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Olfactory sensitivity for putrefaction-associated thiols and indols in three species of non-human primate

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Summary

Using a conditioning paradigm, the olfactory sensitivity of four spider monkeys, three squirrel monkeys and three pigtail macaques to four thiols and two indols, substances characteristic of putrefaction processes and faecal odours, was assessed. With all odorants, the animals significantly discriminated concentrations below 1 p.p.m. (part per million) from the odourless solvent, and in several cases individual animals even demonstrated thresholds below 1 p.p.t. (part per trillion). The detection thresholds of 0.03 p.p.t. for indol in *Saimiri sciureus* and *Macaca nemestrina* and 0.96 p.p.t. for ethanethiol in *Ateles geoffroyi* represent the lowest values among the more than 50 odorants tested so far with these species and are in the same order of magnitude as the lowest detection thresholds reported so far in the rat and the mouse. The results showed (a) all three species of non-human primate to have a highly developed olfactory sensitivity for putrefaction-associated odorants, and (b) a significant correlation between perceptibility in terms of olfactory detection threshold and carbon chain length of the thiols, and a marked effect of the presence *vs* absence of a methyl group on perceptibility of the indols tested in two of the three species. The results support the hypotheses that (a) between-species differences in neuroanatomical or genetic features may not be indicative of olfactory sensitivity, and (b) within-species differences in olfactory sensitivity may reflect differences in the behavioural relevance of odorants.

Key words: olfactory sensitivity, detection thresholds, non-human primates, thiols, indols, putrefaction.

Introduction

A traditional view purports that differences in olfactory capabilities between species can be explained by differences in the relative or absolute size of their respective olfactory brain structures (e.g. Stephan et al., 1988; Walker and Jennings, 1991). A more recent version of this view states that the number of functional olfactory receptor (OR) genes – which some authors believe to co-vary with olfactory brain size (Gilad et al., 2004) – may allow predictions as to the efficiency of a species' sense of smell (Rouquier et al., 2000). Both views, however, have recently been called into question (Shepherd, 2004; Smith and Bhatnagar, 2004). An increasing number of studies now suggest that the behavioural relevance of odour stimuli rather than neuroanatomical features or the size of the repertoire of olfactory receptors may determine both a species' olfactory sensitivity and its discrimination performance (Laska et al., 2000; Laska et al., 2003a; Laska et al., 2006a).

In contrast to the widely held belief that primates are 'microsmatic', for example, several studies have demonstrated that their olfactory detection thresholds for certain odorants such as fruit-associated acetic esters are at least as low as, and in some cases even markedly lower than, those of the rat or dog, both species being commonly regarded as 'macrosmatic' based on the relative size of their olfactory bulbs and cortices (Hernandez Salazar et al., 2003; Laska and Seibt, 2002a). Similarly, honeybees have been shown to be better at discriminating between structurally related odorants such as flower-associated enantiomers compared with species having markedly larger numbers of functional OR genes (Laska and Galizia, 2001). Both findings can be plausibly explained by assuming that the odour stimuli in question differ in their behavioural relevance among the species mentioned, an assumption that is supported by reports on their ecology and behaviour.

Differences in the behavioural relevance of odorants may also explain within-species differences in olfactory performance. In rats, for example, the lowest olfactory detection threshold determined so far has been reported for 2,4,5-trimethylthiazoline (TMT), a volatile compound characteristic of fox faecal odour: the odour of a natural predator of the rat (Laska et al., 2005b).

In addition to predator avoidance, two of the main functions of the sense of smell are food selection and social communication. Avoidance of spoiled food should be of

particular importance to primates as they generally lack specific physiological detoxification mechanisms. Sulphur- and nitrogen-containing odorants such as thiols and indols have been found to be characteristic of putrefaction – that is, of the microbial degradation of proteins (Janzen, 1977; Barker, 1981; Kamiya and Ose, 1984). Interestingly, the same groups of odorants have also been described in the body-borne odours of primates, raising the possibility that they may also play a role in olfactory social communication (Tonzetich et al., 1978; Moore et al., 1987).

The three primate species employed here, spider monkeys, squirrel monkeys and pigtail macaques, are known to differ – at least to some degree – in their dietary habits, with the first-mentioned species showing the highest degree of frugivory and the last-mentioned species the lowest degree of fruit consumption (Caldecott, 1986; Clutton-Brock and Harvey, 1977; Ross, 1992). They also differ in their degree of phylogenetic relatedness to each other, with spider monkeys and squirrel monkeys representing New World primates and pigtail macaques being an Old World primate species, and in their use of olfactory cues for social communication (Caldecott, 1986; Epple, 1985; Kinzey, 1997). These between-species differences allowed us to assess whether such ecological factors might affect olfactory sensitivity to the odorants under investigation.

It was therefore the aim of the present study to determine olfactory detection thresholds for members of the chemical classes of thiols and indols in these three species of non-human primate. By comparing these threshold values with those obtained in earlier studies using the same methods and animals but with other classes of odorants, we aimed at further testing the hypothesis that within-species differences in olfactory sensitivity may reflect differences in the behavioural relevance of odorants. Employing odorants that share certain molecular features such as the type of sulphur- or nitrogen-containing functional group or structure of the carbon chain backbone, and differ from each other in others such as carbon chain length or presence *vs* absence of a methyl group, allowed us to additionally address the question of the impact of these features on the detectability of odorants.

Materials and methods

Animals

Testing was carried out using four adult female spider monkeys (*Ateles geoffroyi* L.), three adult male squirrel monkeys (*Saimiri sciureus* L.), and two adult male and one adult female pigtail macaque (*Macaca nemestrina* L.). All animals had served as subjects in previous olfactory experiments and were completely familiar with the basic test procedure. The animals of a given species were not of close genetic relation. Although only animals of one sex were available for testing in two of the three species, prior research did not indicate gender differences in sensitivity to odorants for either spider monkeys or squirrel monkeys.

Conditions of the animals' maintenance have been described in detail elsewhere (Hernandez Salazar et al., 2003; Laska and Seibt, 2002a). They were fed fresh fruit and vegetables *ad libitum.* The amount of food offered daily to the animals was such that left-overs were still present the next morning and thus it was unlikely that ravenous appetite affected the animals' choice behaviour in the tests. The experiments reported here comply with the 'Principles of animal care', publication no. 86-23, revised 1985, of the National Institutes of Health, and also with current laws in Germany and Mexico, the countries in which the study was performed.

Behavioural test

The experimental procedures for assessing olfactory detection thresholds in the three primate species have been described in detail elsewhere (Laska and Hudson, 1993; Hübener and Laska, 2001; Laska et al., 2003a). Briefly, the animals were tested using a food-rewarded instrumental conditioning paradigm. Olfactory detection thresholds were determined by testing the animals' ability to discriminate between increasing dilutions of an odorant used as the rewarded stimulus (S+), and the odourless solvent diethyl phthalate alone used as the unrewarded stimulus (S–). In each test trial, each monkey sniffed at both options and then decided on one of the alternatives by performing an operant response which, in the case of a correct decision, was food rewarded. Ten such trials were conducted per animal and session, and at least three sessions per experimental condition were performed. To minimize the possibility of adaptation, inter-trial intervals were at least 30 s and only one concentration step was tested per animal per day. Starting with a dilution of 1:10 000 with the thiols, 0.5 g l^{-1} with indol and 0.1 g l^{-1} with 3-methyl indol, each stimulus was successively presented in 10-fold dilution steps until an animal failed to significantly discriminate the odorant from the solvent. Subsequently, an intermediate concentration (0.5 log units between the lowest concentration that was detected above chance and the first concentration that was not) was tested in order to determine the threshold value more exactly.

Odorants

A set of six odorants belonging to the chemical classes of thiols (ethanethiol, 1-propanethiol, 1-butanethiol, and 1-pentanethiol) and indols (indol and 3-methyl indol) was used. The rationale for choosing these substances was to assess the monkeys' sensitivity to odorants that have been shown to be associated with putrefaction (Janzen, 1977; Barker, 1981; Kamiya and Ose, 1984). Additionally, the thiols used here are structurally similar to each other; that is, they share molecular properties such as type and position of the sulphur-containing functional group, and only differ from each other in carbon chain length (Fig. 1). Similarly, the indols used here share type and position of the nitrogen-containing functional group and only differ from each other in the presence *vs* absence of a methyl group, allowing us to assess the impact of these structural features on detectability. All substances were obtained from Fluka (Taufkirchen, Germany) and had a nominal purity of at least 99%. They were diluted using odourless diethyl phthalate as the solvent.

Data analysis

In the method described here, the animal had two options: (1) to correctly open the container that carries the positive stimulus (hit), and (2) to incorrectly open the container that carries the negative stimulus (false alarm). For each individual animal, the

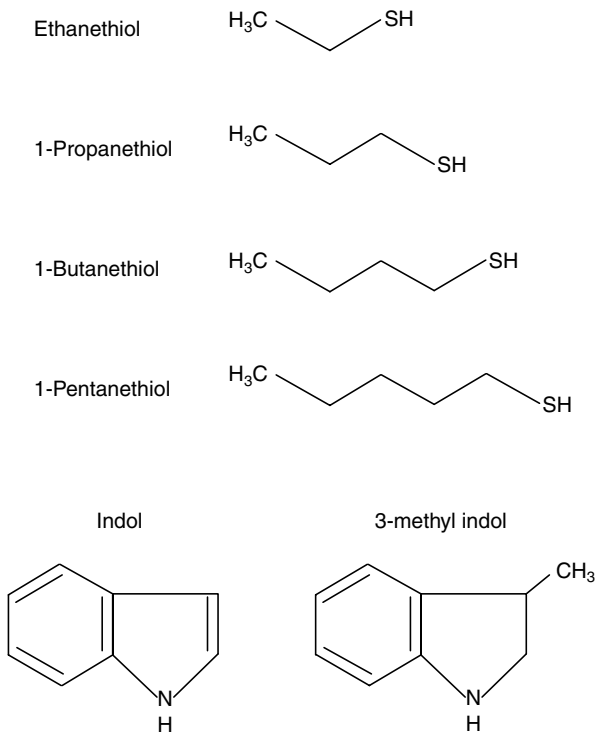

Fig. 1. Chemical structure of the thiols and indols used as odour stimuli.

percentage of hits from the best three consecutive sessions per dilution step, comprising at total of 30 decisions, was calculated and taken as the measure of performance. Significance levels were determined by calculating binomial *z* scores corrected for continuity from the number of correct and false responses for each individual and condition.

Correlations between olfactory threshold values of a given species (individual scores per stimulus were used) and carbon chain length of the thiols tested were calculated using the Spearman rank-correlation test and tested for significance by computing *t* values. Across-species comparisons of performance were conducted using Mann-Whitney *U* tests for independent samples. All tests were two-tailed and, if not otherwise mentioned, the alpha level was set at 0.05.

Results

Spider monkeys

Fig. 2 shows the performance of the spider monkeys in discriminating between various dilutions of a given thiol or indol and the odourless solvent. All four animals significantly distinguished dilutions as low as 1:3 billion ethanethiol, 1:3 million 1-propanethiol and 1-butanethiol, 1:300 000 1-pentanethiol, 15 mg l^{-1} indol and 0.3 mg l^{-1} 3-methyl indol from the solvent (binomial test, $P<0.05$), with single individuals even scoring better.

The individual spider monkeys generally demonstrated similar threshold values and with three of the six odorants (1-propanethiol, indol and 3-methyl indol) they differed only by a dilution factor of 10 between the highest- and the lowest-scoring animal. The largest difference between individuals in sensitivity to a given odorant was for a dilution factor of 100 and was found with ethanethiol, 1-butanethiol and 1-pentanethiol.

Table 1 summarizes the threshold dilutions for the four spider monkeys and shows various measurements of corresponding vapour phase concentrations (Weast, 1987), to enable easy comparison of the data obtained in the present study with those reported by other authors. In all cases, threshold dilutions correspond to vapour phase concentrations below 0.1 p.p.m.; in about half of the cases the animals were even able to detect concentrations below 1 p.p.b.; and with ethanethiol two of the animals detected concentrations below 1 p.p.t.

A significant positive correlation between detection threshold values for the spider monkeys and carbon chain length of the thiols tested was found (Spearman, $r_s=+0.55$, $P=0.0338$). This means that the sensitivity of the animals for the thiols decreased with increasing carbon chain length (see Fig. 5, upper panel).

Squirrel monkeys

Fig. 3 shows the performance of the squirrel monkeys in discriminating between various dilutions of a given thiol or indol and the odourless solvent. All three animals significantly distinguished dilutions as low as 1:300 000 ethanethiol and 1-propanethiol, 1:10 million 1-butanethiol and 1-pentanethiol, 0.05 mg l^{-1} indol, and 1 mg l^{-1} 3-methyl indol from the solvent (binomial test, $P<0.05$), with single individuals even scoring better.

The individual squirrel monkeys generally demonstrated similar threshold values and with three of the six odorants they differed only by a dilution factor of 3 (1-pentanethiol), 10 (3-methyl indol) or 33 (1-butanethiol) between the highest- and the lowest-scoring animal. The largest difference between individuals in sensitivity to a given odorant was for a dilution factor of 300 and was found with indol.

Table 2 summarizes the threshold dilutions for the three squirrel monkeys and shows various measurements of corresponding vapour phase concentrations (Weast, 1987). In all cases, threshold dilutions correspond to vapour phase concentrations below 1 p.p.m.; in about half of the cases the animals were even able to detect concentrations below 1 p.p.b.; and with indol two of the animals detected concentrations below 1 p.p.t.

A significant negative correlation between detection threshold values for the squirrel monkeys and carbon chain length of the thiols tested was found (Spearman, $r_s=-0.85$, $P=0.0051$). This means that the sensitivity of the animals for the thiols increased with increasing carbon chain length (see Fig. 5, middle panel).

Pigtail macaques

Fig. 4 shows the performance of the pigtail macaques in discriminating between various dilutions of a given thiol or indol and the odourless solvent. All three animals significantly distinguished dilutions as low as 1:3 million ethanethiol, 1:30 million 1-propanethiol, 1:300 million 1-butanethiol, 1:10 million 1-pentanethiol, 0.0005 mg l^{-1} indol and 3 mg l^{-1} 3-methyl indol from the solvent (binomial test, $P<0.05$), with single individuals even scoring better.

The individual pigtail macaques generally demonstrated similar threshold values and with three of the six odorants they differed only by a dilution factor of 3 (indol) or 10 (1-pentanethiol and 3-methyl indol) between the highest- and the

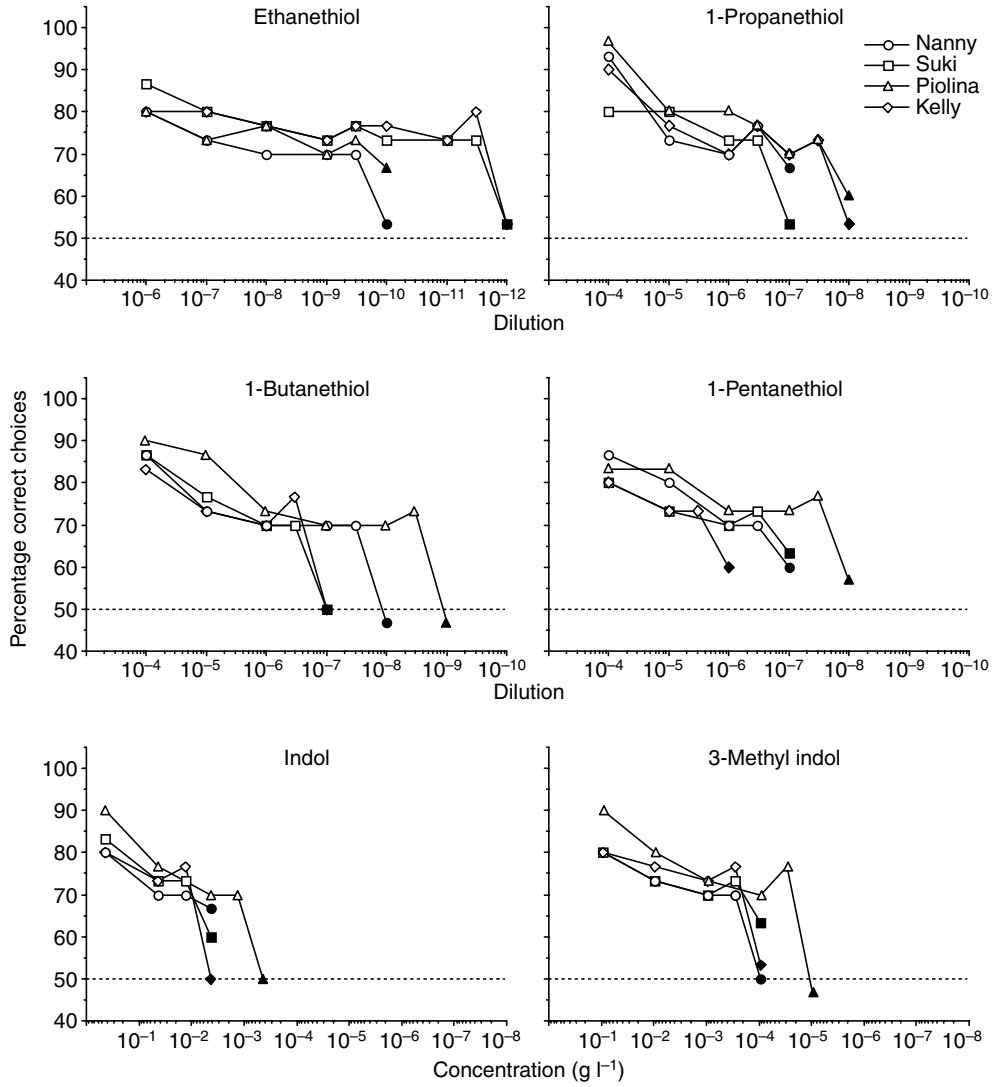

Fig. 2. Performance of four spider monkeys in discriminating between various dilutions of a given odorant and the odourless solvent. Each data point represents the percentage of correct choices from 30 decisions. The four different symbols represent data from each of the four individual animals tested. Filled symbols indicate dilutions that were not discriminated significantly above the chance level (binomial test, $P>0.05$; chance level shown by broken line).

lowest-scoring animal. In the case of 1-propanethiol and of 1-butanethiol, the animals even showed identical threshold values. The largest difference between individuals in sensitivity to a given odorant was for a dilution factor of 1000 and was found with ethanethiol.

Table 3 summarizes the threshold dilutions for the three pigtail macaques and shows various measurements of corresponding vapour phase concentrations (Weast, 1987). In all cases, threshold dilutions correspond to vapour phase concentrations below 0.1 p.p.m., in about half of the cases the animals were even able to detect concentrations below 1 p.p.b., and with indol all animals detected concentrations below 1 p.p.t.

No significant negative correlation between detection threshold values of the pigtail macaques and carbon chain length of the thiols tested was found (Spearman, r_s=–0.40, P=0.19). This means that the sensitivity of the animals for the thiols did not systematically vary as a function of carbon chain length (see Fig. 5, lower panel).

Across-species comparison of performance

Considering all six odorants combined, none of the three species performed significantly better – that is, showed lower detection thresholds – than any of the other two species (Mann-Whitney, $P>0.05$, for all comparisons). The same is true when comparing the performance between species for the thiols and the indols separately (Mann-Whitney, $P>0.05$, for all comparisons). Nevertheless, all four spider monkeys were more sensitive to ethanethiol than all three squirrel monkeys, whereas all three squirrel monkeys and all three pigtail macaques were more sensitive to indol than all four spider monkeys (see Fig. 6).

Discussion

The results of this study demonstrate, for the first time, that spider monkeys, squirrel monkeys and pigtail macaques have a high olfactory sensitivity for monomolecular odorants belonging to the chemical classes of thiols and indols. Further, they show a significant linear correlation between perceptibility

Table 1. *Olfactory detection threshold values in spider monkeys expressed as various measurements of vapour phase concentration*

	N	Dilution	Molecules cm^{-3}	p.p.m.	log p.p.m.	mol l^{-1}	log mol l^{-1}
Ethanethiol	2	1:3 billion	2.6×10^{9}	0.000096	–4.02	4.3×10^{-12}	–11.36
	2	1:300 billion	2.6×10^{7}	0.00000096	–6.02	4.3×10^{-14}	–13.36
1-Propanethiol	2	1:3 million	1.4×10^{12}	0.052	–1.29	2.3×10^{-9}	–8.63
	2	1:30 million	1.4×10^{11}	0.0052	–2.29	2.3×10^{-10}	–9.63
1-Butanethiol	2	1:3 million	4.3×10^{11}	0.016	–1.80	7.1×10^{-10}	–9.15
	1	1:30 million	4.3×10^{10}	0.0016	–2.80	7.1×10^{-11}	–10.15
	1	1:300 million	4.3×10^{9}	0.00016	–3.80	7.1×10^{-12}	–11.15
1-Pentanethiol	1	1:300 000	1.7×10^{12}	0.063	–1.20	2.8×10^{-9}	–8.55
	2	1:3 million	1.7×10^{11}	0.0063	–2.20	2.8×10^{-10}	–9.55
	1	1:30 million	1.7×10^{10}	0.00063	–3.20	2.8×10^{-11}	–10.55
Indol	3	15.0 mg l^{-1}	8.2×10^{10}	0.003	–2.52	1.4×10^{-10}	–9.38
	1	1.5 mg l^{-1}	8.2×10^{9}	0.0003	–3.52	1.4×10^{-11}	–10.87
3-Methyl indol	2	0.3 mg l^{-1}	1.0×10^{9}	0.000037	–4.43	1.7×10^{-12}	–11.78
	1	0.1 mg l^{-1}	3.0×10^{8}	0.000012	–4.95	5.0×10^{-13}	–12.30
	1	0.03 mg l^{-1}	1.0×10^{8}	0.0000037	–5.43	1.7×10^{-13}	–12.78

N indicates the number of animals.

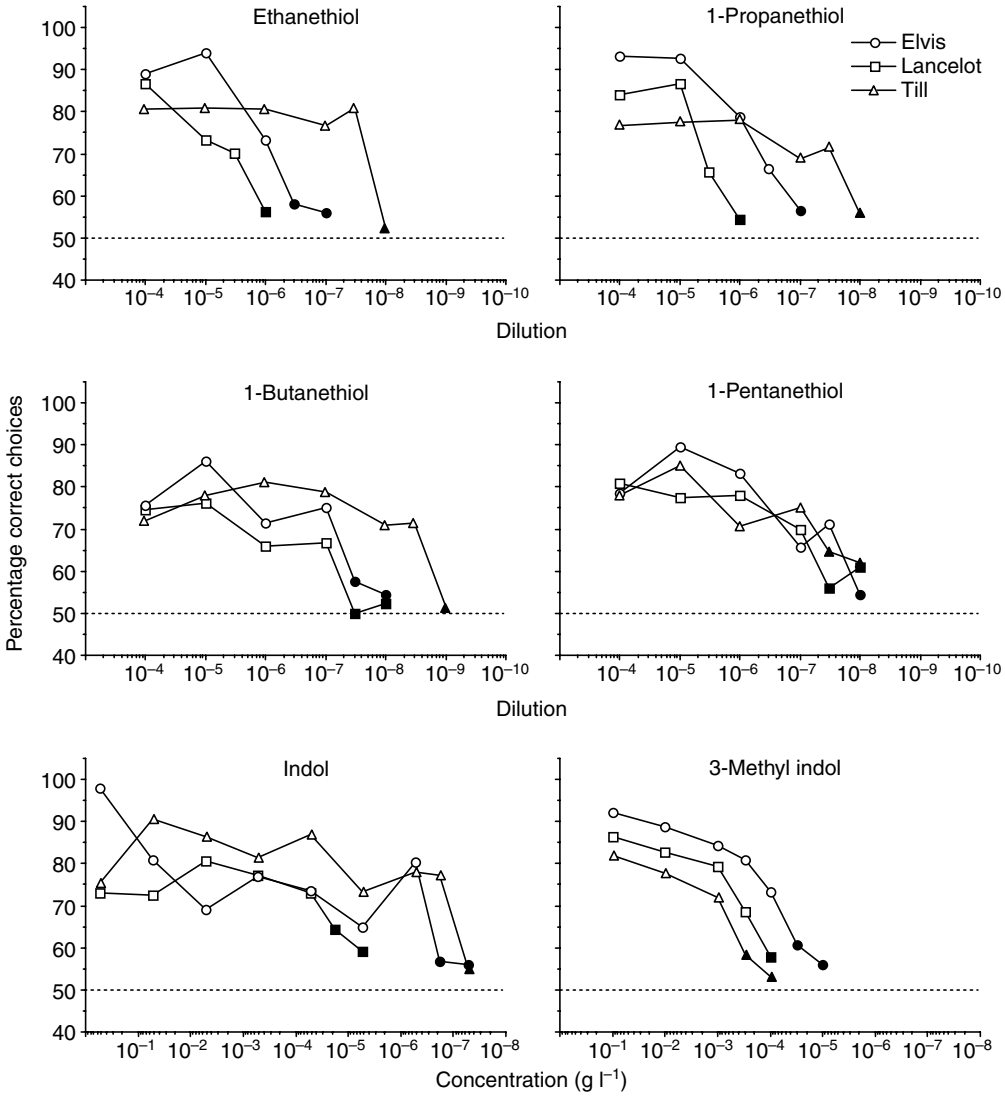

Fig. 3. Performance of three squirrel monkeys in discriminating between various dilutions of a given odorant and the odourless solvent. Each data point represents the percentage of correct choices from at least 30 decisions. The three different symbols represent data from each of the three individual animals tested. Filled symbols indicate dilutions that were not discriminated significantly above the chance level (binomial test, $P>0.05$; chance level shown by dashed line).

Table 2. *Olfactory detection threshold values in squirrel monkeys expressed as various measurements of vapour phase concentration*

	N	Dilution	Molecules cm^{-3}	p.p.m.	log p.p.m.	mol l^{-1}	log mol l^{-1}
Ethanethiol	1	1:300 000	2.6×10^{13}	0.96	–0.02	4.3×10^{-8}	–7.36
	1	1:1 million	7.9×10^{12}	0.29	–0.53	1.3×10^{-8}	–7.88
	1	1:30 million	2.6×10^{11}	0.0096	–2.02	4.3×10^{-10}	–9.36
1-Propanethiol	1	1:300 000	1.4×10^{13}	0.52	–0.29	2.3×10^{-8}	–7.63
	1	1:3 million	1.4×10^{12}	0.052	–1.29	2.3×10^{-9}	–8.63
	1	1:30 million	1.4×10^{11}	0.0052	–2.29	2.3×10^{-10}	–9.63
1-Butanethiol	2	1:10 million	1.3×10^{11}	0.0048	–2.32	2.2×10^{-10}	–9.67
	1	1:300 million	4.3×10^{9}	0.00016	–3.80	7.1×10^{-12}	–11.15
1-Pentanethiol	2	1:10 million	5.2×10^{10}	0.0019	–2.72	8.6×10^{-11}	–10.06
	1	1:30 million	1.7×10^{10}	0.00063	–3.20	2.8×10^{-11}	–10.55
Indol	1	0.05 mg l^{-1}	2.5×10^{8}	0.0000093	–5.03	4.2×10^{-13}	–12.38
	1	0.0005 mg l^{-1}	2.5×10^{6}	0.000000093	–7.03	4.2×10^{-15}	–14.38
	1	0.00015 mg l^{-1}	8.2×10^{5}	0.000000030	–7.52	1.4×10^{-15}	–14.87
3-Methyl indol	1	1.0 mg l^{-1}	3.0×10^{9}	0.00012	–3.95	5.0×10^{-12}	–11.30
	1	0.3 mg l^{-1}	1.0×10^{9}	0.000037	–4.43	1.7×10^{-12}	–11.78
	1	0.1 mg l^{-1}	3.0×10^{8}	0.000012	–4.95	5.0×10^{-13}	–12.30

N indicates the number of animals.

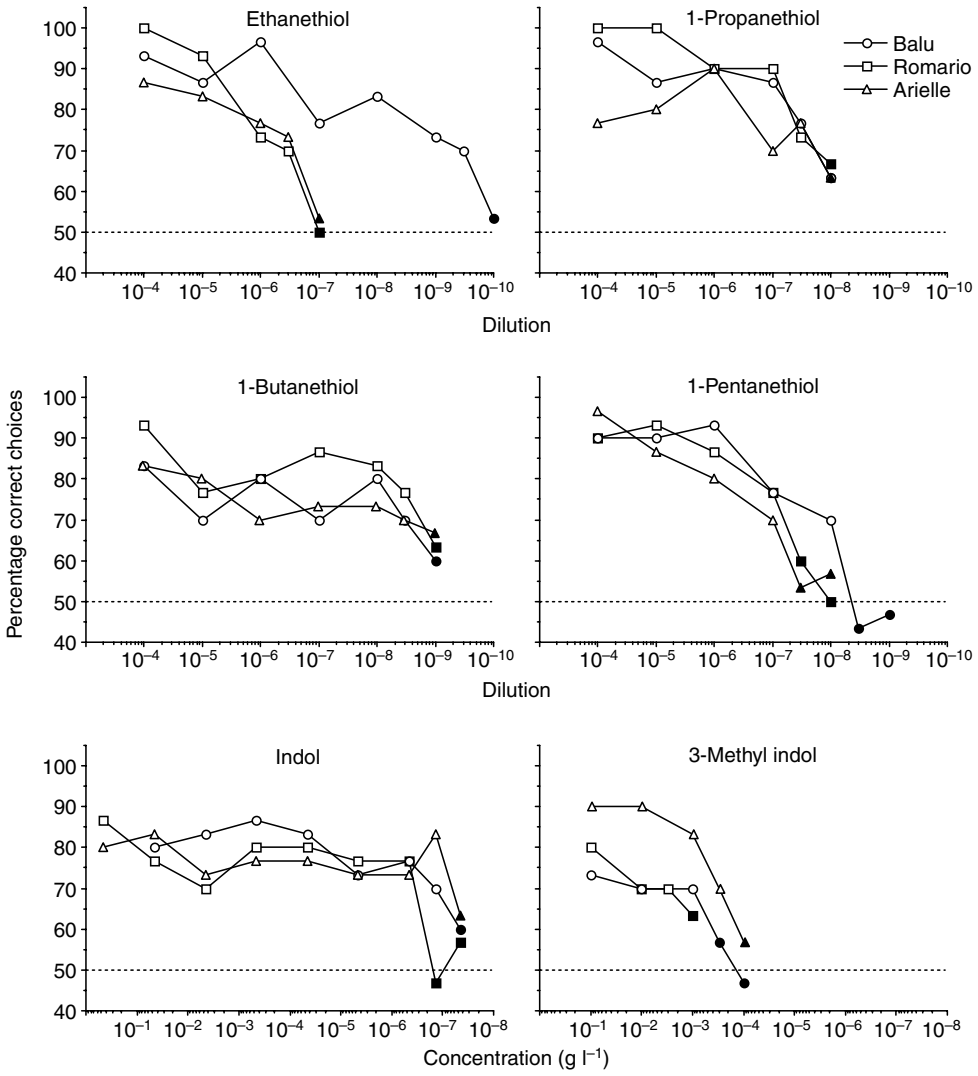

Fig. 4. Performance of three pigtail macaques in discriminating between various dilutions of a given odorant and the odourless solvent. Each data point represents the percentage of correct choices from 30 decisions. The three different symbols represent data from each of the three individual animals tested. Filled symbols indicate dilutions that were not discriminated significantly above the chance level (binomial test, $P>0.05$; chance level shown by broken line).

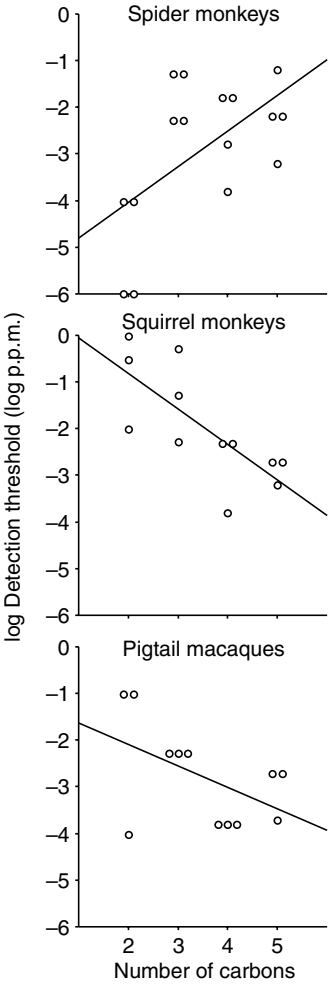

Fig. 5. Olfactory detection threshold values (expressed as vapour phase concentrations) of the spider monkeys, the squirrel monkeys and the pigtail macaques as a function of carbon chain length of the thiols tested. The solid line indicates the regression with the best goodness-of-fit according to the Spearman rank-correlation test.

in terms of olfactory detection thresholds and carbon chain length of the thiols tested, and a marked effect of the presence *vs* absence of a methyl group on the perceptibility of the indols tested in two of the three species.

Although only three or, in the case of the spider monkeys, four animals per species were tested, the results appear robust as interindividual variability was low and generally smaller than the range reported in studies on human olfactory sensitivity; that is, within three orders of magnitude (Stevens et al., 1988). In fact, for the majority of substances tested there was only a factor of 33 or smaller between the threshold values of the highest- and the lowest-scoring animal of a species. Further, with all substances tested, the animals' performance with the lowest concentrations presented dropped to chance level, suggesting that the statistically significant discrimination between higher concentrations of an odorant and the odourless diluent was indeed based on olfactory perception and not on other cues.

Fig. 6 compares the olfactory detection threshold values obtained with spider monkeys, squirrel monkeys and pigtail macaques for the thiols and indols tested with those from human subjects. Although such across-species comparisons should be considered with caution as different methods may lead to widely differing results, it seems admissible to state that human subjects do not generally perform poorer than the non-human primates tested – despite the fact that the relative size of the human brain structures devoted to processing olfactory information is markedly smaller than that of the non-human primates (Stephan et al., 1988), and despite the fact that the number of functional olfactory receptor genes in *Homo sapiens* (~350) is considerably smaller than that of *Macaca nemestrina* (~700) and of *Saimiri sciureus* and *Ateles geoffroyi* (~900) (Rouquier et al., 2000; Glusman et al., 2001; Gilad et al., 2004). Similarly, the pigtail macaques did not generally perform poorer with the odorants tested here compared with the spider monkeys and the squirrel monkeys, again despite the fact that the relative size of the olfactory brain and the size of the repertoire of functional OR types in this Old World primate species is smaller compared with the two other species, which are New World primates. These findings lend additional support to the notion that

Table 3. *Olfactory detection threshold values in pigtail macaques expressed as various measurements of vapour phase concentration*

	N	Dilution	Molecules cm^{-3}	p.p.m.	log p.p.m.	mol l^{-1}	log mol l^{-1}
Ethanethiol	2	1:3 million	2.6×10^{12}	0.096	–1.02	4.3×10^{-9}	–8.36
	1	1:3 billion	2.6×10^{9}	0.000096	–4.02	4.3×10^{-12}	–11.36
1-Propanethiol	3	1:30 million	1.4×10^{11}	0.0052	–2.29	2.3×10^{-10}	–9.63
1-Butanethiol	3	1:300 million	4.3×10^{9}	0.00016	–3.80	7.1×10^{-12}	–11.15
1-Pentanethiol	2	1:10 million	5.2×10^{10}	0.0019	–2.72	8.6×10^{-11}	–10.06
	1	1:100 million	5.2×10^{9}	0.00019	–3.72	8.6×10^{-12}	–11.06
Indol	1	0.0005 mg l^{-1}	2.5×10^{6}	0.000000093	–7.03	4.2×10^{-15}	–14.38
	2	0.00015 mg l^{-1}	8.2×10^{5}	0.000000030	–7.52	1.4×10^{-15}	–14.87
3-Methyl indol	1	3.0 mg l^{-1}	1.0×10^{10}	0.00037	–3.43	1.7×10^{-11}	–10.78
	1	1.0 mg l^{-1}	3.0×10^{9}	0.00012	–3.95	5.0×10^{-12}	–11.30
	1	0.3 mg l^{-1}	1.0×10^{9}	0.000037	–4.43	1.7×10^{-12}	–11.78

N indicates the number of animals.

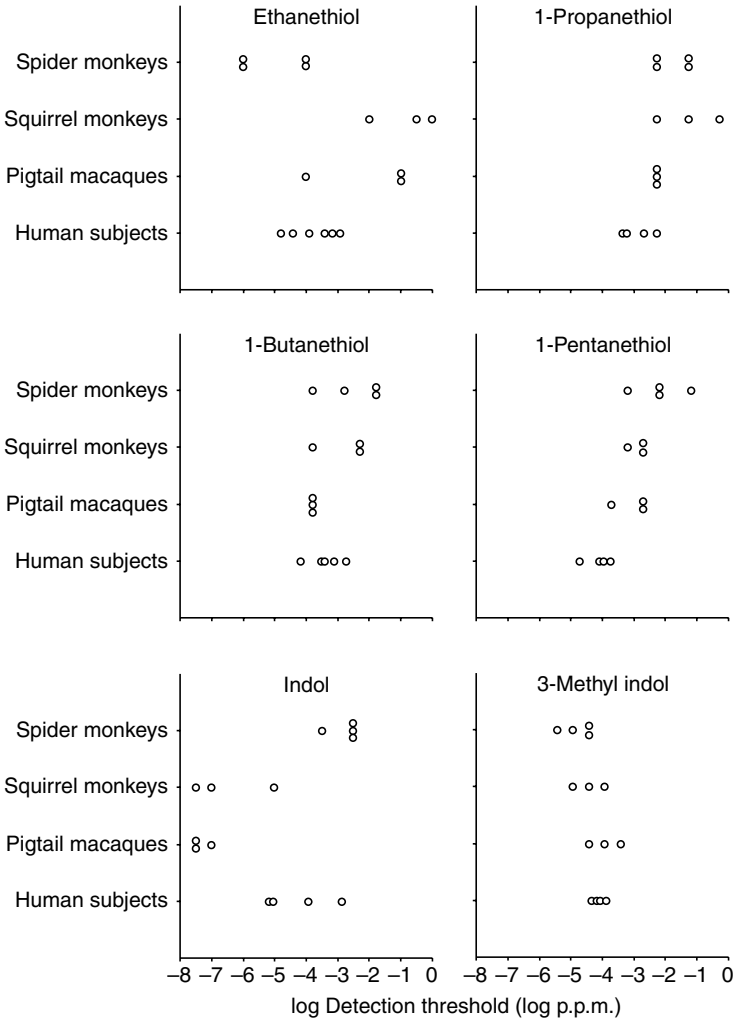

Fig. 6. Comparison of the olfactory detection threshold values (expressed as vapour phase concentrations) of the spider monkeys, the squirrel monkeys and the pigtail macaques for the odorants tested with those of human subjects (van Gemert, 2003). Data points of the three monkey species represent individual threshold values, and data points of the human subjects represent mean values from different studies.

between-species differences in neuroanatomical or genetic features may not be indicative of olfactory sensitivity – at least within the order of primates. Unfortunately, only very few studies so far have tested some of the thiols and indols used here with other species. Snyder and Peterson (Snyder and Peterson, 1979) reported olfactory detection thresholds of blackbilled magpies (*Pica pica*) and pigeons (*Columba livia*) for ethanethiol to be 6 and 8 p.p.m., respectively, and for 1-butanethiol to be 2 p.p.m. in both species. Smith and Paselk (Smith and Paselk, 1986) reported the turkey vulture (*Cathartes aura*) to respond to concentrations of ethanethiol as low as 22 p.p.m. It should be mentioned, however, that these studies employed changes in respiration frequency and heart rate, respectively, to determine olfactory detection thresholds, and both methods are known to be less sensitive than operant conditioning procedures (Hastings, 2003).

A within-species comparison between the detection threshold values of the present study with those obtained in earlier studies using the same methods and animals but with other classes of odorants such as aliphatic esters (Hernandez Salazar et al., 2003; Laska and Seibt, 2002a), alcohols (Laska and Seibt, 2002b; Laska et al., 2006a), aldehydes (Laska et al., 2003b; Laska et al., 2006a), ketones (Laska et al., 2005a), carboxylic acids (Laska et al., 2000; Laska et al., 2004), terpenes (Laska et al., 2006c), thiazoles (Laska et al., 2005b), or steroids (Laska et al., 2005c; Laska et al., 2006b) reveals that in all three species of primate at least one of the putrefaction-associated odorants employed here (indol with squirrel monkeys and pigtail macaques, ethanethiol and 3-methyl indol with the spider monkeys) yielded the lowest detection thresholds among the more than 50 odorants tested so far. This finding is in line with reports showing that human subjects are particularly sensitive to thiols and indols (van Gemert, 2003). It should be emphasized that the 0.03 p.p.t. indol that both *Saimiri sciureus* and *Macaca nemestrina* were able to perceive and the 0.96 p.p.t. ethanethiol that *Ateles geoffroyi* was able to detect are in the same order of magnitude as the lowest detection thresholds determined so far in the rat (0.04 p.p.t. 2,4,5-trimethylthiazoline) (Laska et al., 2005b) and in the mouse (4.8 p.p.t. pentyl acetate) (Walker and O'Connell, 1986), both species presumed to be macrosmatic.

The most plausible explanation for the high sensitivity to thiols and indols found with all three species of non-human primate tested here is that members of these chemical classes may play an important role in controlling their behaviour. Goff and Klee (Goff and Klee, 2006) demonstrated that food-associated volatiles may provide important information about the nutritional makeup and health value of foods. As thiols and indols, in turn, have been found to be major products of the microbial degradation of proteins (Barker, 1981; Kamiya and Ose, 1984) and thus of putrefaction processes, which are usually accompanied by the production of toxins (Janzen, 1977), it seems reasonable to assume that primates should be highly sensitive to such compounds in order to avoid intoxication. Indeed, the food selection behaviour of primates suggests that they use their sense of smell for the evaluation of potential food items (Laska et al., 2007; Visalberghi and Addessi, 2000) and thus supports this assumption.

Indols and thiols have also been found to be major compounds of primate faecal odours (Dehnhard et al., 1991; Moore et al., 1987) and breath odour (Ochiai et al., 2001; Phillips et al., 1999). In both human subjects and non-human mammals, oral breath odour has been demonstrated to be indicative of health status (Eubanks, 2006; Kostelc et al., 1981; Sanz et al., 2001), and in humans the concentrations of volatile sulphur compounds of mouth air have been shown to vary as a function of the menstrual cycle in females (Tonzetich et al., 1978). This raises the possibility that oral breath odour may convey olfactory social information about the health and oestrus status in female primates. Similarly, the composition of human faecal odours is known to vary with health status (Garner et al., 2007) and the obvious interest that a variety of non-human

primate species display in their faeces raises the possibility that faecal odour, too, may convey information about the health status and dietary composition of conspecifics. Additionally, it has been hypothesized that the sulphurous metabolites of meat digestion are important for the repellency of predator urine and faecal odours to potential prey (Nolte et al., 1994), and primates have been shown to actively avoid such odours (Sündermann et al., 2005). Taken together, these findings strongly support the notion that indols and thiols should be of high behavioural relevance for non-human primates.

Differences in dietary habits have repeatedly been shown to plausibly explain differences in chemosensory performance between species (Spector, 2000). Among New World primates, for example, the degree of frugivory has been found to correlate positively with sensitivity for food-associated mono- and disaccharides (Laska, 2000). Similarly, the proportion of animal protein in the diet of primates appears to correlate negatively with their sensitivity for monosodium glutamate (Laska and Hernandez Salazar, 2004). In the olfactory domain with its countless types of stimuli and perceptual qualities, however, such correlations are less easy to establish. The three primate species studied here have been reported to differ markedly in the proportion of animal matter in their diet, with up to 72% of total intake in the squirrel monkey compared with only 1% in the spider monkey, with pigtail macaques (13% of total intake) taking an intermediate position. The spider monkeys' diet, in turn, is known to be composed of up to 90% fruit and seeds whereas the corresponding percentages for pigtail macaques (70%) and squirrel monkeys (26%) are markedly lower (Caldecott, 1986; Clutton-Brock and Harvey, 1977; Ross, 1992). Unfortunately, there is only very little information available on whether the microbial degradation of animal and plant protein leads to different proportions or frequencies of occurrence of the thiols and indols tested here, which might explain the between-species differences in sensitivity for individual odorants found in the present study.

All three species of primate tested here have been reported to display anogenital sniffing (Hopf, 1974; Klein, 1971; Reite and Short, 1980) and thus exposure to conspecific faecal odours that may convey behaviourally relevant information. However, in this context, too, there is too little quantitative information available with regard to both the frequency of such behaviours and possible differences in the composition of faecal odours among the three primate species to draw conclusions that might explain the observed odorant-specific differences in sensitivity. Future studies should therefore aim at analysing the chemical environment of non-human primate species, with particular emphasis on differences in the frequency of occurrence of odorants presumed to play a role in controlling their behaviour.

A second aspect of the present study is our finding of a significant correlation between olfactory detection thresholds and carbon chain length of the thiols in the spider monkeys and the squirrel monkeys (see Fig. 5), and a marked effect of the presence *vs* absence of a methyl group on the detectability of indols in the squirrel monkeys and pigtail macaques (see Fig. 6). Corresponding correlations between olfactory sensitivity and length of the carbon chain backbone have also been found in all three species of non-human primate as well as in human subjects for homologous series of esters, alcohols, aldehydes, ketones and carboxylic acids (Laska and Seibt, 2002a; Laska and Seibt, 2002b; Laska et al., 2000; Laska et al., 2003b; Laska et al., 2004; Laska et al., 2005a; Laska et al., 2006a; Cometto-Muniz and Cain, 1994). This suggests that this type of correlation might not be restricted to classes of odorants with oxygen-containing functional groups but may represent a more general phenomenon. This should not be surprising, considering that the carbon chain length of odorant molecules has been shown to be an important determinant of the specificity of interaction between stimulus and receptor (Gaillard et al., 2002), as well as of the chemotopic organization, and thus odour quality coding within the olfactory bulb (Johnson et al., 2004).

A marked effect of the presence *vs* absence of a methyl group on the detectability of odorants has also been reported in all three species of primate for terpenes (Laska et al., 2006c). Here, too, the present findings suggest that this phenomenon may not be restricted to odorants with oxygen moieties.

Our finding of a lack of correlation between olfactory detection thresholds and carbon chain length of the thiols in the pigtail macaques (see Fig. 5), and between detectability and the presence *vs* absence of a methyl group in the spider monkeys (see Fig. 6) may at first seem difficult to explain. However, with regard to differences in across-odorant patterns of olfactory sensitivity it should be considered that the quantitative distribution of individual receptor types, each responding selectively to a limited range of carbon chain lengths and functional groups, may differ between species. This, in turn, may plausibly explain why one species may show a regular connection between sensitivity and a given molecular structural feature whereas another species does not.

Taken together, the results of the present study support the hypotheses that (a) between-species differences in neuroanatomical or genetic features may not be indicative of olfactory sensitivity, and (b) within-species differences in olfactory sensitivity may reflect differences in the behavioural relevance of odorants.

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