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The Number of Functional Olfactory Receptor Genes and the Relative Size of Olfactory Brain Structures Are Poor Predictors of Olfactory Discrimination Performance with Enantiomers

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Abstract

The ability of four squirrel monkeys and three pigtail macaques to distinguish between nine enantiomeric odor pairs sharing an isopropenyl group at the chiral center was investigated in terms of a conditioning paradigm. All animals from both species were able to discriminate between the optical isomers of limonene, carvone, dihydrocarvone, dihydrocarveole and dihydrocarvyl acetate, whereas they failed to distinguish between the (+)- and (−)-forms of perillaaldehyde and limonene oxide. The pigtail macaques, but not the squirrel monkeys, also discriminated between the antipodes of perillaalcohol and isopulegol. A comparison of the across-task patterns of discrimination performance shows a high degree of similarity among the two primate species and also between these nonhuman primates and human subjects tested in an earlier study on the same tasks. These findings suggest that between-species comparisons of the relative size of olfactory brain structures or of the number of functional olfactory receptor genes are poor predictors of olfactory discrimination performance with enantiomers.

Key words: discrimination ability, enantiomers, nonhuman primates, olfaction

Introduction

Enantiomers appear to be valuable tools 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, at least partially, due to properties such as differing diffusion rates in the mucus covering the olfactory sensory epithelium or differing air/mucus partition coefficients (Hahn *et al*., 1994), enantiomers exhibit identical chemical and physical properties (except for their optical activity, i.e. their rotation of polarized electromagnetic waves), and thus any difference in odor perception must originate from chiral selectivity at the peripheral level (Rossiter, 1996). Therefore, the systematic assessment of 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.

In a recent study, Laska (2004) tested the ability of human subjects to distinguish between nine pairs of enantiomers sharing an isopropenyl group at the chiral carbon atom and found that only five of them were discriminated at the group level. Subsequent analysis of odor structure–activity relationships suggested that only the combined presence of an isopropenyl group at the chiral center, a methyl group at the *para*-position and/or an oxygen-containing group at the *meta*-position allows for the discrimination of members of the tested set of enantiomeric odor pairs in humans.

In the present study, we tested the discriminability of the same nine pairs of enantiomers in two species of nonhuman primates, the squirrel monkey and the pigtail macaque. This allowed us to address (i) whether human and nonhuman primates share common principles of odor structure–activity relationships, and (ii) whether species differing in relative size of their olfactory brain structures and in their number of functional olfactory receptor genes (Stephan *et al*., 1988; Rouquier *et al*., 2000) may also differ in their olfactory discrimination capabilities for structurally related odorants.

Materials and methods

Animals

Testing was carried out using three adult male and one adult female squirrel monkeys (*Saimiri sciureus*), and two adult males and one adult female pigtail macaques (*Macaca nemestrina*).

Conditions of the animals' maintenance have been described in detail elsewhere (Laska and Seibt, 2002). 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 tests

The experimental procedures have been described in detail elsewhere (Laska and Hudson, 1993; Hübener and Laska, 2001). Briefly, the animals were tested using a food-rewarded instrumental conditioning paradigm. Manipulation objects were fitted with absorbent paper strips impregnated with 10 µl of an odorant signalling either that they contained a food reward (S+) or that they did not (S−). In each test trial, each monkey sniffed at both options and then decided to open one of the two manipulation objects. Ten such trials were conducted per animal and session, and at least three sessions per experimental condition were performed. In all experiments, two animals of each species were trained to associate the (+)-form of a given substance as the rewarded stimulus, and the other animals were trained to associate the (−)-form of the same substance as the rewarded stimulus (see Table 1).

Odorants

A set of 18 odorants comprising nine pairs of enantiomers, all sharing an isopropenyl group at the chiral center, was used (Table 1). All substances had a nominal purity of at least 99% (Fluka). 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, intensity matching was performed by a panel of six subjects adopting a standardized psychophysical procedure (ASTM, 1975).

Table 1 Substances and concentrations used

Substance pair		Concentration (g/l)
S-(−)-Limonene	*R*-(+)-Limonene	16.9
R-(−)-Carvone	*S*-(+)-Carvone	96.0
(−)-Dihydrocarvone	(+)-Dihydrocarvone	92.9
(−)-Dihydrocarveol	(+)-Dihydrocarveol	92.6
(−)-Dihydrocarvyl acetate	(+)-Dihydrocarvyl acetate	94.8
(−)-Perillaaldehyde	(+)-Perillaaldehyde	9.7
(−)-Perillaalcohol	(+)-Perillaalcohol	95.8
(−)-Isopulegol	(+)-Isopulegol	30.4
(−)-Limonene oxide	(+)-Limonene oxide	9.3

Data analysis

For each individual animal, the percentage from the best three sessions per odor pair, comprising at total of at least 30 decisions, was calculated. Significance levels were determined by calculating binomial *z*-scores corrected for continuity (Siegel and Castellan, 1988) from the number of correct and false responses for each individual and condition.

Between-species comparisons of the across-task patterns of performance were evaluated using Spearman's rank-correlation coefficient and tested for significance by computing *t*-values. All tests were two-tailed, and the α level was set at 0.05.

Results

Squirrel monkeys

Figure 1 shows the performance of four squirrel monkeys in discriminating between the nine enantiomeric odor pairs. All four animals significantly discriminated between the enantiomers of limonene, carvone, dihydrocarvone, dihydrocarveole and dihydrocarvyl acetate, and all four animals failed to distinguish between the antipodes of perillaalcohol, isopulegol and limonene oxide. Only one out of four animals succeeded in discriminating between the (+)- and the (−)-form of perillaaldehyde. Interindividual variability in performance

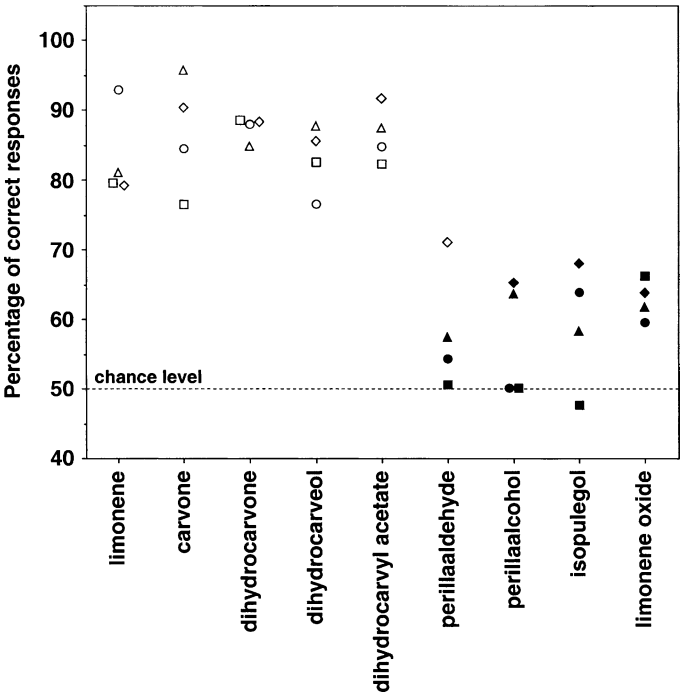

Figure 1 Performance of four squirrel monkeys in discriminating between nine pairs of enantiomers. Each data point represents the percentage of correct choices per odor pair and animal. Filled symbols indicate odor pairs that were not discriminated above chance.

with a given task was generally low and averaged only 12.2% between the best- and the poorest-scoring animal.

Pigtail macaques

Figure 2 shows the performance of three pigtail macaques in discriminating between the nine enantiomeric odor pairs. All three animals significantly discriminated between the enantiomers of limonene, carvone, dihydrocarvone, dihydrocarveole, dihydrocarvyl acetate, perillaalcohol and isopulegol, and all three animals failed to distinguish between the antipodes of limonene oxide. Only one out of three animals succeeded in discriminating between the (+)- and the (−)-form of perillaaldehyde. Interindividual variability in performance with a given task was generally low and averaged only 10.9% between the best- and the poorest-scoring animal.

Between-species comparisons of performance

A comparison of the across-task patterns of performance between squirrel monkeys, pigtail macaques and human subjects tested in an earlier study (Laska, 2004) reveals striking similarities between the three species. All three species were clearly able to discriminate between the enantiomers of limonene, carvone, dihydrocarvone and dihydrocarvyl acetate, and all three species failed to distinguish between the antipodes of perillaaldehyde and limonene oxide. Accordingly, the across-task patterns of performance correlated significantly between all three species (Spearman's rank correlation coefficient, human subjects versus squirrel monkeys: $r_s = 0.82$, $P < 0.03$; human subjects versus pigtail macaques: $r_s = 0.71$, $P < 0.05$; squirrel monkeys versus pigtail macaques: $r_s = 0.82$, $P < 0.02$).

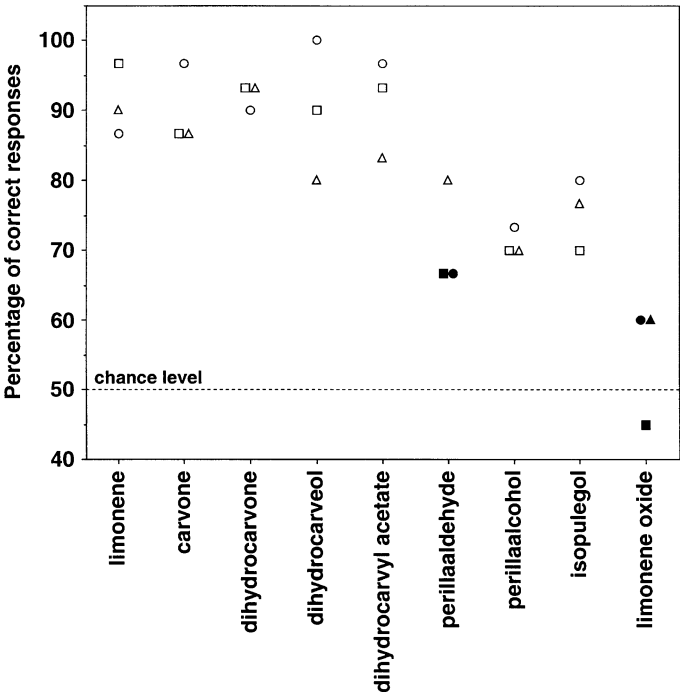

Figure 2 Performance of three pigtail macaques in discriminating between nine pairs of enantiomers. Each data point represents the percentage of correct choices per odor pair and animal. Filled symbols indicate odor pairs that were not discriminated above chance.

Discussion

The results of this study demonstrate that the ability of squirrel monkeys and pigtail macaques to discriminate between enantiomeric odor pairs sharing an isopropenyl group at the chiral center is substance-specific. Further, we found that the across-task patterns of discrimination performance show a high degree of similarity among the two species and also between these nonhuman primates and human subjects tested in an earlier study on the same tasks (Laska, 2004).

Our finding that enantioselectivity of odor perception in human and nonhuman primates appears to be restricted to certain pairs of optical antipodes is in agreement with an earlier report that also showed human subjects and squirrel monkeys capable of distinguishing between only three, respectively four, out of ten enantiomeric odor pairs tested (Laska *et al.*, 1999a,b). Both the results of the present and the earlier study are in contrast with theoretical considerations as olfactory receptors have been identified as proteins, i.e. as chiral molecules (Buck and Axel, 1991), which should always interact differently with the two enantiomeric forms of a chiral odorant (Pickenhagen, 1989) and thus should lead to perceptible differences in odor quality and/or intensity (Brenna *et al.,* 2003).

However, our finding that only the combined presence of an isopropenyl group at the chiral carbon atom, a methyl group at the *para*-position and/or an oxygen-containing group at the *meta*-position allowed for the discrimination of enantiomeric odor pairs in all three species tested (Table 2) is in line with the multipoint 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 even more, dipole–dipole interactions or hydrogen bonds (Afshar *et al.*, 1998; Chastrette and Rallet, 1998).

The second main finding of the present study, a striking similarity in the across-task patterns of discrimination performance between squirrel monkeys, pigtail macaques and human subjects (Table 2), may at first not seem surprising given that all three species belong to the same order of mammals and thus have a long history of evolution in common, suggesting that they are likely to share a large proportion of olfactory receptor types (Rouquier *et al.*, 2000). However, it should be considered that the three primate species differ markedly in the relative size of their olfactory brain structures and in their number of functional olfactory receptor genes. Within the order of primates, the relative size of the olfactory bulbs—the first neuropil of the olfactory pathway—has been demonstrated to show the order of New World primates > Old World monkeys > humans (Stephan *et al.*, 1988). Similarly, humans are said to have only ~350 functional olfactory receptor genes, with the rest being

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)
	Hum	Sai	Mac		*Ortho*	*Meta*	*Para*	
Limonene	+	+	+	+	–	–	–	–
Carvone	+	+	+	+	–	+	–	–
Dihydrocarvone	+	+	+	+	–	+	–	+
Dihydrocarveol	+	+	+	+	–	+	–	+
Dihydrocarvyl acetate	+	+	+	+	–	+	–	+
Perillaaldehyde	–	–	–	–	–	–	+	–
Perillaalcohol	–	–	+	–	–	–	+	–
Isopulegol	–	–	+	+	+	–	–	+
Limonene oxide	–	–	–	+	–	–	–	+

Hum, human subjects (data from Laska, 2004); Sai, *Saimiri sciureus*; Mac, *Macaca nemestrina*.
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.

pseudogenes which are presumed not to be transcribed into proteins, and Old World monkeys such as pigtail macaques are supposed to have only 700 functional genes coding for olfactory receptors, whereas New World primates such as the squirrel monkey are said to have the full mammalian repertoire of ~1000 functional olfactory receptor genes which has also been found in species such as the rat or the dog (Rouquier *et al*., 2000; Glusman *et al*., 2001; Gilad *et al*., 2004).

Despite these marked differences in neuroanatomical and genetic features, all three species displayed very similar capabilities in discriminating between the enantiomeric odor pairs tested. A recent study has shown that the ability of rats to discriminate between the optical antipodes of carvone—one of the enantiomeric odor pairs of the present study—depends on the presence of at least two different receptors selective for D- and L-carvone respectively (Kirner *et al*., 2003). This suggests that the marked reduction in the number of functional olfactory receptor genes from New World primates to Old World monkeys to humans may not have affected those receptor types responsible for the detection and discrimination of the five pairs of optical antipodes that were perceived as qualitatively different by all three primate species tested. A possible reason that may underlie this—hypothetical—conservation of certain enantioselective olfactory receptor types across the order of primates is that there is still sufficient selective pressure acting on the species tested to favor individuals having the ability to distinguish between certain chiral odor pairs. This idea is supported by the fact that carvone and limonene, which both were discriminated by all three species, are widely distributed with both their enantiomers in a large variety of food plant extracts, whereas limonene oxide, which was not discriminated by all three species, is hardly ever found in essential oils (König *et al*., 1990; Mosandl *et al*., 1990). A significant correlation between the frequency of occurrence of enantiomers in flower odors and olfactory discrimination capabilities of honeybees has been reported by Laska and Galizia (2001). They also showed that the honeybees' performance in distinguishing between optical isomers is at least as good as that of squirrel monkeys—despite the fact that the former species has only ~100 different types of olfactory receptors compared to the 1000 found in the latter species.

Taken together, the present findings lend additional support to the growing body of evidence that between-species comparisons of neuroanatomical features or of the number of functional olfactory receptor genes are poor predictors of olfactory discrimination performance (Laska and Freyer, 1997; Laska and Teubner, 1998; Laska *et al*., 1999a,b). In order to further corroborate this idea, future studies should include additional species differing in relative size of olfactory brain structures and/or absolute size of olfactory receptor gene repertoire that should be tested using the same sets of odorants.

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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 E, 544–575.

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

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

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

Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D. and **Pääbo, S.** (2004) *Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates*. PLoS Biol., 2, 120–125.

Glusman, G., Yanai, I., Rubin, I. and **Lancet, D.** (2001) *The complete human olfactory subgenome*. Genome Res., 11, 685–702.

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

Hübener, F. and **Laska, M.** (2001) *A two-choice discrimination method to assess olfactory performance in pigtailed macaques,* Macaca nemestrina. Physiol. Behav., 72, 511–519.

Kirner, A., Deutsch, S., Weiler, E., Polak, E. H., and **Apfelbach, R.** (2003) *Concanavalin A application to the olfactory epithelium revels different sensory neuron poulations for the odour pair D- and L-carvone*. Behav. Brain Res., 138, 201–206.

König, W.A., Krebber, R., Evers, P. and **Bruhn, G.** (1990) *Stereochemical analysis of constituents of essential oils and flavor compounds by enantioselective capillary gas chromatography.* J. High Res. Chromatogr., 13, 328–332.

Laska, M. (2004) *Olfactory discrimination ability of human subjects for enantiomers with an isopropenyl group at the chiral center.* Chem. Senses, 29, 143–152.

Laska, M. and **Freyer, D.** (1997) *Olfactory discrimination ability for aliphatic esters in squirrel monkeys and humans*. Chem. Senses, 22, 457–465.

Laska, M. and **Hudson, R.** (1993) *Assessing olfactory performance in a New World primate,* Saimiri sciureus. Physiol. Behav., 53, 89–95.

Laska, M. and **Seibt, A.** (2002) *Olfactory sensitivity for aliphatic esters in squrrel monkeys and pigtail macaques*. Brain Res., 138, 201–206.

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 **Galizia, C.G.** (2001) *Enantioselectivity of odor perception in honeybees (*Apis mellifera carnica*)*. Behav. Neurosci., 115, 632–639.

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

Laska, M., Trolp, S. and **Teubner, P.** (1999b) *Odor structure–activity relationships correspond between human and non-human primates*. Behav. Neurosci., 113, 998–1007.

Mosandl, A., Deger, W., Gessner, M., Günther, C., Singer, G., Kustermann, A. and **Schubert, V.** (1990) *Chiral fruit flavour compounds: stereodifferentiation and fruit-specific distribution of enantiomers*. In Holmstedt, B., Frank, H. and Testa B. (eds), Chirality and Biological Activity. Alan R. Liss, New York, pp. 119–127.

Ohloff, G. (1994) Scent and Fragrances. The Fascination of odors and their Chemical Perspectives. Springer Verlag, 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, DC, pp. 151–157.

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

Rouquier, S., Blancher, A. and **Giorgi, D.** (2000) *The olfactory receptor gene repertoire in primates and mouse: evidence for reduction of the functional fraction in primates*. Proc. Natl. Acad. Sci. USA, 97, 2870–2874.

Siegel, S. and **Castellan, N.J.** (1988) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York.

Stephan, H., Baron, G. and **Frahm, H.D.** (1988) *Comparative size of brains and brain structures*. In Steklis, H. and Erwin, J. (eds), Comparative Primate Biology, Vol. 4. Alan R. Liss, New York, pp. 1–38.

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