carbonyl group on the detectability of the enantiomers; and 4) that spider monkeys detected the racemic mixtures of both carvone and limonene at lower concentrations compared to the unmixed compounds, whereas the mice failed to do so.

Although only six mice and five spider monkeys were tested per stimulus, the results appear robust as interindividual variability was generally low and smaller than the range reported in studies on human olfactory sensitivity, that is, within three orders of magnitude (Doty, 1991). Further, for all substances, the animals' performance with the lowest concentrations presented dropped to chance level, suggesting that the statistically significant discrimination between higher concentrations of a stimulus and the odorless diluent was indeed based on chemosensory perception and not on other cues.

Figure 6 compares the olfactory detection threshold values obtained with the mice and the spider monkeys for the stimuli tested to those from rats and human subjects. Although such across-species comparisons should be considered with caution as different methods may lead to widely differing results (Hastings, 2003), it seems admissible to state that the spider monkeys did not generally perform poorer than the mice. Rather, with the exception of (−)-limonene, the spider monkeys' sensitivity for the stimuli tested here was at least as high as that of the mice, despite the fact that the relative size of the olfactory brain structures in *A. geoffroyi* is considerably smaller than that in *M. musculus* (Stephan *et al.*, 1988). Similarly, human subjects did not generally perform poorer compared to the mice and spider monkeys,

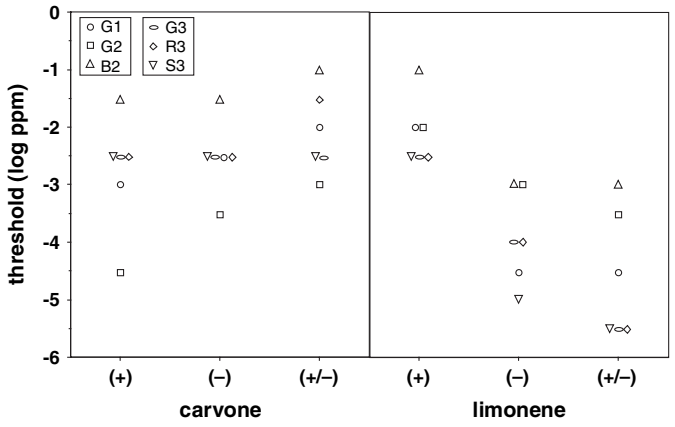

Figure 3 Olfactory detection threshold values (expressed as vapor-phase concentrations) of the CD-1 mice for the stimuli tested. The six different symbols represent data from each of the six individual animals tested per odorant.

despite the fact that mice (Godfrey *et al.*, 2004) and spider monkeys (Gilad *et al.*, 2004) have been shown to possess ≈1000 functional genes coding for olfactory receptors, whereas human subjects have only ≈350 such genes (Glusman *et al.*, 2001), with the rest being pseudogenes that are presumed not to be transcribed into proteins. However, it should be mentioned that the threshold values of the human subjects for the enantiomers of carvone and limonene as depicted in Figure 6 represent mean values across groups of subjects (van Gemert, 2003), whereas all animal data represent individual threshold values. Rats that were tested using a method and olfactometer similar to the one employed in the present study appear to be more sensitive for the optical isomers of carvone (Kirner *et al.*, 2003) than mice and spider monkeys but not for (+)-limonene (Youngentob *et al.*, 1997). Unfortunately, no threshold data for the racemic mixtures of carvone and limonene are at

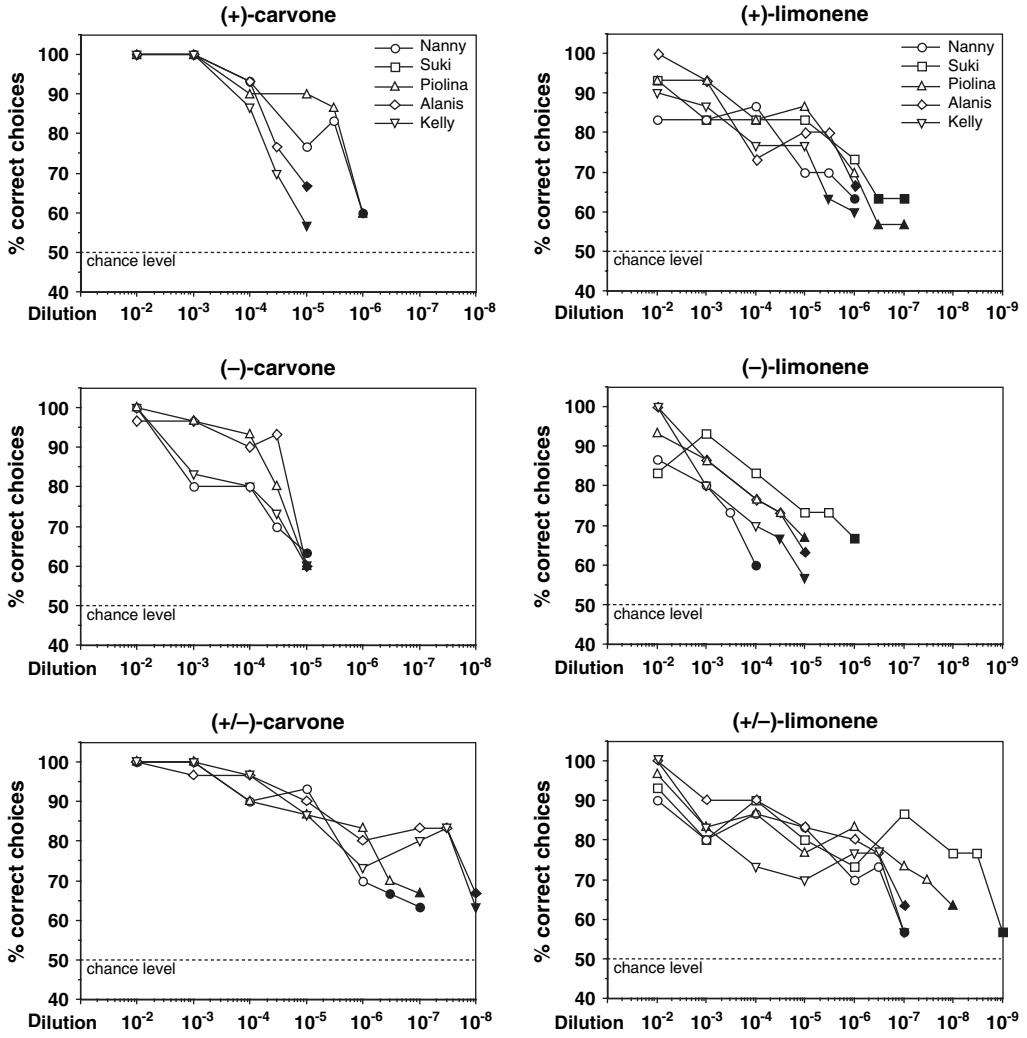

Figure 4 Performance of spider monkeys in discriminating between various dilutions of an enantiomer or its racemic mixture and the odorless solvent diethyl phthalate. Each data point represents the percentage of correct choices from a total of 30 decisions per individual animal. The five different symbols represent data from each of the five individual animals tested per stimulus. Filled symbols indicate dilutions that were not discriminated significantly above chance level (binomial test, $P > 0.05$).

Table 2 Olfactory detection threshold values for the enantiomers of carvone and limonene plus their racemic mixtures in spider monkeys, expressed in various measures of vapor-phase concentrations

	n	Liquid dilution	Vapor-phase concentrations				
			Molecules/cm^3	ppm	Log ppm	mol/l	Log mol/l
(+)-Carvone	2	1:30,000	4.0×10^{11}	0.015	−1.83	6.6×10^{-10}	−9.18
	2	1:300,000	4.0×10^{10}	0.0015	−2.83	6.6×10^{-11}	−10.18
(−)-Carvone	4	1:30,000	4.0×10^{11}	0.015	−1.83	6.6×10^{-10}	−9.18
(±)-Carvone	1	1:1 million	1.2×10^{10}	0.00044	−3.35	2.0×10^{-11}	−10.70
	1	1:3 million	4.0×10^{9}	0.00015	−3.83	6.6×10^{-12}	−11.18
	2	1:30 million	4.0×10^{8}	0.000015	−4.83	6.6×10^{-13}	−12.18
(+)-Limonene	1	1:100,000	7.9×10^{11}	0.029	−1.53	1.3×10^{-9}	−8.88
	2	1:300,000	2.6×10^{11}	0.0096	−2.02	4.3×10^{-10}	−9.36
	2	1:1 million	7.9×10^{10}	0.0029	−2.53	1.3×10^{-10}	−9.88
(−)-Limonene	1	1:3000	2.6×10^{13}	0.96	−0.02	4.3×10^{-8}	−7.36
	1	1:10,000	7.9×10^{12}	0.29	−0.53	1.3×10^{-8}	−7.88
	2	1:30,000	2.6×10^{12}	0.096	−1.02	4.3×10^{-9}	−8.36
	1	1:300,000	2.6×10^{11}	0.0096	−2.02	4.3×10^{-10}	−9.36
(±)-Limonene	3	1:3 million	2.6×10^{10}	0.00096	−3.02	4.3×10^{-11}	−10.36
	1	1:30 million	2.6×10^{9}	0.000096	−4.02	4.3×10^{-12}	−11.36
	1	1:300 million	2.6×10^{8}	0.0000096	−5.02	4.3×10^{-13}	−12.36

n, number of animals.

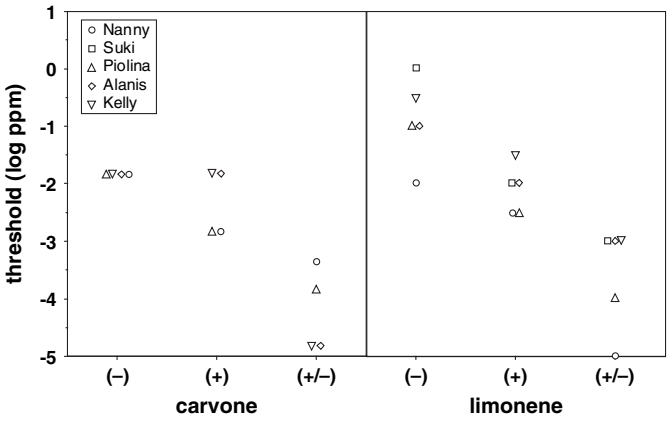

Figure 5 Olfactory detection threshold values (expressed as vapor-phase concentrations) of the spider monkeys for the stimuli tested. The five different symbols represent data from each of the five individual animals tested per odorant.

hand for rats. Taken together, these across-species comparisons support the idea that both the number of functional olfactory receptor genes and the relative size of olfactory brain structures are not reliable predictors of a species' olfactory sensitivity.

It is also interesting to note that none of the animals tested here showed a specific anosmia to either of the carvones or the structurally similar limonenes, whereas 8% of the human population has been reported to be anosmic to (−)-carvone and some of its structural analogues (Pelosi and Viti, 1978). Specific anosmias in mice have so far been reported for isovaleric acid (Griff and Reed, 1995), geraniol (Price, 1977), and androstenone (Wang *et al.*, 1993), and a gender-specific anosmia has recently been found in spider monkeys for androstadienone and estratetraenol (Laska *et al.*, 2006c). Further screening for specific anosmias, and for species differences in such substance-specific inabilities to detect odorants, may be a useful approach to assess the ligand specificity of olfactory receptors and its genetic basis.

Our finding that both CD-1 mice and spider monkeys were clearly more sensitive to one of the optical isomers of limonene—the (−)-form in the case of the mice and the (+)-form in the case of the spider monkeys—but failed to show a corresponding difference in sensitivity for the (+)- and (−)-forms of carvone (see Figures 3 and 5) is in line with the idea that the effect of chirality on detectability of enantiomers is substance specific and not a generalizable phenomenon. This idea is also supported by human psychophysical studies showing that the enantiomers of some substances such as carvone (Leitereg *et al.*, 1971; Laska and Teubner, 1999) or gamma-ionone (Brenna *et al.*, 2002) are detected at different concentrations, whereas the optical isomers of limonene (Padrayuttawat *et al.*, 1997; Laska and Teubner,

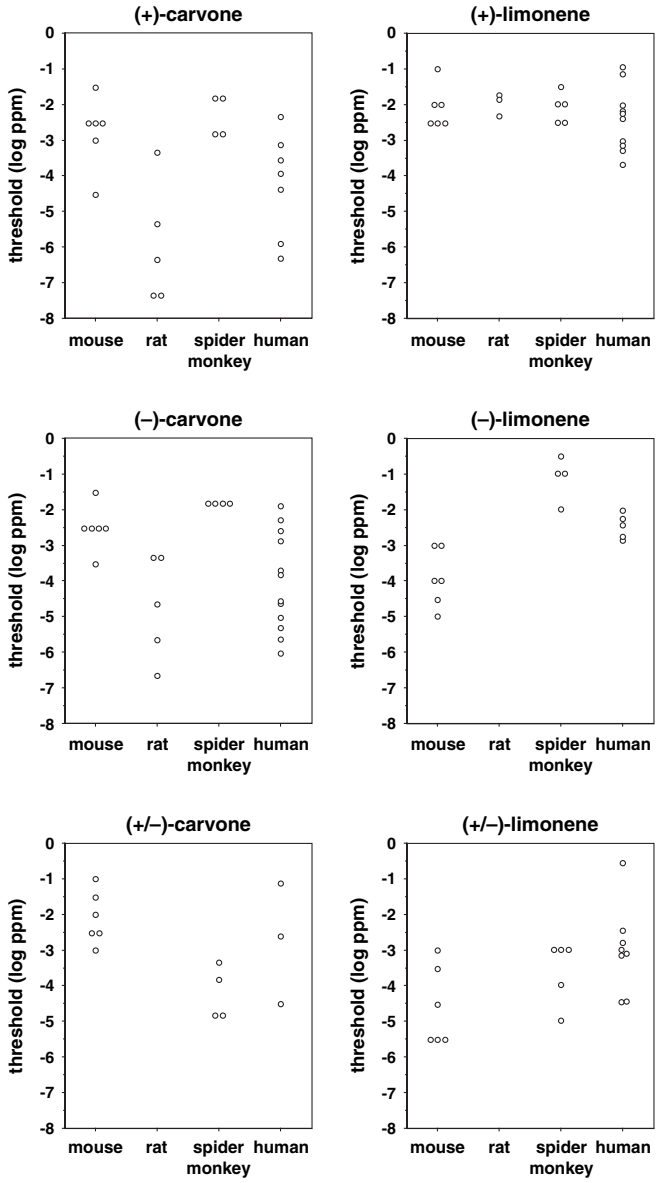

Figure 6 Comparison of the olfactory detection threshold values (expressed as vapor-phase concentrations) of the CD-1 mice and the spider monkeys for the six stimuli tested here and those of human subjects and rats. (Human data: van Gemert, 2003; rat data: Kirner *et al.*, 2003, for carvone; Youngentob *et al.,* 1997, for limonene.) Data points of the three animal species represent threshold values of individual animals. Data points of the human subjects represent mean values from different studies.

1999) or alpha-ionone (Brenna *et al.*, 2002), for example, yield similar if not identical thresholds. One possible explanation underlying this phenomenon is that the frequency of occurrence of the two forms of a chiral odorant in a species' chemical environment may differ markedly between substances (Knudsen *et al.*, 1993; Kubeczka, 2002), which, in turn, may lead to differences in the expression of chiral-specific olfactory receptors. This idea is supported by findings that showed the discriminability of enantiomeric odor pairs to correlate with their frequency of occurrence in flower odors (Laska and Galizia, 2001) and fruit odors (Laska *et al.*, 1999a).

Our finding that the CD-1 mice were clearly more sensitive to (−)-limonene compared to (−)-carvone (except for one individual) and the spider monkeys showed the reverse pattern of sensitivity (except for one individual), whereas both species failed to show a clear difference in sensitivity for (+)-carvone and (+)-limonene (see Figures 3 and 5), suggests that the presence (carvone) or absence (limonene) of a functional carbonyl group (see Figure 1) has no generalizable effect on the detectability of these enantiomers in these two species. Human subjects, in contrast, have repeatedly been shown to be more sensitive for (+)-carvone compared to (+)-limonene and for (−)-carvone compared to (−)-limonene (Padrayuttawat *et al.*, 1997; Laska and Teubner, 1999), suggesting that the presence or absence of the functional carbonyl group may systematically affect detectability of these enantiomers in humans. Future studies should further elucidate if and how the presence or absence of oxygen-containing functional groups affects detectability and discriminability of enantiomers and whether such effects are substance and/or species specific.

A final aspect of the present study is our finding that spider monkeys detected the racemic mixtures of both carvone and limonene at lower concentrations compared to the unmixed compounds, whereas the mice failed to do so. There is general agreement that combining odorants at suprathreshold concentrations usually results in some form of suppression in which the mixture is perceived as less intense than the sum of the component intensities would predict (Laffort, 1989; Laing, 1995). Combining odorants at concentrations near or below threshold, in contrast, has repeatedly been reported to lead to a variety of positive interaction effects, including partial additivity, full additivity, and in some cases even enhancement, meaning that mixtures may gain in detectability compared to its constituents (Laska and Hudson, 1991; Patterson *et al.*, 1993; Cometto-Muniz *et al.*, 2005). The mechanism thought to underlie this phenomenon is an increasing recruitment of receptor types with increasing complexity of the mixture. However, the degree to which different receptor types involved in mixture perception overlap in their molecular receptive ranges may be critical for the occurrence and degree of positive interaction effects at the perceptual level. In the present case, it could be hypothesized that the receptor types activated by the racemic mixtures of carvone and of limonene, respectively, might overlap in their molecular receptive ranges to a lesser degree in the spider monkeys compared to those activated by the same mixtures in the mouse. A recent study demonstrated that more than 80% of mouse olfactory sensory neurons responsive to carvone were nondiscriminating between the (+)- and (−)-forms of this enantiomer, leaving less than 20% of chiral-specific carvone receptors that might be recruited additionally when presented with a racemic mixture rather than with one of the optical isomers alone

(Hamana *et al.*, 2003). Future studies should elucidate whether this ratio is substance and/or species specific and whether it is predictive of interaction effects at the threshold level.

Taken together, the findings of the present study suggest that CD-1 mice and spider monkeys are highly sensitive to nonpheromonal odorants that are abundant in plant odors (Knudsen *et al.*, 1993; Burdock, 2005). These threshold data may provide useful information for the choice of adequate stimulus concentrations in electrophysiological or imaging studies of the olfactory system or investigations of the discriminative abilities of mice and spider monkeys.

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