Enantioselectivity of odor perception in squirrel monkeys and humans

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Laska, Matthias, Anne Liesen, and Peter Teubner. Enantioselectivity of odor perception in squirrel monkeys and humans. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1098–R1103, 1999.—With use of a conditioning paradigm, the ability of six squirrel monkeys to distinguish between 10 pairs of enantiomers, i.e., odorants that are identical except for chirality, was investigated. As a group, the animals were only able to discriminate between the optical isomers of α-pinene, carvone, limonene, and fenchone, whereas they failed to distinguish between the (+) and (−) forms of β-citronellol, menthol, rose oxide, 2-butanol, α-terpinel, and camphor. With use of a triple forced-choice procedure, 10 human subjects were tested for their ability to discriminate between the same enantiomeric odor pairs in parallel and, with the exception of fenchone, showed a very similar pattern of performance compared with the squirrel monkeys. These findings support the assumption that human and nonhuman primates may share common principles of odor quality perception. Furthermore, the results suggest that, in both species, enantioselective molecular odor receptors may only exist for some, but not all volatile enantiomers and thus that chiral recognition of odorants is not a general phenomenon, but may be restricted to some substances.

olfaction; odor discrimination; enantiomers; chirality; nonhuman primates

Chiral recognition of ligands is a fundamental feature of biological systems (11). The ability to distinguish a molecular structure from its mirror image plays an important role in fields such as drug effectiveness (4), insect chemical communication (25), and taste perception (33). The optical isomers of pharmacologically active compounds are frequently characterized by different physiological activities and different toxicological risks and side effects. Specific enantiomers often appear as insect pheromone-active compounds (35), and chiral substances such as L-amino acids have been shown to generally elicit bitter taste sensations, whereas the D-enantiomers are mostly sweet (33).

A variety of volatile optical isomers have also been described as having different odor qualities and/or different odor intensities for humans (29, 30). This should not be surprising given that the first event in odor perception is the interaction of an odor molecule with an olfactory receptor (10). As olfactory receptors have been identified as proteins, i.e., chiral molecules (2), 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 (31). However, there are also reports of identically smelling enantiomeric odor pairs (37) that 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 (36).

Most of the human studies reporting qualitative differences between enantiomers have employed odor profiling or scaling procedures that are presumed to be particularly susceptible to cognitive influences (5). Only a few studies, on the other hand, have directly tested the discriminability of (+) and (−) forms of such odorants, although this method not only avoids the disadvantages of poor resolution and semantic ambiguity (3), but is also applicable to nonhuman species.

Given the possible importance of enantioselectivity for our understanding of the molecular mechanisms underlying the interaction between odor stimulus and olfactory receptor and the possibility to draw conclusions as to the nature and generality of odor structure-activity relationships across species, we decided to test the ability of squirrel monkeys and human subjects to distinguish between 10 pairs of enantiomers. Substances were chosen on the basis of earlier studies that reported qualitative attributes of antipodes to range from “identical” to “very different” (30), allowing us to present odor pairs presumed to differ in their degrees of perceptual similarity and thus discriminability.

Thus the aims of this study are twofold: 1) to provide first data on the olfactory discrimination ability of squirrel monkeys for enantiomeric odor pairs, and 2) by testing a group of human subjects in parallel, to assess whether human and nonhuman primates share common principles of odor quality perception.

MATERIALS AND METHODS

Animals. Testing was carried out using two adult female and four adult male squirrel monkeys (Saimiri sciureus) maintained as part of an established breeding colony. All animals had served as subjects in previous olfactory experiments and were completely familiar with the basic test procedure (13, 18–23). The colony was housed in a double enclosure made up of a 23-m² home cage joined to a 7-m² test cage by two tunnels that could be closed by sliding doors to allow the temporary separation of animals for individual testing. Animals were provided with marmoset pellets (Ssniff, Soest, Germany), fresh fruit, vegetables, and water ad libitum.

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 also with current German laws.
Behavioral test. In a task designed to simulate olfactory-guided foraging, opaque 1.5-ml Eppendorf flip-top reagent cups were equipped with absorbent paper strips (35 × 7 mm; Sugi, Kettenbach, Germany) impregnated with 10 μl of an odorant signaling either that they contained a peanut food reward (S+) or that they did not (S−). The odor strips were attached to the vials by cutting a slit in each strip and slipping it over the flip-up lid, which was connected to the vial by a narrow band. Eighteen such cups, nine positive and nine negative, were inserted in pseudorandom order in holes along the horizontal bars of a climbing frame in such a way that some effort was required for the animals to remove them. The frame was mounted to one of the enclosure walls and consisted of a 2.5-m vertical pole (40 mm diameter) fitted with seven cross bars (20 mm diameter) 30 cm apart, the middle three of which extended 50 cm to either side and were equipped with conically bored holes to hold the cups (13).

In each test trial, each monkey was allowed 1 min to harvest as many baited cups from the frame as possible. Five such trials were conducted per animal per session, and usually two sessions were conducted per day. Cups were used only once, and the odorized strips were prepared fresh at the start of each session.

In all experiments, three animals were trained to associate the (+) form of a given substance as the rewarded stimulus, and the other three animals were trained to associate the (−) form of the same substance as the rewarded stimulus (see Table 1). To allow an animal to build a robust association between a given odor and its significance as rewarded stimulus, each monkey received 10 sessions of five 1-min trials, using lavender oil as unrewarded stimulus. Subsequently, each animal received eight sessions of five 1-min trials with the familiar antipode as rewarded stimulus versus the unfamiliar antipode as unrewarded stimulus. To prevent the more challenging conditions leading to extinction or to a decline in the animals’ motivation, the eight sessions of critical trials were subdivided into two blocks of four sessions each and interspersed with two sessions of trials using lavender as S− again.

Human subjects. Ten healthy, unpaid volunteers (7 females and 3 males), 23–38 yr of age, participated in the study. All were right-handed and had a history of olfactory dysfunction. All subjects had previously served in olfactory 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/Hong Kong.

Test procedure. A 40-ml aliquot of each odorant was presented in a 250-ml polyethylene squeeze bottle equipped with a flip-up spout that for testing was fitted with a custom-made Teflon nosepiece. 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 nosepiece was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant to allow the stimulus to enter both nostrils.

In a forced-choice triangular test procedure, subjects were asked to compare three bottles and 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 two times, with an interstimulus interval of at least 10 s. Sampling duration was restricted to 1 s per presentation to minimize adaptation effects. The sequence of presenting the stimulus pairs was systematically varied between sessions and individual subjects while taking care to avoid successive presentations of the same combinations and while systematically varying the order in which the stimuli were sampled. The presentation of a given substance as an odd or even stimulus was balanced within and between sessions. Approximately 30 s were allowed between trials, and no feedback regarding the correctness of the subjects’ choice was given.

Different stimulus pairs, the same ones as for the squirrel monkeys, were presented two times per session so as to give a total of 20 judgments. Testing was repeated in four more sessions, each 1–3 days apart, enabling 10 judgments per stimulus pair and panelist to be collected.

Odorants. A set of 21 odorants was used (Table 1). All substances had a nominal purity of at least 99%. They were diluted using diethyl phthalate (Merck) as the solvent. The enantiomers of a given pair were presented at equal concentrations. In an attempt to ensure that the different enantiomeric odor pairs were of approximately equal strength when presented on the absorbant paper strips, intensity matching was performed by a panel using freshly prepared strips impregnated with 10 μl of an 8.7 g/l solution of isomyl acetate as the standard and adopting a standardized psycho-physical procedure (1). This was chosen 1) because this odorant and concentration had been successfully used in previous studies (18, 19, 23) and 2) to provide odor concentrations that could be reliably detected by the animals but weak enough to prevent contamination of the test cage and to force the animals to sniff closely.

Likewise, 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 using a 8.7 g/l solution of isomyl acetate as the standard and adopting a standardized psycho-physical procedure (1). Because the mode of presentation of odorants differed between squirrel monkeys (absorbant paper strips, i.e., an open system allowing odorants to diffuse freely) and humans (squeeze bottles, i.e., a closed system allowing

<table>
<thead>
<tr>
<th>Table 1. Substances and concentrations used</th>
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<tr>
<td>Substance</td>
</tr>
<tr>
<td>(1R, 5S)-(-)-menthol</td>
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<tr>
<td>(1S, 2R, 5S)-(+)-menthol</td>
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<tr>
<td>(1R)-(-)-pinene</td>
</tr>
<tr>
<td>(1S)-(+)-pinene</td>
</tr>
<tr>
<td>R-(-)-carvone</td>
</tr>
<tr>
<td>S-(-)-carvone</td>
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<tr>
<td>S-(-)-limonene</td>
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<tr>
<td>R-(-)-limonene</td>
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<tr>
<td>(-)-Camphor</td>
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<tr>
<td>(-)-Camphor</td>
</tr>
<tr>
<td>(-)-β-Citronellol</td>
</tr>
<tr>
<td>(+)-β-Citronellol</td>
</tr>
<tr>
<td>(-)-Fenchone</td>
</tr>
<tr>
<td>(+)-Fenchone</td>
</tr>
<tr>
<td>(-)-α-Terpineol</td>
</tr>
<tr>
<td>(+)-α-Terpineol</td>
</tr>
<tr>
<td>(-)-Rose oxide</td>
</tr>
<tr>
<td>(+)-Rose oxide</td>
</tr>
<tr>
<td>R-(-)-2-butanol</td>
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<tr>
<td>S(-)-2-butanol</td>
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| Lavender oil | 19.6 | *(+)- and (-) denote different forms of odorants. *According to Ohloff (30). R and S, clockwise or counterclockwise sequence, respectively, of substituents around a chiral element [according to an arbitrary ranking (35)].
optical isomers of limonene, carvone, and animals were only able to discriminate between the 10 enantiomeric odor pairs. As a group, the performance of the squirrel monkeys in discriminating was low and generally <20% between the highest- and lowest-scoring animal (see SDs in Fig. 1). In the remaining tasks, either one (carvone and fenchone) or three (β-citronellol and menthol) out of six animals, respectively, failed to distinguish between the optical isomers of a given substance, and accordingly interindividual variability in these tasks was high (see SDs in Fig. 1). However, ANOVA detected significant differences in the animals’ performance between tasks (Friedman’s test, P < 0.001), and subsequent pairwise tests revealed that the enantiomers of limonene and α-pinene were significantly less difficult to discriminate compared with the other tasks (Wilcoxon’s test, P < 0.05).

At the individual level, the best animal was able to significantly distinguish 6 out of 10 enantiomeric odor pairs, whereas the poorest-forming animal failed to do so with seven of the tasks. Nevertheless, the across-task patterns of performance were generally quite similar among animals, with all six subjects scoring better with limonene, α-pinene, and fenchone than with rose oxide, 2-butanol, α-terpineol, and camphor.

Human subjects. Figure 2 shows the mean performance of the human subjects in discriminating among the 10 enantiomeric odor pairs. As a group, the human subjects were only able to discriminate between the optical isomers of limonene, carvone, and α-pinene, whereas they failed to distinguish between the (+) and (−) forms of the seven other substances.

Interindividual variability was high, particularly in tasks that were not significantly discriminated at the group level (see SDs in Fig. 2). However, ANOVA detected significant differences in the group’s perfor-
formance between tasks (Friedman, \( P < 0.001 \)), and subsequent pairwise tests revealed that the enantiomers of fenchone, \( \beta \)-citronellol, menthol, rose oxide, 2-butanol, \( \alpha \)-terpineol, and camphor were significantly more difficult to discriminate compared with limonene, carvone, and \( \alpha \)-pinene (Wilcoxon, \( P < 0.01 \)). Accordingly, between 5 and 9 out of 10 subjects failed to significantly distinguish between the antipodes of the former group of substances, whereas only 1 out of 10 subjects, respectively, was unable to discriminate the enantiomers of the latter group of substances.

At the individual level, 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 two. Nevertheless, the across-task patterns of performance were very similar between subjects, with all individuals scoring better with limonene, carvone, and \( \alpha \)-pinene compared with the other tasks.

Between-species comparisons. A comparison of the across-task patterns of performance between squirrel monkeys and human subjects reveals striking similarities between the two species (see Figs. 1 and 2). Both in human and nonhuman primates, the discrimination of the enantiomers of limonene, carvone, and \( \alpha \)-pinene yielded the highest scores, and the discrimination of the antipodes of rose oxide, 2-butanol, \( \alpha \)-terpineol, and camphor resulted in the lowest scores. Thus the across-task patterns of discrimination performance of squirrel monkeys and human subjects correlated significantly (Spearman's rank correlation coefficient, \( r_s = 0.81, P < 0.01 \)).

**DISCUSSION**

The results of this study demonstrate 1) that the ability of both human and nonhuman primates to discriminate between enantiomeric odor pairs is substance specific and thus not a generalizable phenomenon, and 2) that squirrel monkeys showed a very similar across-task pattern of discrimination performance compared with human subjects.

Our finding of substance specificity in chiral odor discrimination is in line with earlier reports that assigned verbal descriptors to optical antipodes (see Table 1) (30) as well as with the few studies so far that employed discrimination procedures to assess the ability of humans to detect differences between enantiomeric odor pairs (6, 12, 15, 16). It is interesting to note, however, that several of our results and findings of other authors who tested the discriminability of enantiomers in humans are in contradiction with results of descriptive studies. The antipodes of \( \alpha \)-terpineol, for example, have been assigned different verbal labels (see Table 1). Nevertheless, squirrel monkeys and human subjects both failed to discriminate between this pair of chiral odorants. The same discrepancy between the results of descriptive studies and our, as well as other, discrimination studies can be found with the enantiomers of menthol, \( \alpha \)-pinene, \( \beta \)-citronellol, and rose oxide (see Table 1 and Figs. 1 and 2). This suggests that discrimination procedures might be more reliable to assess qualitative differences between odorants compared with descriptive methods with their inherent problem of semantic ambiguity.

Numerous studies have shown that chirality is often essential for the specificity of pheromone perception in insects (e.g., 24, 25, 35, 39). Only few studies, on the other hand, have assessed discrepant enantiomer effects in nonhuman vertebrates. Müller-Schwarze et al. (28) reported that black-tailed deer showed slightly stronger behavioral responses to the \((-\) form of "deer lactone," the main component of this species' tarsal gland secretion, compared with the \((+) form. More recently, Heth et al. (9) assessed the behavior of mole rats near pairs of enantiomeric compounds in two-choice tests. The results indicated that mole rats respond differentially to odors of the enantiomers of carvone, citronellol, and fenchone. However, the authors could not determine whether indifference to or anosmia for one of the antipodes of a given odor pair accounted for the observed differences in behavior.

In our study, we can exclude the possibility that an inability to perceive an odorant might have contributed to discrimination performance. None of the human subjects reported a lack of odor sensation with any given odor stimulus, and all of our squirrel monkeys not only readily learned within two sessions to correctly assign a given enantiomer as rewarded stimulus when tested against lavender, but they were also clearly able to do so when tested against an odorless control (unpublished data).

Although the possibility that differences in perceived odor intensity might have contributed to the ability of both species to discriminate between some of the enantiomeric odor pairs tested cannot be ruled out completely, this seems quite unlikely as the reliability of our intensity-matching procedure was confirmed by the fact that, during the critical discrimination tasks, \( >90\% \) of the human subjects' decisions were reported to be based on perceived differences in odor quality rather than odor intensity (see Test procedure). Furthermore, 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.

In an earlier study (21), we could show that the discrimination scores of our squirrel monkeys remained quite stable even when the concentration of the \( S^– \) was one order of magnitude higher or lower than the concentration matched to the \( S^+ \). Therefore, we believe the discrimination scores of both species to reflect the ability of their olfactory systems to distinguish between the odor qualities of the \((+) \) and \((–) \) forms of limonene, carvone, \( \alpha \)-pinene, and, in the squirrel monkeys, of fenchone.

Our finding of a high degree of conformity in the across-task patterns of performance between squirrel monkeys and human subjects in discriminating enantiomeric odor pairs is in agreement with earlier reports of striking similarities in the relative discrimination abilities of these two species with aliphatic esters (19),
carboxylic acids (23), and artificial odor mixtures (21). This suggests that the conformity in relative performance found between the two species in the present study is not restricted to the particular set of odorants tested here but may represent a more generalizable phenomenon that holds true for a larger part or even the whole spectrum of odors. Thus our findings lend support to the assumption that closely related species, such as, for example, human and nonhuman primates, might have a larger proportion of molecular odor receptors in common compared with phylogenetically more distant species (14). However, honeybees have also been shown to score better with limonene and carvone than with menthol when tested for their ability to discriminate between the enantiomers of these substances (R. Menzel, personal communication), and thus insects seem to display a similar pattern of relative discrimination performance compared with primates.

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 substances whose optical isomers were significantly distinguished by both species (carvone and limonene) share a propenyl group at the chiral center, and thus it would be worthwhile to include other enantiomeric odor pairs that show this structural feature in future studies of olfactory discrimination performance. Our finding that the enantiomers of α-pinene were also discriminated despite their lack of a propenyl group, on the contrary, 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 enantiomers of a substance are discriminable as, for example, carvone, fenchone, and camphor are all carbonyl compounds but differ significantly in their discriminability (see Figs. 1 and 2).

A more biological approach trying to explain why some enantiomeric odor pairs were discriminated whereas others were not is that enantioselectivity of the primate or perhaps even all olfactory systems 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 this stimulus (32, 38). Thus it might be that chiral odorants, for which only one of their enantiomers is naturally occurring, cannot be discriminated from their mirror images due to a lack of an appropriate enantioselective receptor. Analytical studies of essential oils (17, 27) and fruit flavors (8, 26) showed that the relative amounts found with the optical isomers of a chiral substance can vary widely. With menthol, for example, the dextro form is prevailing in all essential oils containing this compound, whereas the laevo form is only found in trace amounts (7).

Carvone, α-pinene, and limonene, on the other hand, are widely distributed with both their enantiomeric forms, although in different ratios, in a large variety of plant extracts (17, 27). Our finding that the optical isomers of the latter three substances were discriminable for both species, whereas those of menthol were not supports the hypothesis that a widespread occurrence of both enantiomeric forms of a substance in our odorous environment might be a prerequisite for the ability to distinguish between these. However, 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 squirrel monkeys and 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. Furthermore, they suggest that human and nonhuman primates may share common principles of odor quality perception.

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