Chem. Senses 29: 143–152, 2004 DOI: 10.1093/chemse/bjh019

Olfactory Discrimination Ability of Human Subjects for Enantiomers with an Isopropenyl Group at the Chiral Center

Matthias Laska

Institut für Medizinische Psychologie, Ludwig-Maximilians-Universität München, Goethestr. 31, D-80336 München, Germany

Correspondence to be sent to: Matthias Laska, Institut für Medizinische Psychologie, Ludwig-Maximilians-Universität, Goethestr. 31, D-80336 München, Germany. e-mail: Laska@imp.med.uni-muenchen.de

Abstract

The ability of 20 human subjects to distinguish between nine enantiomeric odor pairs sharing an isopropenyl group at the chiral center was tested in a forced-choice triangular test procedure. I found (i) that as a group, the subjects were only able to significantly discriminate the optical isomers of limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate, whereas they failed to distinguish between the (+)- and (–)-forms of perillaalcohol, perillaaldehyde, isopulegol and limonene oxide; (ii) marked interindividual differences in discrimination performance, ranging from subjects who were able to significantly discriminate between eight of the nine odor pairs to subjects who failed to do so with six of the nine tasks; and (iii) that with none of the nine odor pairs the antipodes were reported to differ significantly in subjective intensity when presented at equal concentrations. Additional tests of the chemesthetic potency and threshold measurements of the optical isomers of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate suggest that the discriminability of these three enantiomeric odor pairs is indeed due to differences in odor quality. Analysis of structure–activity relationships suggest that the combined presence of (i) an isopropenyl group at the chiral center; (ii) a methyl group at the para-position; and/or (iii) an oxygen-containing group at the meta-position allows for the discrimination of enantiomeric odor pairs.

Key words: discrimination ability, enantiomers, humans, odor structure–activity relationships, olfaction

Introduction

When four different ligands are attached to the same carbon atom, two different structural arrangements emerge that are nonsuperimposable but are mirror images of each other. The recognition of such molecules, called optical isomers or enantiomers, has been shown to play an important role in many interactions with biological sites such as drug response (e.g. Caldwell and Hutt, 1996), enzyme specificity (e.g. Faber and Griengl, 1991), taste perception (e.g. Shallenberger, 1993) and insect chemical communication (e.g. Mori, 1996).

A variety of volatile optical isomers have been described as having different odor qualities and/or different odor intensitites for humans (Ohloff, 1994; Brenna *et al.*, 2003). This should not be surprising given that the first event in odor perception is the interaction of an odor molecule with an olfactory receptor (Hildebrand and Shepherd, 1997). As olfactory receptors have been identified as proteins, i.e. chiral molecules (Buck and Axel, 1991), this interaction should also be enantioselective, meaning that odor receptors should react differently with the two enantiomeric forms of a chiral odorant, leading to differences in perceived odor intensity and/or quality (Kraft and Frater, 2001; Nandi, 2003). However, there are also reports of identically smelling enantiomeric odor pairs (Theimer *et al.*, 1977; Boelens *et al.*, 1993) that seem inconsistent with the assumption that olfactory receptors should be enantioselective. The situation is even more complicated by findings of chiral isomers in which one form has a distinct odor quality whereas the other form is odorless (Pickenhagen, 1989; Simmons *et al.*, 1992).

Most of the human studies reporting qualitative and/or quantitative differences between enantiomers have employed odor profiling or scaling procedures that are presumed to be particularly susceptible to cognitive influences (Corwin, 1992). Only a few studies, on the other hand, have directly tested the discriminability of (+)- and (–)-forms of such odorants, although this method largely avoids the disadvantages of poor resolution and semantic ambiguity (Wise *et al.*, 2000). Laska and Teubner (1999a) tested human subjects for their ability to distinguish between 10 pairs of enantiomers. They found that, as a group, the subjects were only able to significantly discriminate between three of these odor pairs whereas they failed with the remainder. Further, they reported that qualitative attributes assigned to several of these enantiomeric odor pairs in verbal profiling studies

(Ohloff, 1994) do not allow us to predict whether a given stimulus pair is discriminable or not (Laska and Teubner, 1999a). Interestingly, two of the three enantiomeric odor pairs that were discriminable for humans—the antipodes of carvone and limonene, respectively—share the same functional isopropenyl group at the chiral carbon atom, whereas none of the non-discriminated optical isomers had this structural feature. This raises the question as to whether enantiomers having an isopropenyl group at the chiral center might generally differ from each other in odor quality.

Given the possible importance of enantioselectivity for our understanding of the molecular mechanisms underlying the interaction between odor stimulus and olfactory receptor, and thus of odor quality coding at the peripheral level, I therefore decided to test the ability of human subjects to discrimininate between the members of nine pairs of enantiomers sharing an isopropenyl group at the chiral center.

Experiment 1: discrimination of enantiomers

In this experiment, the ability of human subjects to distinguish between nine enantiomeric odor pairs was assessed. Substances were chosen on the basis of an earlier study which reported the antipodes of carvone and limonene—which both share an isopropenyl group at the chiral center—to be discriminable, whereas the majority of enantiomeric odor pairs lacking this structural feature were not discriminated (Laska *et al.*, 1999).

To further elucidate whether enantiomeric odor pairs with an isopropenyl group at the chiral center generally differ in odor quality, carvone, limonene and seven other chiral odor pairs sharing this structural feature were tested for their discriminability.

Materials and methods

Subjects

Twenty healthy, unpaid volunteers (18 females and two males), 24–39 years of age, participated in the study. All were non-smokers and none had any history of olfactory dysfunction. All subjects had previously participated in a clinical test of olfactory function and were found to be normosmic. All subjects had also previously served in olfactory discrimination tests and were familiar with the basic test procedure. They were informed as to the aim of the experiment and provided written consent. The study was performed in accordance with the Declaration of Helsinki.

Odorants

A set of 18 odorants comprising nine pairs of enantiomers was used (Table 1, Figure 1). All substances had a nominal purity of at least 99% (Fluka, Taufkirchen, Germany). They were diluted using diethyl phthalate (Merck, Darmstadt, Germany) as the solvent. The enantiomers of a given pair were presented at equal concentrations in order to assess whether differences in perceived intensity rather than differences in perceived odor quality contributed to discrimination performance (see Test procedure). In an attempt to ensure that the different enantiomeric odor pairs were of approximately equal strength when presented in squeeze bottles, intensity matching was performed by a panel of six subjects adopting a standardized psychophysical procedure (ASTM, 1975).

Table 1 Substances and concentrations used (g/l)

Substance	Concentration
R-(–)-Carvone	96.0
S-(+)-Carvone	96.0
S-(–)-Limonene	16.9
R-(+)-Limonene	16.9
(–)-Dihydrocarvone	92.9
(+)-Dihydrocarvone	92.9
(–)-Dihydrocarveol	92.6
(+)-Dihydrocarveol	92.6
(–)-Dihydrocarvyl acetate	94.8
(+)-Dihydrocarvyl acetate	94.8
(–)-Perillaaldehyde	19.7
(+)-Perillaaldehyde	19.7
(–)-Perillaalcohol	95.8
(+)-Perillaalcohol	95.8
(–)-Isopulegol	30.4
(+)-Isopulegol	30.4
(–)-Limonene oxide	19.3
(+)-Limonene oxide	19.3

Test procedure

A 40 ml aliquot of each odorant was presented in a 250 ml polyethylene squeeze bottle equipped with a flip-up spout which for testing was fitted with a hand-made Teflon nose-piece. Subjects were instructed as to the manner of sampling and at the start of the first session were allowed time to familiarize themselves with the bottles and the sampling technique. Care was taken that the nose-piece was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to allow the stimulus to enter both nostrils.

In a forced-choice triangular test procedure 20 subjects were asked to compare three bottles and to identify the one containing the odd stimulus. Additionally, after each decision, subjects were asked whether their choice was predominantly based on perceived differences in odor quality or on perceived differences in odor intensity. Each

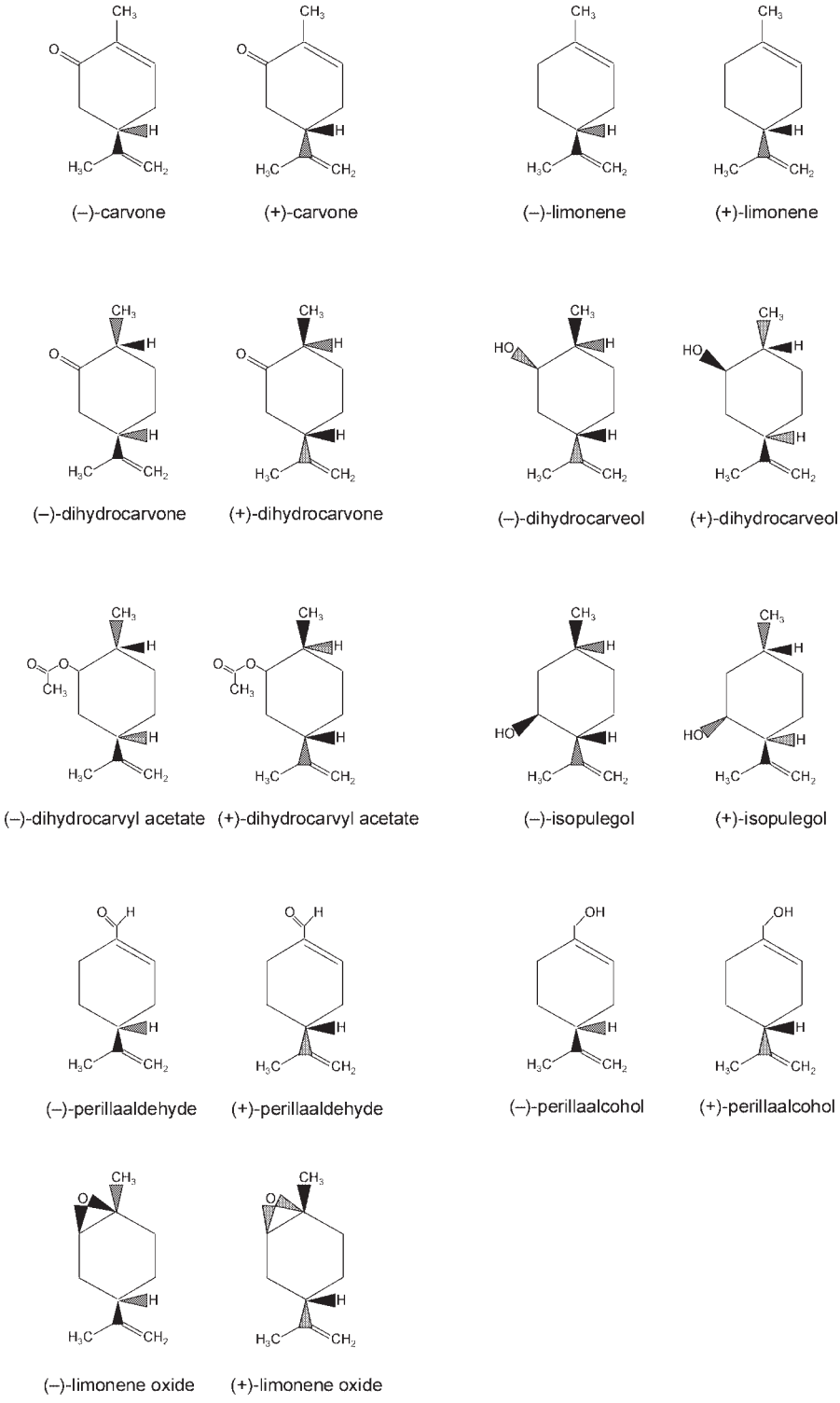

Figure 1 Molecular structures of the nine enantiomeric odor pairs.

bottle could be sampled twice, with an inter-stimulus interval of at least 10 s. Sampling duration was restricted to 1 s per presentation in order to minimize adaptation effects. The sequence of presenting the stimulus pairs was systematically varied between sessions and individual subjects while taking care that the presentation of a given odorant as odd or even stimulus was balanced within and between sessions. In order to control for possible cross-adaptation effects, the order in which the stimuli of a given triad were sampled was systematically varied between sessions.

Approximately 30 s was allowed between trials and no feedback regarding the correctness of the subjects' choice was given.

The nine stimulus pairs were presented twice per session and testing was repeated in four more sessions each 1–3 days apart, enabling 10 judgements per stimulus pair and panelist to be collected.

Data analysis

The criterion for an individual subject to be regarded as capable of discriminating a given odor pair was set at seven or more out of 10 decisions correct (two-tailed binomial test, $P < 0.05$). Accordingly, the criterion for the group of subjects to be regarded as capable of discriminating a given odor pair was set at 12 or more out of 20 subjects performing significantly above chance (two-tailed binomial test, $P < 0.05$).

Comparisons of group performance across tasks or sessions were made using the Friedman two-way analysis of variance (ANOVA). When ANOVA detected differences between tasks, this was then followed by pairwise Wilcoxon signed-rank tests for related samples to evaluate which tasks were responsible (Siegel and Castellan, 1988). All data are reported as means ± SD.

Results

Figure 2 summarizes the mean performance of 20 subjects in discriminating between the nine enantiomeric odor pairs. As a group, the human subjects performed significantly above chance in five tasks—involving the discrimination of the enantiomers of limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate—whereas they failed to do so with the four other tasks. However, the distribution of group discrimination scores across tasks suggests a continuum of performance rather than a clear-cut division between odor pairs that were discriminable and odor pairs that were not. Accordingly, application of less conservative statistics would suggest that all odor pairs were discriminated at the group level.

Interindividual variability was high, particularly in tasks that were not significantly discriminated at the group level (see SDs in Figure 2). However, ANOVA detected significant differences in the group's performance between tasks (Friedman two-way ANOVA, $P < 0.001$) and subsequent pairwise tests revealed that the enantiomers of perillaalcohol, perillaaldehyde, isopulegol and limonene oxide were significantly more difficult to discriminate compared to limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate (Wilcoxon, $P < 0.01$). Accordingly, 12–17 out of 20 subjects failed to significantly distinguish between the antipodes of the former group of substances, whereas only 2–4 out of 20 subjects were unable to discriminate the enantiomers of the latter group of substances.

With only one exception (limonene versus dihydrocarvone), discrimination scores within these two groups of substances did not differ significantly from each other (Wilcoxon, $P > 0.05$).

Figure 3 shows the distribution of individual performance in discriminating between the nine enantiomeric odor pairs. The percentage of errors ranged from 12% for the best-performing subject up to 47%. Accordingly, the best panelists were able to significantly distinguish eight out of nine enantiomeric odor pairs whereas the poorest-performing subjects failed to do so with six out of nine tasks.

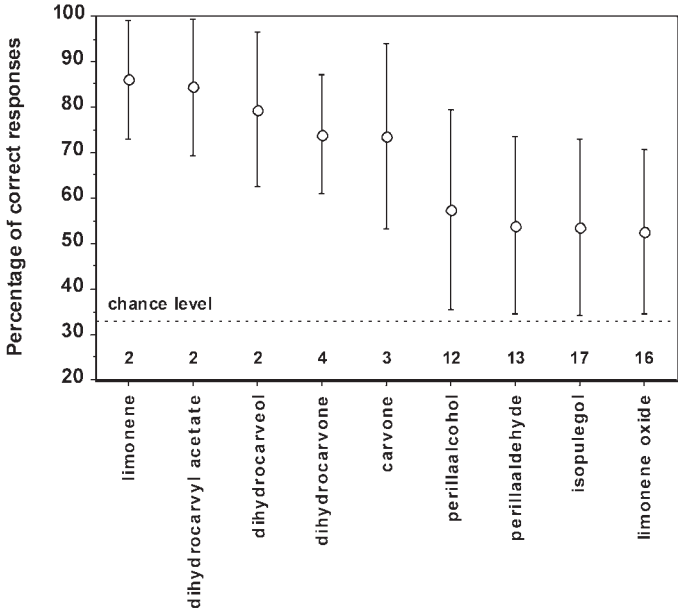

Figure 2 Performance of 20 subjects in discriminating between nine pairs of enantiomers. Each data point represents the percentage (means ± SD) of correct choices from 10 decisions per odor pair and subject. The figures above the abscissa indicate the number of subjects that failed to perform significantly above chance in the corresponding task.

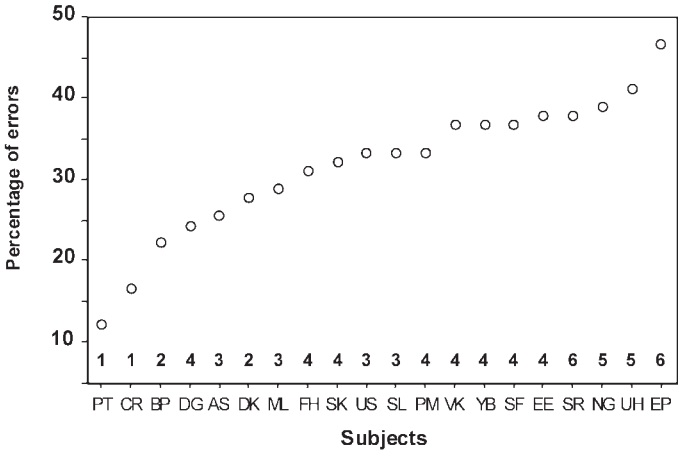

Figure 3 Distribution of individual performance in discriminating between nine pairs of enantiomers. Each data point represents the percentage of errors from 90 decisions per subject. The figures above the abscissa indicate the number of odor pairs that a subject failed to discriminate significantly above chance.

Nevertheless, the across-task patterns of performance were very similar between subjects with the majority of individuals scoring better with limonene and dihydrocarvyl acetate compared to the other tasks.

It is interesting to note, however, that there were also cases in which some subjects did better with one odor pair relative to another odor pair, whereas other subjects showed the reverse pattern of performance.

Figure 4 shows the mean performance of the 20 subjects across the five test sessions. Error rates were quite stable and did not differ significantly between sessions (Friedman two-way ANOVA, $P > 0.05$) and thus no significant learning or training effects at the group level were found.

With all nine odor pairs, <10% of decisions were reported to be based upon perceived differences in odor intensity rather than odor quality (see Test procedure). The five enantiomeric odor pairs that were significantly discriminated at the group level yielded the lowest percentages of perceived intensity as the choice criterion, with 4.5% (for carvone) to 9.0% (for dihydrocarvone), whereas the percentages with the four odor pairs that were not significantly distinguished at the group level ranged from 14.0% (for limonene oxide) to 16.0% (for perillaaldehyde). Thus, a significant negative correlation between discriminability of the enantiomeric odor pairs and the frequency of perceived differences in odor intensity as the choice criterion was found (Spearman, $r = -0.73$; $P < 0.05$).

With none of the nine odor pairs did discriminability differ as a function of whether the (+)-form or the (–)-form of an odorant was presented as the odd stimulus in a given triad (Wilcoxon, $P > 0.05$ for all pairs).

Experiment 2: chemesthetic potency of enantiomers

The results of experiment 1 showed that human subjects are able to discriminate between the enantiomers of limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate when presented at equal concentrations. In order to elucidate whether the nasal trigeminal system (or other systems mediating chemesthesis) contributed to this performance, I assessed whether the antipodes of the latter three substances differ in their chemesthetic potency by testing subjects' ability to localize the side of monorhinal stimulation. This simple method has been shown adequate to reliably quantify the chemesthetic impact of odorants (Berg *et al.*, 2000). Carvone and limonene were not included in this experiment as discriminability of both enantiomeric odor pairs has already been shown not to be mediated by chemesthetic information (Laska and Teubner, 1999a).

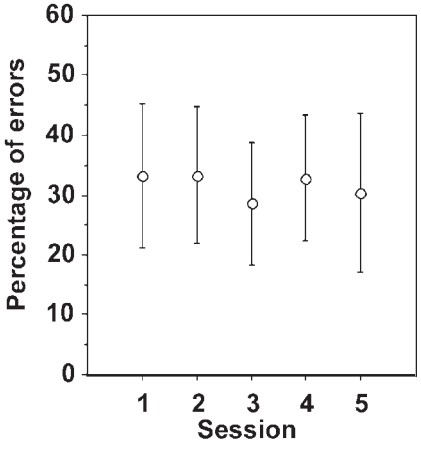

Figure 4 Performance of 20 subjects across the five test sessions in experiment 1. Each data point represents the percentage (means ± SD) of errors from 18 decisions per subject.

Materials and methods

Subjects

Ten healthy, unpaid volunteers (nine females and one male), 22–37 years of age, participated in the study. All subjects had already participated in experiment 1.

Odorants

A set of six odorants comprising the enantiomers of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate was used (Table 1). The substances were diluted using diethyl phthalate (Merck) as the solvent to the same concentrations as in experiment 1.

Test procedure

Using a custom-made squeezer, air from two 250 ml polyethylene squeeze bottles was applied to the right and the left nostril of a subject. One bottle contained 40 ml of an odorant whereas the other bottle contained 40 ml of the odorless solvent. Both bottles were equipped with a flip-up spout which for testing was fitted with a hand-made Teflon nose-piece. Care was taken that the nose-pieces were in direct contact with the nostrils during sampling in order to ensure that each stimulus entered one nostril only. Presentation of an odorant was synchronized with a subject's inhalation and the squeezer was calibrated to deliver 20 ml of air to each nostril.

In a forced-choice test procedure 10 subjects were asked to identify the side of stimulation with an odorant. The sequence of presenting the stimuli was systematically varied between sessions and individual subjects while taking care that the presentation of a given odorant to the left or the right nostril was balanced within and between sessions. Approximately 30 s was allowed between trials and no feedback regarding the correctness of the subjects' choice was given. The six stimuli were presented five times per session and testing was repeated in three more sessions, each 1–3 days apart, enabling 20 judgements per stimulus and panelist to be collected.

Data analysis

The criterion for an individual subject to be regarded as capable of localizing the side of monorhinal stimulation with a given odorant was set at 14 or more out of 20 decisions correct (two-tailed binomial test, $P < 0.05$). Accordingly, the criterion for the group of subjects to be regarded as capable of localizing a given odorant was set at eight or more out of 10 subjects performing significantly above chance (two-tailed binomial test, $P < 0.05$).

Comparisons of group performance across sessions were made using the Friedman two-way ANOVA, and comparisons of group performance between tasks involving the antipodes of a given substance were made using the Wilcoxon signed-rank test for related samples (Siegel and Castellan, 1988). All data are reported as means ± SD.

Results

Figure 5 summarizes the mean performance of 10 subjects in localizing the side of monorhinal stimulation with the enantiomers of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate when presented at the same concentrations as in experiment 1.

As a group, the human subjects failed to perform significantly above chance in all six tasks, with between nine and 10 out of 10 individuals not reaching the criterion of at least 14 out of 20 decisions correct.

Interindividual variability was low (see SDs in Figure 5) and altogether there were only two cases of individual subjects scoring 70% correct choices (corresponding to a 5% level of significance), one with (+)-dihydrocarveol and one with (–)-dihydrocarvyl acetate.

Pairwise comparisons of performance between the two antipodes of a substance revealed that the enantiomers of dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate, respectively, did not differ significantly in their chemesthetic potency at the concentrations tested (Wilcoxon, $P > 0.10$).

Figure 6 shows the distribution of individual performance in localizing the side of monorhinal stimulation with the (+)- and (–)-forms of dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate. The percentage of correct choices ranged from 57% for the best-performing subject to 38%. Even the two best panelists were only able to significantly localize the side of stimulation with one out of six enantiomers at a 5% level of significance whereas all other subjects failed to do so with all six tasks.

Figure 7 shows the mean performance of the 10 subjects across the four test sessions. Localization scores were quite stable and did not differ significantly between sessions

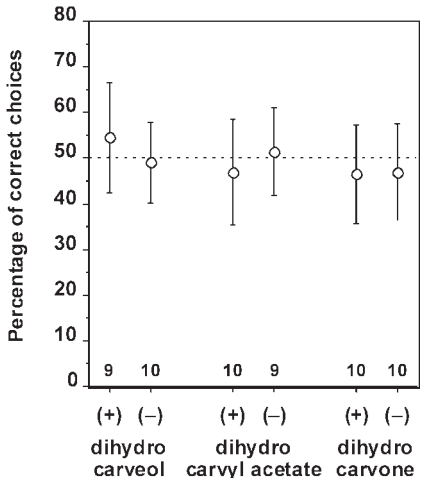

Figure 5 Performance of 10 subjects in correctly localizing the side of monorhinal stimulation. Each data point represents the percentage (means ± SD) of correct choices from 20 decisions per odor and subject. The figures above the abscissa indicate the number of subjects that failed to perform significantly above chance in the corresponding task.

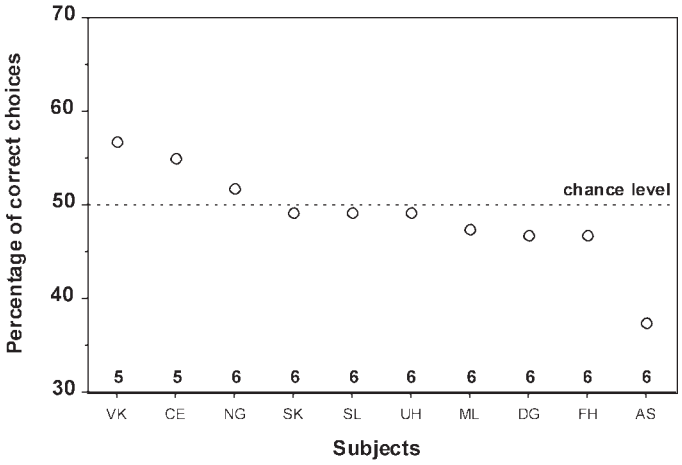

Figure 6 Distribution of individual performance in correctly localizing the side of monorhinal stimulation with the enantiomers of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate. Each data point represents the percentage of correct choices from 120 decisions per subject. The figures above the abscissa indicate the number of odors that a subject failed to correctly localize significantly above chance.

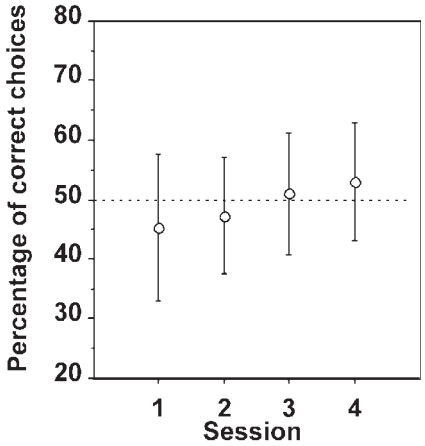

Figure 7 Performance of 10 subjects across the four test sessions in experiment 2. Each data point represents the percentage (means ± SD) of errors from 30 decisions per subject.

(Friedman two-way ANOVA, $P > 0.05$) and thus no significant learning or training effects at the group level were found.

Experiment 3: Detection thresholds of enantiomers

The results of experiment 2 showed that the nasal trigeminal system (or other systems mediating chemesthesis) is unlikely to contribute to the ability of human subjects to discriminate between the enantiomers of dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate at the concentrations tested. In order to get a further indication of whether differences in perceived intensity rather than quality of the odorants contributed to the discrimination performance—despite the subjects' self-reports in experiment 1, which suggest this not to be the case—I determined olfactory detection thresholds for the optical isomers of these three substances. Here, too, carvone and limonene were not included in this experiment as an earlier study showed that sensitivity for the antipodes of both substances did not to differ significantly from each other (Laska and Teubner, 1999a).

Materials and methods

Subjects

Ten healthy, unpaid volunteers (eight females and two males), 22–37 years of age, participated in the study. All subjects had already participated in experiment 1 and/or in experiment 2.

Odorants

A set of six odorants comprising the enantiomers of dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate was used (Table 1). For each stimulus, a geometric dilution series using diethyl phthalate (Merck) as the solvent was prepared starting at a concentration of 9.3 g/l (for dihydrocarvone and dihydrocarveol) and 9.5 g/l (for dihydrocarvyl acetate), and progressing by a factor of 5. Stem dilutions were designated step 1, and subsequent dilutions step 2, 3 and so forth.

Test procedure

A 40 ml aliquot of each odorant was presented in a 250 ml polyethylene squeeze bottle equipped with a flip-up spout which for testing was fitted with a hand-made Teflon nose-piece. Bottles containing the pure diluent served as blanks. Subjects were instructed as to the manner of sampling and at the start of the first session were allowed time to familiarize themselves with the bottles and the sampling technique. Care was taken that the nose-piece was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to allow the stimulus to enter both nostrils.

Detection thresholds were determined using a triangular test procedure in which panelists were presented with three randomly arranged bottles, two of which contained pure diluent and the third the stimulus (Laska and Hudson, 1991; Laska and Teubner, 1999a). In order to minimize adaptation effects, testing followed an ascending staircase procedure. At the first testing, stimuli were presented two concentration steps below the investigator's threshold and in subsequent sessions one concentration step below the threshold previously determined for the panelist.

Each bottle could be sampled twice per trial, with an interstimulus interval of at least 10 s. Sampling duration was restricted to 1 sec per presentation in order to minimize adaptation effects. Panelists were required to decide whether there was no difference between the bottles or identify one as containing the stimulus. In the case of 'no difference', testing proceeded to the next dilution step; otherwise, the bottles were rearranged and the panelist allowed to sample a second time. If both choices were correct, this was provisionally recorded as the threshold dilution. However, if these had been preceded by one correct and one incorrect choice, the previous dilution was again tested, and if both choices were then correct this was taken as threshold. In this way, thresholds for the six odorants were determined for each panelist. Testing was repeated in two more sessions, each 1–3 days apart, taking care to systematically vary the order in which the six odorants were presented across sessions.

Data analysis

Comparisons of group performance across sessions were made using the Friedman two-way ANOVA. When ANOVA detected differences between tasks, this was then followed by pairwise Wilcoxon signed-rank tests for related samples to evaluate which sessions were responsible. Comparisons of group performance between tasks involving the antipodes of a given substance were made using the Wilcoxon signed-rank test for related samples (Siegel and Castellan, 1988). All data are reported as means ± SD.

Results

Figure 8 shows the mean detection thresholds of 10 subjects for each of the six odorants tested across three sessions. With all six odorants, threshold values differed significantly across sessions (Friedman two-way ANOVA $P < 0.05$), and accordingly a significant increase in performance from the first to the third session was found with all six odorants (Wilcoxon signed-rank test, $P < 0.05$).

Interindividual variability was comparatively low as can be inferred from the SDs in Figure 8 which ranged from 0.70 dilution steps (i.e. a factor of 3) for (–)-dihydrocarveol in session 1 to 2.11 dilution steps (i.e. a factor of 30) for (+)-dihydrocarvyl acetate in session 3.

Detectability of the (+)- and the (–)-form of dihydrocarveol did not differ significantly from each other in any session (Wilcoxon signed-rank test, $P > 0.05$). The same is true for the antipodes of dihydrocarvone. Detectability of the enantiomers of dihydrocarvyl acetate was found to differ significantly in one of the three sessions, with the (–)-form

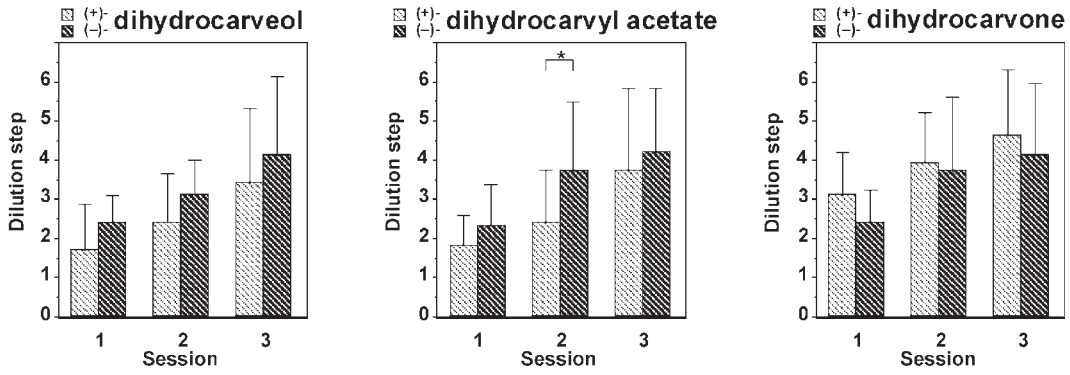

Figure 8 Detection thresholds for the enantiomers of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate. Means ± SD ($n = 10$ subjects) for each of the three test sessions are given. Significant differences in performance within a given session are indicated by asterisks with $*P < 0.05$ (Wilcoxon signed-rank test).

yielding lower threshold values compared to the (+)-form (Wilcoxon signed-rank test, $P < 0.05$ in session 2).

Discussion

The results of this study demonstrate that the ability of human subjects to discriminate between enantiomeric odor pairs sharing an isopropenyl group at the chiral center is substance-specific and thus not a generalizable phenomenon. Whereas almost all subjects had little difficulty in distinguishing the (+)- and the (–)-forms of limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate, most panelists failed to discriminate between the antipodes of perillaalcohol, perillaaldehyde, isopulegol and limonene oxide when presented at equal concentrations.

Prior to a discussion of these findings it seems appropriate to consider whether the performance of the human subjects shown in the present study was indeed based on the ability of the olfactory system to discern between the odor qualities of some optical isomers or whether other sensory systems or other talents of the olfactory system may have been involved.

It is well-established that both the olfactory and trigeminal systems contribute to the perception of the majority of odorants (Doty and Cometti-Muñiz, 2003). This raises the possibility that the nasal trigeminal system (or other systems mediating chemesthesis) might have contributed to the discrimination of the enantiomers of limonene, carvone, dihydrocarvone, dihydrocarveol and dihydrocarvyl acetate, a possibility which is supported by the finding that congenitally anosmic subjects possess at least a coarse ability to distinguish between odorants using sensory information provided by their fifth cranial nerve (Laska *et al.*, 1997). The results of experiment 2 and of an earlier study that employed limonene and carvone (Laska and Teubner, 1999a), however, strongly suggest that the substances used here had only little, if any, chemesthesis-stimulating properties at the concentrations tested and that in any case the antipodes of a given substance did not differ in their chemesthetic potency. Thus, the possibility of involvement of chemesthesis in the discrimination of the five enantiomeric odor pairs in question can be excluded.

The possibility that differences in perceived odor intensity might have contributed to the discrimination performance seems also quite unlikely as >90% of the subjects' decisions involving the five odor pairs that were significantly discriminated at the group level in experiment 1 were reported to be based on perceived differences in odor quality rather than odor intensity (see Test procedure). Further, the comparatively few instances in which perceived differences in odor intensity were reported seem to reflect a subjects' difficulty to discriminate at all, as error rates in such cases tended to be higher compared to the regular case of reported differences in odor quality. The same tendency for higher error rates with reports of perceived differences in odor intensity rather than odor quality as a choice criterion has been found in studies assessing the discriminability of members of homologous series of aliphatic (Laska and Teubner, 1998, 1999b; Laska *et al.*, 2000; Laska and Hübener, 2001) and aromatic substances (Laska, 2002). The results of experiment 3 lend additional support to the assumption that possible differences in odor intensity did not contribute to discrimination performance as detection thresholds for the antipodes of dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate, respectively, did not differ significantly from each other (see Figure 8).

Taken together, the results of experiments 2 and 3 suggest the discrimination scores found with carvone, limonene, dihydrocarvone, dihydrocarveol, and dihydrocarvyl acetate to reflect the ability of the human olfactory system to distinguish the odor qualities of these enantiomeric odor pairs. Nevertheless, the possibility that trace impurities of the substances used might also have contributed to discrimination performance should be considered and future studies testing discriminability of enantiomeric odor pairs should try to establish their purity.

The finding of the present study that the presence of an isopropenyl group at the chiral carbon atom is not sufficient for enantiomeric odor pairs to be generally discriminated is

Table 2 Discrimination performance with and structural features of the nine enantiomeric odor pairs

Odor pair	Discrimination performance	Methyl group at para-position	Oxygen-containing functional group at			Additional chiral center(s)
			Ortho	Meta	Para	
Carvone	+	+	–	+	–	–
Limonene	+	+	–	–	–	–
Dihydrocarvone	+	+	–	+	–	+
Dihydrocarveol	+	+	–	+	–	+
Dihydrocarvyl acetate	+	+	–	+	–	+
Perillaaldehyde	–	–	–	–	+	–
Perillaalcohol	–	–	–	–	+	–
Isopulegol	–	+	+	–	–	+
Limonene oxide	–	+	–	–	–	+

With regard to discrimination performance, a '+' indicates that the corresponding odor pair was discriminated at the group level and a '–' indicates failure to do so. With regard to the other columns, a '+' indicates the presence and a '–' the absence of the corresponding structural feature.

in line with the mulitpoint attachment theory (Ohloff, 1994). This theory predicts that the interaction of an odor molecule with an olfactory receptor is a process that involves at least two, and probably many more, dipole-dipole interactions or hydrogen bonds (Afshar *et al.*, 1998; Chastrette and Rallet, 1998).

In order to explain the present results it therefore seems reasonable to consider additional structural features of the enantiomers tested here. Table 2 summarizes some of their molecular properties. It is apparent that all five optical isomers that were discriminated at the group level share a methyl group at the para-position, i.e. at the opposite side of the ring structure relative to the chiral carbon atom bearing the isopropenyl group (see Figure 1). However, two of the four enantiomeric odor pairs that were not discriminated (isopulegol and limonene oxide) also show this structural feature. Four of the five discriminable pairs of optical isomers additionally share an oxygen-containing functional group at the meta-position, i.e. at the carbon atom of the ring structure adjacent to the para-position, whereas none of the enantiomeric odor pairs that the human subjects failed to distinguish shows this feature (see Figure 1). The presence or absence of additional chiral centers, on the other hand, does not appear to affect discriminability in a regular manner as three out of five discriminable and two out of four non-discriminable enantiomeric odor pairs have more than one carbon atom with four different ligands (see Figure 1). Thus, it appears that the combined presence of (i) an isopropenyl group at the chiral center; (ii) a methyl group at the para-position; and/or (iii) an oxygen-containing group at the meta-position allow for the discrimination of enantiomeric odor pairs. In order to corroborate this supposition, future studies should assess discrimination performance with other pairs of optical isomers sharing one or more of these molecular features.

It is commonly agreed that both the type and position of functional group(s) are important determinants of the quality, and concomitantly of the discriminability, of odorants (Rossiter, 1996; Uchida *et al.*, 2000). With regard to odorants bearing a ring-like structure, Mori *et al.* (1999) have shown that certain mitral cells of the rabbit olfactory bulb respond selectively to disubstituted benzenes sharing a functional group at the para-, but not at the meta- or ortho-position. Other mitral cells, on the contrary, were found to respond selectively to a certain type of functional group, irrespective of its position at the benzene ring (Mori *et al.*, 1999). Buchbauer and Shafii-Tabatabai (2003) reported the qualitative attributes of cyclic enantiomers as perceived by human subjects to depend on both an odorant's chirality and the type of functional group(s) at carbon atoms other than the chiral center, which is in agreement with the present findings.

In conclusion, enantiomers appear to be valuable tools to investigate the olfactory primary process, and, in particular, to assess how molecular structure is encoded by the olfactory system finally leading to discriminable odor qualities. Whereas perceptual differences between non-enantiomeric odorants can be partially due to properties such as differing diffusion rates in the mucus or differing air/mucus partition coefficients (Hahn *et al.*, 1994), 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. Therefore, the systematic assessment of the discriminability of sets of enantiomeric odor pairs that share certain molecular features and differ from each other in only one structural property may contribute to our understanding of odor quality coding.

Acknowledgements

I thank my panelists for their willingness to participate in this study, and the Deutsche Forschungsgemeinschaft for financial support (La 635/10-2).

References

Afshar, M., Hubbard, R.E. and **Demaille, J.** (1998) *Towards structural models of molecular recognition in olfactory receptors*. Biochimie, 80, 129–135.

ASTM (1975) *Standard recommended practice for referencing suprathreshold odor intensity*. Am. Soc. Testing Materials (Phil.) E, 544–575.

Berg, J., Hummel, T., Huang, G. and **Doty, R.L.** (2000) *Trigeminal impact of odorants assessed with lateralized stimulation.* Chem. Senses, 25, 793–794.

Boelens, M.H., Boelens, H. and **van Gemert, L.J.** (1993) *Sensory properties of optical isomers.* Perf. Flavor., 18, 1–16.

Brenna, E., Fuganti, C. and **Serra, S.** (2003) *Enantioselective perception of chiral odorants.* Tetrahedron Asym., 14, 1–42.

Buchbauer, G. and **Shafii-Tabatabai, A.** (2003) *Enones of (+)- and (–)-3-pinanones: influence of chirality on flavour.* Flavour Fragr. J., 18, 441–445.

Buck, L. and **Axel, R.** (1991) *A novel multigene family may encode odorant receptors*. Cell, 65, 175–187.

Caldwell, J. and **Hutt, A.J.** (1996) Chirality and Analgesia. Adis, Auckland.

Chastrette, M. and **Rallet, E.** (1998) *Structure–minty odour relationships: suggestion of an interaction pattern*. Flavor Fragr. J., 13, 5–18.

Corwin, J. (1992) *Assessing olfaction: cognitive and measurement issues.* In Serby, M.J. and Chobor, K.L. (eds), Science of Olfaction. Springer, Berlin, pp. 335–354.

Doty, R.L. and **Cometti-Muñiz, J.E.** (2003) *Trigeminal chemosensation.* In Doty, R.L. (ed.), Handbook of Olfaction and Gustation, 2nd ed. Marcel Dekker, New York, pp. 981–999.

Faber, K. and **Griengl, H.** (1991) *Chirality in organic synthesis: the use of biocatalysts.* In Janoschek, R. (ed.), Chirality: From Weak Bosons to the Alpha-helix. Springer, Berlin, pp. 103–139.

Hahn, I., Scherer, P.W. and **Mozell, M.M.** (1994) *A mass transport model of olfaction.* J. Theor. Biol., 167, 115–128.

Hildebrand, J.G. and **Shepherd, G.M.** (1997) *Mechanisms of olfactory discrimination: Converging evidence for common principles across phyla*. Annu. Rev. Neurosci., 20, 595–631.

Kraft, P. and **Frater, G.** (2001) *Enantioselectivity of the musk odor sensation.* Chirality, 13, 388–394.

Laska, M. (2002) *Olfactory discrimination ability for aromatic odorants as a function of oxygen moiety*. Chem. Senses, 27, 23–29.

Laska, M. and **Hudson, R.** (1991) *A comparison of the detection thresholds of odour mixures and their components*. Chem. Senses, 16, 651–662.

Laska, M. and **Hübener, F.** (2001) *Olfactory discrimination ability for homologous series of aliphatic ketones and acetic esters*. Behav. Brain Res., 119, 193–201.

Laska, M. and **Teubner, P.** (1998) *Odor structure–activity relationships of carboxylic acids correspond between squirrel monkeys and humans*. Am. J. Physiol., 274, R1639–R1645.

Laska, M. and **Teubner, P.** (1999a) *Olfactory discrimination ability of human subjects for ten pairs of enantiomers*. Chem. Senses, 24, 161–170.

Laska, M. and **Teubner, P.** (1999b) *Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes*. Chem. Senses, 24, 263–270.

Laska, M., Distel, H. and **Hudson, R.** (1997) *Trigeminal perception of odorant quality in congenitally anosmic subjects*. Chem. Senses, 22, 447–456.

Laska, M., Liesen, A. and **Teubner, P.** (1999) *Enantioselectivity of odor perception in squirrel monkeys and humans.* Am. J. Physiol., 277, R1098–R1103.

Laska, M., Ayabe-Kanamura, S., Hübener, F. and **Saito, S.** (2000) *Olfactory discrimination ability for aliphatic odorants as a function of oxygen moiety.* Chem. Senses, 25, 189–197.

Mori, K. (1996) *Molecular asymmetry and pheromone science.* Biosci. Biotechnol. Biochem., 60, 1925–1932.

Mori, K., Nagao, H. and **Yoshihara, Y.** (1999) *The olfactory bulb: coding and processing of odor molecule information*. Science, 286, 711–715.

Nandi, N. (2003) *Molecular origin of the recognition of chiral odorant by chiral lipid: interaction of dipalmitoyl phosphatidyl choline and carvone*. J. Phys. Chem. A, 107, 4588–4591.

Ohloff, G. (1994) *Scent and fragrances. The fascination of odors and their chemical perspectives*. Springer, Berlin.

Pickenhagen, W. (1989) *Enantioselectivity in odor perception.* In Teranishi, R., Buttery, R.G. and Shahidi, F. (eds), Flavor Chemistry. Trends and Developments. American Chemical Society, Washington, ACS Symposium Series 388, pp. 151–157.

Rossiter, K.J. (1996) *Structure–odor relationships.* Chem. Rev., 96, 3201–3240.

Shallenberger, R.S. (1993) Taste Chemistry. Chapman & Hall, London.

Siegel, S. and **Castellan, N.J.** (1988) Nonparametric Statistics for the Behavioral Sciences. McGraw Hill, New York.

Simmons, D.P., Reichlin, D., Skuy, D. and **Margot, C.** (1992) *Stereoselectivity of odor perception : odorless enantiomers of strong perfumes.* Chem. Senses, 17, 881.

Theimer, E.T., Yoshida, T. and **Klaiber, E.M.** (1977) *Olfaction and molecular shape: chirality as a requisite for odor.* J. Agric. Food Chem., 25, 1168–1177.

Uchida, N., Takahashi, Y.K., Tanifuji, M. and **Mori, K.** (2000) *Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features*. Nat. Neurosci., 3, 1035–1043.

Wise, P.M., Olsson, M.J. and **Cain, W.S.** (2000) *Quantification of odor quality.* Chem. Senses, 25, 429–443.

Accepted December 17, 2003