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Olfactory Discrimination Ability of Human Subjects for Ten Pairs of Enantiomers

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Abstract

We tested the ability of human subjects to distinguish between enantiomers, i.e. odorants which are identical except for chirality. In a forced-choice triangular test procedure 20 subjects were repeatedly presented with 10 enantiomeric odor pairs and asked to identify the bottle containing the odd stimulus. We found (i) that as a group, the subjects were only able to significantly discriminate the optical isomers of α-pinene, carvone and limonene, whereas they failed to distinguish between the (+)- and (–)-forms of menthol, fenchone, rose oxide, camphor, α-terpineol, β-citronellol and 2-butanol; (ii) marked individual differences in discrimination performance, ranging from subjects who were able to significantly discriminate between 6 of the 10 odor pairs to subjects who failed to do so with 9 of the 10 tasks; (iii) that with none of the 10 odor pairs were the antipodes reported to differ significantly in subjective intensity when presented at equal concentrations; and (iv) that error rates were quite stable and did not differ significantly between sessions, and thus, we observed a lack of learning or training effects. Additional tests of the degree of trigeminality and threshold measurements of the optical isomers of α-pinene, carvone and limonene suggest that the discriminability of these three enantiomeric odor pairs is indeed due to differences in odor quality. These findings support the assumption that enantioselective molecular odor receptors may only exist for some but not all volatile enantiomers and thus that chiral recognition of odorants may not be a general phenomenon but is restricted to some substances.

Introduction

Chiral recognition of substances, i.e. the ability to distinguish a molecular structure from its mirror image, is one of the most important and widespread principles of biological activity (Holmstedt *et al.*, 1990). Discrepant enantiomer effects are well-established, with numerous examples in drug effectiveness (e.g. Caldwell, 1996), taste perception (e.g. Siertsema *et al.*, 1998) and insect chemical communication (e.g. Silverstein, 1979).

The first molecular event in odor perception is the interaction of an odorant with a receptor. As olfactory receptors have been identified as proteins, i.e. chiral molecules (Buck and Axel, 1991; Hildebrand and Shepherd, 1997), 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 odor strength and/or quality (Pickenhagen, 1989).

A variety of optical isomers have been described as having different odor qualities and/or different odor intensities for humans (e.g. Ohloff, 1994), although the number of cases reported in which the differences are small seems inconsistent with the large differences found in other biological interactions between body tissues and dextro- and levoforms of the same compounds. There are also reports of identically smelling enantiomeric odor pairs (Theimer *et al.*, 1977) which seem inconsistent with the assumption that optically active 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 (Simmons *et al.*, 1992).

Most of the studies reporting qualitative and/or quantitative differences between enantiomers, however, have employed odor profiling or scaling procedures which are presumed to be particularly susceptible to cognitive influences (Corwin, 1992). Surprisingly few studies, on the other hand, have directly tested the discriminability of chiral odorants, although this method largely avoids the disadvantages of comparatively poor resolution, subjectivity, likely context dependence and semantic ambiguity (Cain and Olsson, 1995). Even fewer studies using discrimination procedures have assessed whether inter- or intraindividual variability in discrimination performance rather than perceptual differences between antipodes may at least partly account for the sometimes widely differing findings with the

same chiral odor pairs. Further, studies on discriminability of enantiomers have so far largely been restricted to testing the ability of subjects to distinguish between (+)- and (–)-carvone (Jones and Velasquez, 1974; Pike *et al.*, 1987, 1988; Cowart, 1990; Hormann and Cowart, 1993), one of the first substances for which both chiral isomers could be synthesized selectively and with high purity rather than extracted from plant matter, thereby excluding the possibility of trace impurities as a source of qualitative differences (Friedman and Miller, 1971; Russell and Hills, 1971).

To the best of our knowledge, only one study so far has investigated the discrimination performance of humans for an array of enantiomeric odorants (Jones and Elliot, 1975). Unfortunately, the authors of this study reported only the total number of correct discriminations pooled from all their subjects—drawing statistically invalid conclusions as to discriminability of a given chiral odor pair due to an inflated number of observations—and gave only cursory information with regard to inter- or intraindividual variability of performance.

Given the continuing uncertainty in the field of chiral recognition of odorants and the possible importance of enantioselectivity for our understanding of the molecular mechanisms underlying the interaction between odor stimulus and olfactory receptor, we decided to test the ability of human subjects to distinguish between 10 pairs of enantiomers.

Experiment 1: discrimination of enantiomers

In this experiment, we assessed the ability of human subjects to distinguish between 10 enantiomeric odor pairs. Substances were chosen on the basis of earlier studies which reported qualitative attributes of antipodes to range from 'identical' to 'very different', allowing us to (i) present odor pairs presumed to differ in their degrees of perceptual similarity and thus discriminability and (ii) test whether reported differences in qualitative attributes assigned to substances predict discriminability.

Materials and methods

Subjects

Twenty healthy, unpaid volunteers (14 females and 6 males), 22–37 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 about the aim of the experiment and provided written consent. The study was performed in accordance with the Declaration of Helsinki/Hong Kong.

Table 1 Substances and concentrations used (g/l)

	Substance	Conc.	Odor quality*
1.	(1R, 2S, 5R)-(–)-menthol[a]	66.7	peppermint, strong cooling
2.	(1S, 2R, 5S)-(+)-menthol[a]	66.7	peppermint, less cooling
3.	(1R)-(–)-α-pinene[b]	86.0	pine-like
4.	(1S)-(+)-α-pinene[b]	86.0	pine-like
5.	R-(–)-carvone[b]	96.0	spearmint
6.	S-(+)-carvone[b]	96.0	caraway
7.	S-(–)-limonene[c]	421.5	turpentine
8.	R-(+)-limonene[c]	421.5	orange
9.	(–)-camphor[c]	133.3	camphoraceous
10.	(+)-camphor[c]	133.3	camphoraceous
11.	(–)-β-citronellol[c]	85.4	geranium oil-type
12.	(+)-β-citronellol[c]	85.4	citronella oil-type
13.	(–)-fenchone[c]	94.5	camphoraceous, sweet
14.	(+)-fenchone[c]	94.5	camphoraceous, sweet
15.	(–)-α-terpineol[c]	311.6	tarry, cold pipe
16.	(+)-α-terpineol[c]	311.6	flowery, lilac
17.	(–)-rose oxide[c]	87.3	green, herbal
18.	(+)-rose oxide[c]	87.3	green, sweet
19.	R-(-)–2-butanol[c]	268.6	oily-vinous
20.	S-(+)–2-butanol[c]	268.6	oily-vinous

Obtained from [a]Aldrich, [b]Merck, [c]Fluka. *According to Ohloff (1994).

Odorants

A set of 20 odorants comprising 10 pairs of enantiomers was used (Table 1). All substances had a nominal purity of at least 99%. 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 (cf. 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).

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 handmade 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 to ensure 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 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 ensuring 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. The inter-trial interval was ~30 s and no feedback regarding the correctness of the subjects' choice was given.

The 10 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 7 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. 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 1 summarizes the mean performance of 20 subjects in discriminating between the 10 enantiomeric odor pairs. As a group, the human subjects performed significantly above chance in only three tasks—involving the discrimination of the enantiomers of α-pinene, carvone and limonene—whereas they failed to do so with the seven other tasks.

Interindividual variability was high, particularly in tasks that were not significantly discriminated at the group level (cf. SDs in Figure 1). However, ANOVA detected significant differences in the group's performance between tasks (Friedman, $P < 0.001$) and subsequent pairwise tests revealed that the enantiomers of β-citronellol, menthol, fenchone, rose oxide, camphor, α-terpineol and 2-butanol were significantly more difficult to discriminate than α-pinene, carvone and limonene (Wilcoxon, $P < 0.01$).

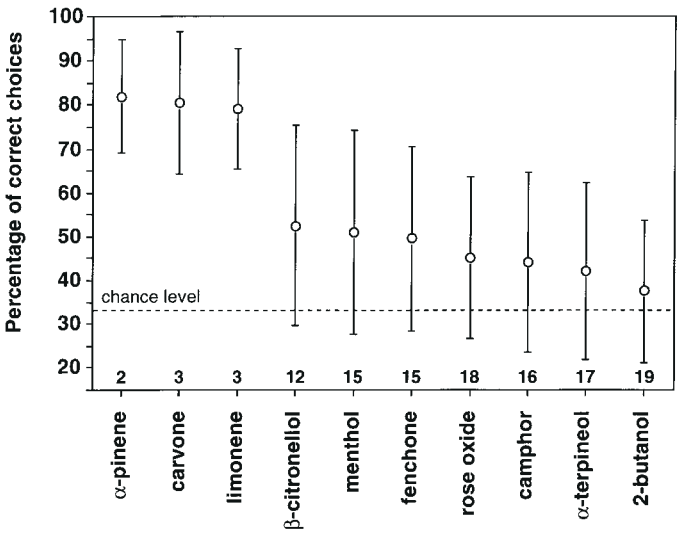

Figure 1 Performance of 20 subjects in discriminating between 10 pairs of enantiomers. Each data point represents the percentage (means ± SD) of correct choices from 10 decisions per odor pair and subject. The figures above the abscissa indicate the number of subjects that failed to perform significantly above chance in the corresponding task.

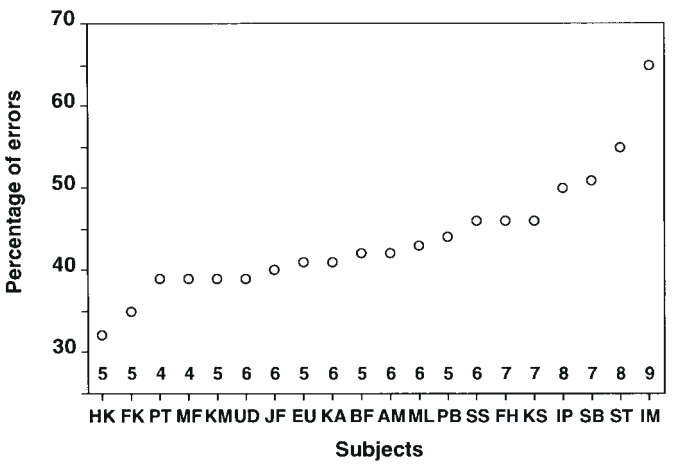

Figure 2 Distribution of individual performance in discriminating between 10 pairs of enantiomers. Each data point represents the percentage of errors from 100 decisions per subject. The figures above the abscissa indicate the number of odor pairs that a subject failed to discriminate significantly above chance.

Accordingly, between 12 and 19 out of 20 subjects failed to significantly distinguish between the antipodes of the former group of substances, whereas only 2 or 3 out of 20 subjects were unable to discriminate the enantiomers of the latter group of substances.

Discrimination scores within these two groups of substances did not differ significantly from each other (Wilcoxon, $P > 0.05$).

Figure 2 shows the distribution of individual performance in discriminating between the 10 enantiomeric odor pairs.

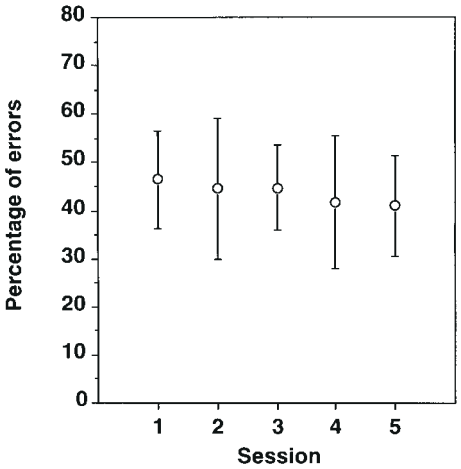

Figure 3 Performance of 20 subjects across the five test sessions in experiment 1. Each data point represents the percentage (means ± SD) of errors from 20 decisions per subject.

The percentage of errors ranged from 32% for the subject performing best up to 65% for the worst. Accordingly, the best panelists were able to significantly distinguish 6 out of 10 enantiomeric odor pairs whereas the poorest-performing subject failed to do so with all tasks but one. Nevertheless, the across-task patterns of performance were very similar between subjects, with virtually all individuals scoring better with α-pinene, carvone and limonene than with the other tasks.

Figure 3 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, $P > 0.05$), and thus no significant learning or training effects at the group level were found.

With all 10 odor pairs <17% of decisions were reported to be based upon perceived differences in odor intensity rather than odor quality (cf. Test procedure). The three enantiomeric odor pairs that were significantly discriminated at the group level yielded the lowest percentages of perceived intensity as the choice criterion, with 3.5, 5.0 and 5.5% for limonene, carvone and α-pinene respectively, whereas the percentages with the seven odor pairs that were not significantly distinguished at the group level ranged from 7.5% for α-terpineol to 16.0% for fenchone. Thus, a negative correlation between discriminability of the enantiomeric odor pairs and the frequency of perceived differences in odor intensity as the choice criterion was found ($r = –0.76$).

With none of the 10 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: trigeminality of enantiomers

The results of experiment 1 showed that human subjects are able to discriminate between the enantiomers of α-pinene, carvone and limonene when presented at equal concentrations. In order to elucidate whether the nasal trigeminal system contributed to this performance, we assessed whether the antipodes of these substances differ in their degree of trigeminality by testing subjects' ability to localize the side of monorhinal stimulation. This simple method has been shown to reliably quantify the trigeminal impact of odorants (Berg *et al.*, 1998).

Materials and methods

Subjects

Ten healthy, unpaid volunteers (seven females and three males), 22–37 years of age, participated in the study. Two of the subjects had already participated in experiment 1.

Odorants

A set of six odorants comprising the enantiomers of α-pinene, carvone and limonene was used (Table 1). The substances were diluted, using diethyl phthalate 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 to 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 handmade 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 ensuring that the presentation of a given odorant to the left or the right nostril was balanced within and between sessions. The inter-trial interval was ~30 s 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 8 or more out of 10 subjects performing significantly above chance (two-tailed binomial test, $P < 0.05$).

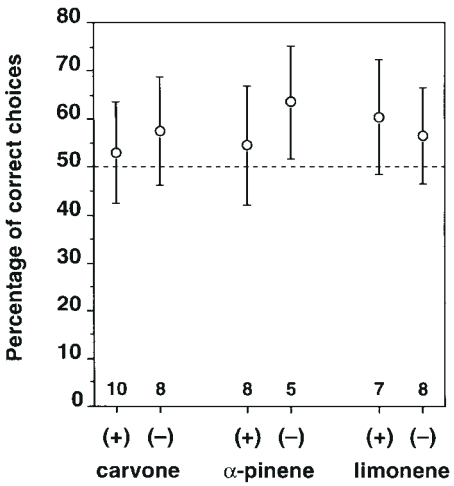

Figure 4 Performance of 10 subjects in correctly localizing the side of monorhinal stimulation. Each data point represents the percentage (means ± SD) of correct choices from 20 decisions per odor pair and subject. The figures above the abscissa indicate the number of subjects that failed to perform significantly above chance in the corresponding task.

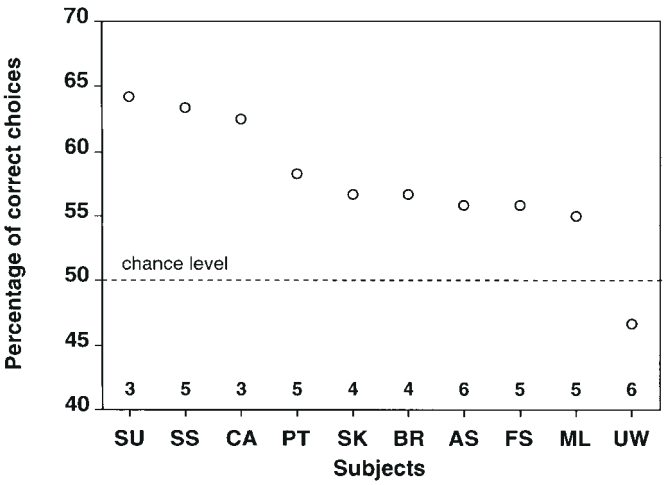

Figure 5 Distribution of individual performance in correctly localizing the side of monorhinal stimulation with the enantiomers of carvone, α-pinene and limonene. Each data point represents the percentage of correct choices from 120 decisions per subject. The figures above the abscissa indicate the number of odor pairs that a subject failed to discriminate significantly above chance.

Comparisons of group performance across sessions were made using the Friedman two-way analysis of variance, 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 4 summarizes the mean performance of 10 subjects in localizing the side of monorhinal stimulation with the enantiomers of α-pinene, carvone and limonene 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 5 and 10 out of 10 individuals not reaching the criterion of at least 14 out of 20 decisions correct.

Interindividual variability was low (cf. SDs in Figure 4) and altogether there were only two cases of individual subjects scoring 80% correct choices (corresponding to a 1% level of significance), one with (–)-α-pinene and one with (+)-limonene.

Pairwise comparisons of performance between the two antipodes of a substance revealed that the enantiomers of α-pinene, carvone and limonene did not differ significantly in their degree of trigeminality at the concentrations tested (Wilcoxon, $P > 0.10$).

Figure 5 shows the distribution of individual performance in localizing the side of monorhinal stimulation with the (+)- and (–)-forms of α-pinene, carvone and limonene. The percentage of correct choices ranged from 64% for the best-performing subject to 47% for the worst. Even the best panelists were only able to significantly localize 3 out of 6 enantiomers at a 5% level of significance whereas the poorest-performing subject failed to do so with all six tasks.

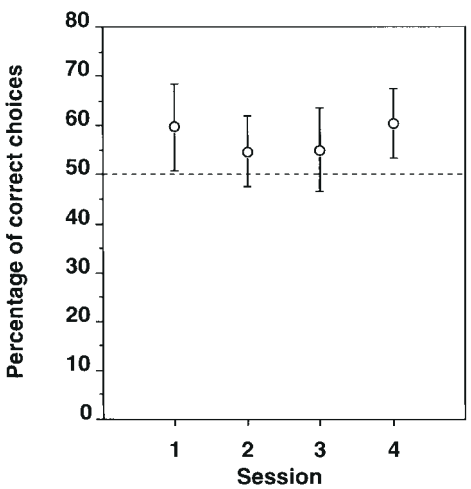

Figure 6 Performance of 10 subjects across the four test sessions in experiment 2. Each data point represents the percentage (means ± SD) of errors from 30 deci- sions per subject.

Figure 6 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 (Friedman, $P > 0.05$), 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 is unlikely to contribute to the ability of human subjects to discriminate between the enantiomers of α-pinene, carvone and limonene at the concentrations tested. In order to get a further indication of whether differences in perceived odor intensity rather than odor quality of the discriminants contributed to this performance—despite the subjects' self-reports in experiment 1, which suggest this not to be the case—we determined olfactory detection thresholds for the optical isomers of these three substances.

Materials and methods

Subjects

Ten healthy, unpaid volunteers (seven females and three 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 α-pinene, carvone and limonene was used (Table 1). For each stimulus, a geometric dilution series using diethyl phthalate as the solvent was prepared, starting at a concentration of 1.0 g/l 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 handmade 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 *et al.*, 1996, 1997). 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 inter-stimulus interval of at least 10 s. Sampling duration was restricted to 1 s 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 was 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 the threshold. In this way, thresholds for the six odorants were determined for each panelist. Testing was repeated in four 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 analysis of variance. 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 7 shows the mean detection thresholds of 10 subjects for each of the six odorants tested across five sessions. With the exception of (+)-carvone, for which a significant increase in performance from the first to the third session was found (Wilcoxon $P < 0.05$), threshold values were quite stable and did not differ significantly across sessions (Friedman $P > 0.1$).

Interindividual variability was comparatively low, as can be inferred from the SDs in Figure 7, which ranged from 0.52 dilution steps (i.e. a factor of 2.3) for (+)-limonene in session 4 to 2.72 dilution steps (i.e. a factor of 80) for (–)-α-pinene in session 5.

Detectability of the (+)- and the (–)-form of α-pinene did not differ significantly from each other in any session (Wilcoxon $P > 0.05$). The same is true for the antipodes of limonene. In contrast, detectability of the enantiomers of carvone was found to differ significantly in three of the five sessions, with the (–)-form yielding lower threshold values than the (+)-form (Wilcoxon $P < 0.05$ in session 2, and $P < 0.01$ in sessions 1 and 5).

Discussion

The results of this study demonstrate that the ability of human subjects to discriminate between enantiomeric odor pairs is substance-specific and thus not a generalizable phenomenon. Whereas almost all subjects had few difficulties in distinguishing the (+)- and the (–)-forms of α-pinene, carvone and limonene, most panelists failed to discriminate between the antipodes of β-citronellol, menthol, fenchone, rose oxide, camphor, α-terpineol and 2-butanol when presented at equal concentrations.

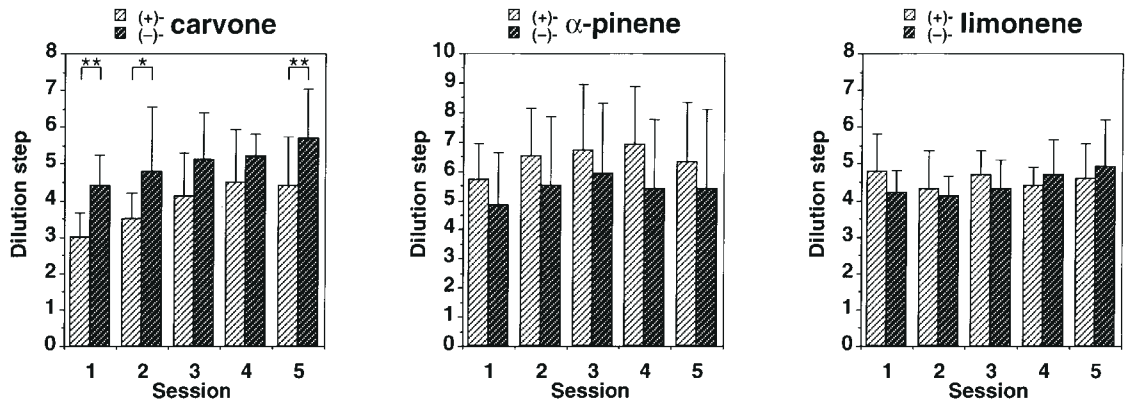

Figure 7 Detection thresholds for the enantiomers of carvone, α-pinene and limonene. Means and standard deviations ($n = 10$ subjects) for each of the five test sessions are given. Significant differences in performance within a given session are indicated by asterisks with $*P < 0.05$, $**P < 0.01$ (Wilcoxon).

These findings are in accordance with earlier reports which assigned different verbal descriptors to the enantiomers of carvone (Russell and Hills, 1971; Friedman and Miller, 1971; Leitereg *et al.*, 1971a,b; Pickenhagen, 1989; Koppenhoefer *et al.*, 1994; Ohloff, 1994), limonene (Koppenhoefer *et al.*, 1994; Ohloff, 1994) and α-pinene (Beets, 1978).

They are also in line with reports which assigned the same verbal labels to the antipodes of menthol (Doll and Bournot, 1949; Beets, 1978; Eccles, 1990), citronellol (Maas *et al.*, 1993), camphor (Theimer *et al.*, 1977; Simmons *et al.*, 1992; Ohloff, 1994), fenchone (Ohloff, 1994) and 2-butanol (Ohloff, 1994).

On the contrary, our findings do not agree with reports which assigned different verbal labels to the enantiomers of menthol and α-terpineol (Beets, 1978; Koppenhoefer *et al.*, 1994), and to the optical isomers of citronellol (Ohloff, 1972, 1994) and rose oxide (Ohloff, 1972; Pickenhagen, 1989). They also differ from reports which assigned the same verbal labels to the antipodes of α-pinene (Ohloff, 1994).

The fact that different authors came to contradictory conclusions with regard to the equality or inequality of qualitative attributes assigned to several of the enantiomeric odor pairs employed here (α-pinene, menthol and citronellol) reflects the fundamental problem of semantic ambiguity in the verbal description of odor quality and illustrates the need for more unequivocal means of assessing qualitative similarities and differences between odorants.

The few studies which have so far used discrimination procedures to assess the ability of humans to detect differences between enantiomeric odor pairs are generally in agreement with our findings. Jones and Velasquez (1974), Pike *et al.* (1987, 1988), Cowart (1990) and Hormann and Cowart (1993) all reported the (+)- and (–)-forms of carvone to be readily discriminable both when presented at equal concentrations and when stimulus intensity of one of the discriminants was intentionally altered. Using a triangular test procedure similar to the one employed here, Cowart (1990) also found that humans are unable to discriminate between the antipodes of fenchone.

In the only study so far that has employed an array of chiral odor pairs, Jones and Elliot (1975) reported the ability of human subjects to discriminate between enantiomers to be both substance-specific and subject-specific. In line with our results, the majority of their subjects were able to distinguish the antipodes of carvone and of α-pinene. Their finding of 2-butanol—which was significantly discriminated by only 1 out of 20 subjects in our study—to be discriminable from its mirror image, however, was based on invalid statistics as the authors applied binomial tests to the total number of correct responses pooled from all subjects. Converted to percentages, their summed score for this odor pair corresponds to 40.3% decisions correct, which compares favorably with our finding of an average score of 37.5%.

The same authors reported large differences in discrimination performance between subjects. Unfortunately, they gave no detailed information but only stated that 7 of their 31 subjects failed to reach a significant overall score which the authors discussed as a 'general chiral anosmia' (Jones and Elliot, 1975). We also found considerable interindividual variability both with individual odor pairs (cf. SDs in Figure 1) and across tasks (cf. Figure 2). However, the across-task patterns of performance were very similar between subjects, with virtually all individuals scoring better with α-pinene, carvone and limonene than with the other tasks. This suggests that the substance-specificity of the ability to discriminate between enantiomeric odor pairs is a robust phenomenon.

It is well-established that both the olfactory and trigeminal systems contribute to the perception of the majority of odorants (Doty, 1995). This raises the possibility that the nasal trigeminal system might have contributed to the discrimination of the enantiomers of α-pinene, carvone and limonene, 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, however, strongly suggest that the substances used here had little if any trigeminal-stimulating properties at the concentrations tested and that in any case the antipodes of a given substance did not differ in their degree of trigeminality. Thus, the possibility of trigeminal involvement in the discrimination of the three enantiomeric odor pairs in question can be excluded.

The possibility that differences in perceived odor intensity might have contributed to the discrimination performance also seems quite unlikely as >90% of the subjects' decisions involving the three 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 (cf. Test procedure). Further, the comparatively few instances in which perceived differences in odor intensity were reported seem to reflect a subject's difficulty to discriminate at all, as error rates in such cases tended to be higher compared with 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 alcohols (Laska and Trolp, 1998) and carboxylic acids (Laska and Teubner, 1998). 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 enantiomers of α-pinene and the antipodes of limonene did not differ from each other (cf. Figure 7). Our finding that (–)-carvone yielded significantly lower threshold values than (+)-carvone in three of the five test sessions is in line with earlier studies (Leitereg *et al.*, 1971a,b; Cowart, 1990; Hormann and Cowart, 1993) reporting the same discrepancy with these stimuli. However, Cowart (1990) also reported suprathreshold concentrations of (+)-carvone to be more intense than its mirror image and discriminability to be largely unaffected by changes in the concentration of one of the discriminants.

Taken together, the results of experiments 2 and 3 suggest that the discrimination scores found with α-pinene, carvone and limonene reflect the ability of the human olfactory system to distinguish the odor qualities of these enantiomeric odor pairs.

A final aspect of the present study is the finding that no generalizable conclusions can be drawn from our data as to odor structure–activity relationships which would allow us to predict whether or not a given pair of enantiomers can be olfactorily discriminated. However, it was apparent that two of the three substances whose optical isomers were significantly distinguished (carvone and limonene) share a propenyl group at the chiral center and thus it would be worthwhile to include other enantiomeric odor pairs which show this structural feature in future studies of olfactory discrimination performance. Our finding that the antipodes of α-pinene were also discriminable despite their lack of a propenyl group, on the other hand, illustrates that the presence or absence of a certain functional group at the chiral carbon atom is not a sufficient predictor of enantioselectivity. Similarly, membership of a certain chemical class is not a predictor of whether or not the antipodes of a substance are discriminable as, for example, carvone, fenchone and camphor are all carbonyl compounds but differ significantly in their discriminability (cf. Figure 1).

A more biological explanation of why some enantiomeric odor pairs can be discriminated whereas others cannot is that enantioselectivity of the human olfactory system may be restricted to substances for which both optical isomers are widely present in our natural odor world. There is accumulating evidence that the mammalian olfactory system, analogous to the immune system, may be capable of increasing the expression of molecular receptors that are responsive to a given odorant after repeated exposure to that stimulus (Wang *et al.*, 1993; Semke *et al.*, 1995). Thus it might be that chiral odorants for which only one of their antipodes is naturally occurring cannot be discriminated from their mirror images due to a lack of an appropriate enantioselective receptor. Analytical studies of essential oils (König *et al.*, 1990; Mosandl *et al.*, 1990b) and fruit flavours (Gessner *et al.*, 1988; Mosandl *et al.*, 1990a) have shown that the relative amounts found with the optical isomers of a chiral substance can vary widely. With menthol, for example, the levo-form prevails in all essential oils containing this compound whereas the dextro-form is found only in trace amounts (Eccles *et al.*, 1988). Carvone, α-pinene and limonene, on the other hand, are widely distributed with both their enantiomeric forms—although in different ratios—in a wide variety of plant extracts (König *et al.*, 1990; Mosandl *et al.*, 1990b). Our finding that the optical isomers of the latter three substances were discriminable while those of menthol were not supports the hypothesis that a widespread occurrence of both enantiomeric forms of a substance in our odorous environment is a prerequisite for our ability to distinguish between these. However, in order to further corroborate this hypothesis it is clearly important to include other enantiomeric odor pairs in studies of olfactory discrimination performance and to compare these findings with the natural occurrence and distribution of such substances.

So far, the results of the present study provide evidence that the ability of humans to discriminate between enantiomeric odor pairs is substance-specific and thus support the assumption that enantioselective molecular odor receptors may only exist for some but not all volatile enantiomers.

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