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ORIGINAL ARTICLE

Olfactory sensitivity for six amino acids: a comparative study in CD-1 mice and spider monkeys

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Abstract Using a conditioning paradigm, the olfactory sensitivity of five CD-1 mice for the L- and D-forms of cysteine, methionine, and proline was investigated. With all six stimuli, the animals discriminated concentrations $\leq$0.1 ppm (parts per million) from the odorless solvent, and with three of the six stimuli the best-scoring animals were even able to detect concentrations <0.1 ppb (parts per billion). Three spider monkeys tested in parallel were found to detect the same six stimuli at concentrations <1 ppm, and with four of the six stimuli the best-scoring animals detected concentrations $\leq$1 ppb. Both CD-1 mice and spider monkeys displayed a higher olfactory sensitivity with the L- and D-forms of cysteine and methionine than with the prolines, suggesting an important role of the sulfur-containing functional groups for detectability. Accordingly, the across-odorant patterns of detection thresholds obtained with mice and spider monkeys showed a significant positive correlation. A comparison of the detection thresholds between the two species tested here and those obtained in human subjects suggests that neither the number of functional olfactory receptor genes nor the absolute or the relative size of the olfactory bulbs reliably predicts a species' olfactory sensitivity for amino acids.

Keywords Olfactory detection thresholds · Cysteine · Methionine · Proline

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Introduction

Amino acids are known to evoke specific taste sensations in a wide variety of vertebrate species ranging from fishes (Caprio 1977) to humans (Schiffman et al. 1981). In a series of landmark studies, Caprio and co-workers demonstrated that fishes are able to perceive amino acids not only via the gustatory system but also via the olfactory system and that the latter is more sensitive than the former for this group of stimuli (Caprio 1977; Caprio and Byrd 1984; Nikonov and Caprio 2001, 2007a). Electrophysiological studies revealed that certain molecular properties of amino acids such as chirality, functional groups, and side chain polarity are encoded by the fish olfactory system and form the basis for the odorant-specific detection and olfactory discrimination of amino acids (Nikonov and Caprio 2007b). Recent genetic studies have shown that the olfactory receptors interacting with amino acids are not confined to fishes but can be found in all classes of vertebrates (Niimura and Nei 2006). Surprisingly little, however, is known about the olfactory properties of amino acids in non-aquatic vertebrates. This is surprising given that amino acids are widely present in free form in the food of land-living vertebrates (Maarse 1991) and given that amino acids are known to play a crucial role as olfactory cues in some vertebrates other than fishes (Ferrer and Zimmer 2007; Inoue and Nakatani 2010). A recent study now demonstrated that human subjects are capable of detecting and discriminating the odor of certain amino acids (Laska 2010).

In order to gain information about the olfactory detectability of and sensitivity for amino acids in nonhuman mammals, it was therefore the aim of the present study to determine olfactory detection thresholds for six amino acids in a rodent species, the mouse, and in a nonhuman primate

species, the spider monkey. Using the L- and D-forms of cysteine, methionine, and proline allowed us to additionally assess the impact of chirality and other molecular structural features on olfactory perception of these odorants. Comparing the olfactory detection thresholds determined here with those obtained in human subjects (Laska 2010) allowed us to assess whether neuroanatomical features such as the size of olfactory brain structures or genetic features such as the number of functional olfactory receptor genes correlate with olfactory sensitivity for amino acids.

Materials and methods

Animals

Testing was carried out using five adult male CD-1 mice *(Mus musculus)* and three adult female spider monkeys *(Ateles geoffroyi,* Kuhl 1820). The rationale for choosing this outbred strain of mice was to use animals with a genetic background that is more similar to wild-type mice than that of inbred strains. Furthermore, data on olfactory detection thresholds for a homologous series of aliphatic aldehydes (Laska et al. 2006b), structurally related alkylpyrazines (Laska et al. 2009) and monoterpenes (Joshi et al. 2006) were obtained in earlier studies using the same mouse strain. The rationale for choosing spider monkeys, in addition to the aforementioned reasons, was that data on olfactory detection thresholds for homologous series of aliphatic esters (Hernandez Salazar et al. 2003), carboxylic acids (Laska et al. 2004), alcohols, and aldehydes (Laska et al. 2006c), as well as for thiols and indols (Laska et al. 2007), monoterpenes (Joshi et al. 2006), monoterpene alcohols (Laska et al. 2006a), steroids (Laska et al. 2005, 2006d), and alkylpyrazines (Laska et al. 2009) were obtained in earlier studies using the same animals, allowing us to compare their performance between the amino acids tested here and members of other chemical classes. Maintenance of both species has been described in detail elsewhere (mice: Laska et al. 2006b; spider monkeys: Laska et al. 2003).

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 Swedish and Mexican laws.

Stimuli

A set of six odorants comprising the L- and D-forms of cysteine, methionine, and proline was used. All substances were of the highest available purity (>99.5% with the three L-amino acids, and >99.0% with the three D-amino acids) and were obtained from Sigma-Aldrich (St. Louis, MO). They were diluted using demineralized water. Gas phase concentrations for the headspace above the diluted odorants were calculated using published vapor pressure data (Dykyi et al. 2001) and corresponding formulae (Weast 1987). Figure 1 shows the molecular structure of the odorants.

Behavioral tests

The mice were tested using a water-rewarded instrumental conditioning paradigm (Bodyak and Slotnick 1999). The test apparatus consisted of an automated liquid-dilution olfactometer (Knosys, Tampa, FL) connected to an operant chamber (15 × 20 × 15 cm) made of transparent plexiglass. A 17-mm inside diameter glass tube, mounted

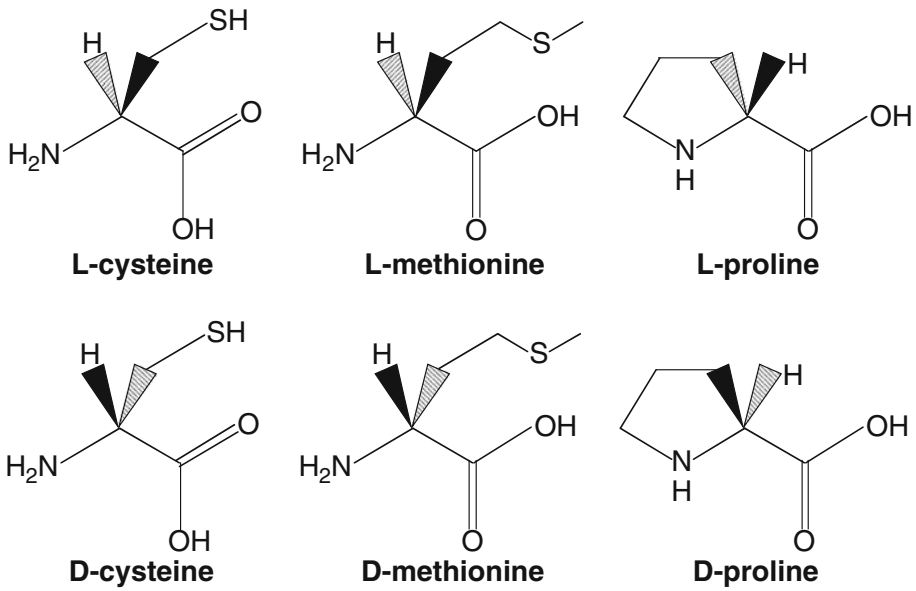

Fig. 1 Chemical structure of the amino acids used

vertically on the outside of one of the operant chamber's walls served for the delivery of odor stimuli. A 15-mm diameter hole through the plexiglass wall and the glass tube (from here on this hole is named "odor port") allowed the mouse to insert its snout into the glass tube and to sample the airstream provided by the olfactometer. An infrared photo cell was used to detect snout insertions into the odor port and to trigger the presentation of odor stimuli. A 13-gauge stainless steel tube ending in a 3-mm diameter ball served to deliver water reinforcement. The outlet of the steel tube was located on the inner wall of the glass tube opposite the odor port. Thus, a mouse that had inserted its snout into the odor port was only millimeters away from the outlet of the steel tube and could easily reach it in order to lick the water. A ventilator mounted on the wall of the operant chamber opposite the odor port provided a permanent positive air pressure inside the operant chamber and thus prevented the odorized air from the glass tube bearing the odor port to enter and contaminate the operant chamber. A second ventilator connected to the far end of the glass tube bearing the odor port served as an exhaust pump which prevented the odorized air to enter and contaminate the test room in which the operant chamber and olfactometer were located.

The olfactometer provided a constant airstream of 1,950 cm^3/min. Eight odor saturator bottles of 250 ml volume containing 10 ml of a given odorant at desired dilutions served as the source for the odor stimuli delivered to the odor port. Upon opening of a pinch valve, the saturated headspace above the odorant dilution was transported at a rate of 50 cm^3/min and mixed into the constant airstream of 1,950 cm^3/min, thus producing a 40-fold dilution of the saturated headspace when the odor bolus arrived at the odor port. Gas chromatographic analyses performed by the manufacturer of the olfactometer confirmed that the odorant concentration at the odor port is indeed a factor of 40 lower than the concentration of the saturated headspace above the odorant in the corresponding saturator bottle.

Using standard instrumental conditioning procedures the animals were trained to insert their snout into the odor port. This triggered a 2-s presentation of either an odorant used as the rewarded stimulus (S+) or a blank (headspace of the solvent) used as the unrewarded stimulus (S−). Licking at the steel tube which provided 2.5 µl of water reinforcement in response to presentation of the S+ served as the operant response.

Testing started at a concentration of 400 mM of a given odorant in the saturator bottles. The air dilution provided by the olfactometer (see explanation above) led to a gas phase concentration at the odor port (where the animal sampled the stimulus) equivalent to the headspace above a 10-mM solution of the odorant. This dilution was tested on two subsequent days (i.e. for a total of 10 blocks of 20 trials) to allow the animals to build a robust association between a given odorant and its reward value. Each block of 20 trials was composed of 10 presentations of the S+ and 10 presentations of the S-, in pseudorandomized order.

To determine detection thresholds for the odorants, the mice were then presented with tenfold increasing dilutions (i.e. lower concentrations) of the rewarded stimulus (S+) for two blocks of 20 trials (i.e. a total of 40 trials) per stimulus concentration until they failed to discriminate it from the unrewarded stimulus (S−). Subsequently, they were presented (again, for two blocks of 20 trials) with an intermediate concentration (0.5 log units between the lowest concentration that was detected and the first concentration that was not) in order to determine the threshold value more exactly.

The mice were maintained on a 1.5 ml/day water deprivation schedule.

The spider monkeys were tested using a food-rewarded two-choice instrumental conditioning paradigm (Laska et al. 2003). The test apparatus consisted of a 50-cm long and 6-cm wide metal bar with two cube-shaped opaque PVC boxes with a side length of 5.5 cm attached to it at a distance of 22 cm from each other. Each container was equipped with a tightly closing hinged metallic lid, hanging 2 cm down the front of the container. From the center of the front part of the lid, a pin of 3 cm length extended towards the animal and served as a lever to open the lid. On top of each lid was a metal clip attached. This clip held a 70 × 10 mm absorbent paper strip (Schleicher & Schuell, Einbeck, Germany) which was impregnated at its distal end with 10 µl of an odorant used as rewarded stimulus (S+) or with 10 µl of the odorless solvent used as unrewarded stimulus (S−). The paper strips extended approximately 3 cm into the cage when the apparatus was presented to the animals. The box with the odorized paper strip attached to the lid contained a food reward, a Kellogg's Honey Loop®, while the one with the odorless paper strip did not.

When presented with the test apparatus the monkeys sniffed both paper strips as much as they liked and then decided to open one of the boxes. In the rare cases when a monkey tried to open a box without prior sniffing or tried to open both boxes, the experimenter held a chain connected to the lid tight so that the animal could not move the lid. After the decision and, in the case of a correct choice, after food retrieval the apparatus was immediately removed and prepared for the next presentation out of sight from the monkeys. Each monkey received three blocks of ten trials (i.e. three sessions) per day. In five of the ten trials of a session, the left box was baited and in the other five trials the right box was baited. The order of the ''correct'' and the ''wrong'' sides was randomized with the limitation that one box was not baited more often than three times in a row.

Control tests without a food reward being present in the box bearing the paper strip with the S+ resulted in the same high level of correct choices as tests with a food reward being present in the box. Further, the animals consistently failed to perform above chance level when the odorant was presented at subthreshold (i.e. undetectable) concentrations, despite a food reward being present in the box bearing the paper strip with the S+. Together, this excludes the possibility that the monkeys smelled the food reward inside the box.

The animals were tested individually to avoid distraction from conspecifics. To this end, an animal voluntarily entered a small test cage (80 × 50 × 50 cm) adjacent to the group enclosure which could be closed by a sliding door for temporary separation. The animal sat on a bar mounted horizontally and parallel to the front side of the test cage. This front side of the test cage consisted of a stainless steel mesh with a width of 1 cm and had two openings of 5 × 5 cm allowing the animal to reach through the mesh, open the lid of one of the boxes of the test apparatus and retrieve the food reward. The test apparatus could be attached to the outside of the front side of the test cage in such a way that the lids of the boxes were at a height consistent with the reach-through openings.

Testing started at a dilution of 100 mM of a given odorant. This dilution was tested on three subsequent days (i.e. for nine sessions comprising a total of 90 trials) to allow the animals to build a robust association between a given odorant and its reward value. In the rare cases when an animal failed to score more than 70% correct choices on the third day, a threefold higher concentration of the same odorant was tested on the next day.

To determine detection thresholds for the odorants, the monkeys were then presented with tenfold increasing dilutions (i.e. lower concentrations) of the rewarded stimulus (S+) for three sessions (i.e. a total of 30 trials) per dilution step until they failed to discriminate it from the unrewarded stimulus (S−). Subsequently, they were presented with an intermediate dilution step (0.5 log units between the lowest concentration that was detected and the first concentration that was not) in order to determine the threshold value more exactly.

The spider monkeys were not maintained on a food deprivation schedule but were tested in the morning prior to the presentation of their daily ration of food.

Data analysis

For each individual animal, the percentage of correct choices from 40 (mice) and 30 (spider monkeys) trials per dilution step was calculated. With the mice, correct choices consisted both of licking in response to presentation of the S+ and not licking in response to the S−, and errors consisted of animals showing the reverse pattern of operant responses, i.e. not licking in response to the S+ and licking in response to the S−. With the spider monkeys, correct choices consisted both of animals opening a box equipped with the S+ and failing to open a box equipped with the S−. Conversely, errors consisted of animals opening a box equipped with the S− or failing to open a box equipped with the S+.

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. All tests were two-tailed and the alpha level was set at 0.05.

Results

CD-1 mice

Figure 2 shows the performance of the mice in discriminating between various dilutions of a given stimulus and the odorless solvent. All five animals significantly distinguished dilutions as low as 0.01 mM L-cysteine, 0.03 mM D-cysteine, 3.3 mM L-methionine, 0.3 mM D-methionine, and 10 mM L- and D-proline, respectively, from the solvent (binomial test, $p < 0.05$), with some individuals even scoring better.

The individual mice generally demonstrated similar detection threshold values with a given stimulus. With two of the six stimuli (L-cysteine and D-proline) they differed only by a dilution factor of 3 between the highest- and the lowest-scoring animal, and with L-proline all five animals even scored identical threshold values. In the case of D-cysteine and D-methionine, the individual threshold values differed by a factor of 33. The largest difference in sensitivity for a given stimulus between individuals was a dilution factor of 100 and was found with L-methionine.

Table 1 summarizes the threshold dilutions of the mice and shows various measures of corresponding gas phase concentrations (Weast 1987) allowing readers to easily compare the data obtained in the present study to those reported by other authors using one of these convertible measures. In all cases, threshold dilutions correspond to gas phase concentrations <0.1 ppm, and with three of the six stimuli the best-scoring animals were even able to detect concentrations <0.1 ppb.

Figure 4 compares the detection threshold values of the mice (circles) for the six stimuli tested. All five animals were less sensitive to the L- and D-forms of proline than to the L- and D-forms of cysteine and proline, respectively. Similarly, all five animals were more sensitive to L-cysteine than to L-methionine, and to D-cysteine than to D-methionine.

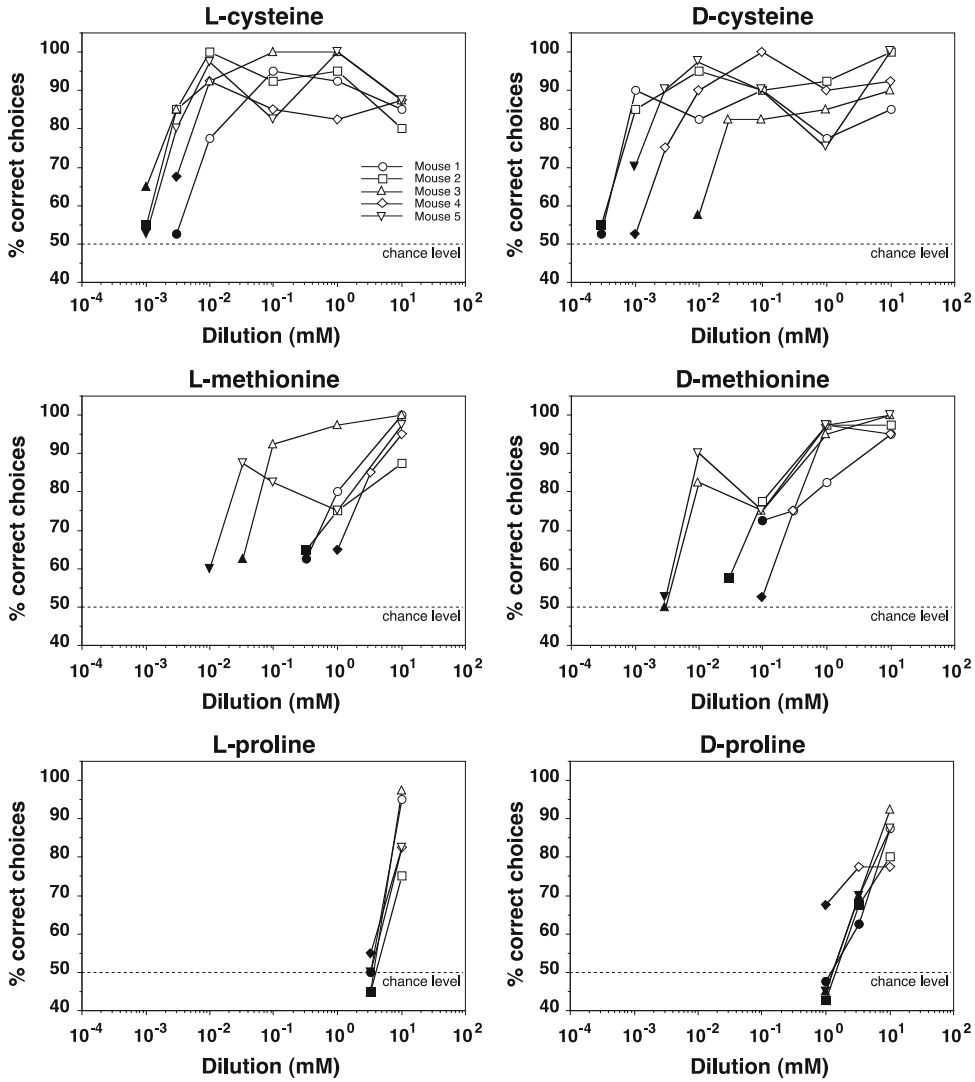

Fig. 2 Performance of CD-1 mice in discriminating between various dilutions of an amino acid and demineralized water used as odorless solvent. Each data point represents the percentage of correct choices from a total of 40 decisions per individual animal. The *five different symbols* represent data from each of the five individual animals tested per stimulus. *Filled symbols* indicate dilutions that were not discriminated significantly above chance level (binomial test, $p > 0.05$)

Spider monkeys

Figure 3 shows the performance of the spider monkeys in discriminating between various dilutions of a given stimulus and the odorless solvent. All three animals significantly distinguished dilutions as low as 1 mM L-cysteine, 0.3 mM D-cysteine, 3 mM L-methionine, 1 mM D-methionine, 30 mM L-proline, and 0.3 mM D-proline from the solvent (binomial test, $p < 0.05$), with some individuals even scoring better.

The individual spider monkeys generally demonstrated similar detection threshold values with a given stimulus. With two of the six stimuli (L-cysteine and D-methionine) they differed only by a dilution factor of 3 between the highest- and the lowest-scoring animal, and with D-cysteine and D-proline all three animals even scored identical threshold values. The largest difference in sensitivity for a given stimulus between individuals was a dilution factor of 10 and was found with L-methionine and L-proline.

Table 2 summarizes the threshold dilutions of the spider monkeys and shows various measures of corresponding gas phase concentrations. In all cases, threshold dilutions correspond to gas phase concentrations <1 ppm, and with four of the six stimuli the best-scoring animals were even able to detect concentrations ≤1 ppb.

Figure 4 compares the detection threshold values of the spider monkeys (squares) for the six stimuli tested. All three animals were less sensitive to L-proline than to D-proline and the L- and D-forms of cysteine and methionine, respectively. No clear differences in sensitivity were found between the remaining five amino acids.

Comparison between species

With L- and D-cysteine all five mice were more sensitive than all three spider monkeys. Conversely, with D-proline all three spider monkeys displayed lower detection thresholds (i.e. were more sensitive) compared with all five

Table 1 Olfactory detection threshold values for the six amino acids in CD-1 mice, expressed in various measures of gas phase concentrations

	Liquid dilution		Gas phase concentration				
	n	mM	molec./cm^3	ppm	log ppm	mol/l	log mol/l
L-cysteine	2	0.01	1.2×10^{9}	0.000044	−4.35	2.0×10^{-12}	−11.70
	3	0.03	3.6×10^{8}	0.000013	−4.88	6.0×10^{-13}	−12.22
D-cysteine	1	0.03	3.6×10^{9}	0.00013	−3.88	6.0×10^{-12}	−11.22
	2	0.003	3.6×10^{8}	0.000013	−4.88	6.0×10^{-13}	−12.22
	2	0.001	1.2×10^{8}	0.0000044	−5.35	2.0×10^{-13}	−12.70
L-methionine	1	3.3	3.0×10^{11}	0.011	−1.95	5.0×10^{-10}	−9.30
	2	1.0	9.8×10^{10}	0.0036	−2.44	1.6×10^{-10}	−9.79
	1	0.1	9.8×10^{9}	0.00036	−3.44	1.6×10^{-11}	−10.79
	1	0.03	3.0×10^{9}	0.00011	−3.95	5.0×10^{-12}	−11.30
D-methionine	2	0.3	3.0×10^{10}	0.0011	−2.95	5.0×10^{-11}	−10.30
	1	0.1	9.8×10^{9}	0.00036	−3.44	1.6×10^{-11}	−10.79
	2	0.01	9.8×10^{8}	0.000036	−4.44	1.6×10^{-12}	−11.79
L-proline	5	10	1.8×10^{12}	0.067	−1.18	3.0×10^{-9}	−8.52
D-proline	4	10	1.8×10^{12}	0.067	−1.18	3.0×10^{-9}	−8.52
	1	3.3	6.1×10^{11}	0.023	−1.65	1.0×10^{-9}	−8.99

n indicates the number of animals

mice. With L-methionine, D-methionine, and L-proline the range of threshold values with a given stimulus overlapped between the two species (Fig. 4).

A comparison of the across-odorant patterns of detection thresholds obtained with the two species demonstrates a high degree of similarity. Both mice and spider monkeys scored higher threshold values (i.e. displayed a lower olfactory sensitivity) with L-proline than with the L- and D-forms of cysteine and proline. Similarly, both species displayed overlapping threshold values (i.e. a similar sensitivity) with the L- and D-form of cysteine, and with the L- and D-form of methionine, respectively. Accordingly, a significant positive correlation in the across-odorant patterns of detection thresholds obtained with mice and spider monkeys was found (Spearman $r_s = 0.67$, $p < 0.01$).

The across-odorant pattern of detection thresholds obtained with human subjects (Fig. 4, triangles) in an earlier study (Laska 2010) also correlates significantly with those of the mice (Spearman $r_s = 0.82$, $p < 0.01$) and those of the spider monkeys (Spearman $r_s = 0.77$, $p < 0.01$) tested here.

Discussion

The results of this study demonstrate that both mice and spider monkeys are able to detect the odors of the L- and D-forms of cysteine, methionine, and proline at gas phase concentrations <1 ppm. Further, the results show that mice are not generally more sensitive for the odorants tested than spider monkeys.

Although only five mice and three spider monkeys were tested per stimulus, the results appear robust as interindividual variability was generally low and smaller than the range reported in studies on human olfactory sensitivity, that is, within three orders of magnitude (Doty 1991). In fact, the largest difference between the highest- and the lowest-scoring animal with a given stimulus was a factor of 100 and with the majority of stimuli it was a factor of 10 or even smaller (see Figs. 2, 3). Further, for all odorants, 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 odorless diluent was indeed based on chemosensory perception and not on other cues.

It should be mentioned that the gas phase concentrations reported here were not determined empirically, e.g. via gas chromatographic analyses, but were calculated using published vapor pressure data (Dykyi et al. 2001) and corresponding formulae based on the classical gas laws (Weast 1987). Thus, these derived measures may not be exact but should be regarded as an approximation. However, as it is the gas phase, and not the liquid phase, which constitutes the odor stimulus, we report both (see Tables 1, 2) to allow readers to easily compare the data obtained in the present study to those reported by other authors using one of these measures.

Olfactory sensitivity for amino acids

Figure 4 compares the olfactory detection threshold values obtained with the mice and the spider monkeys for

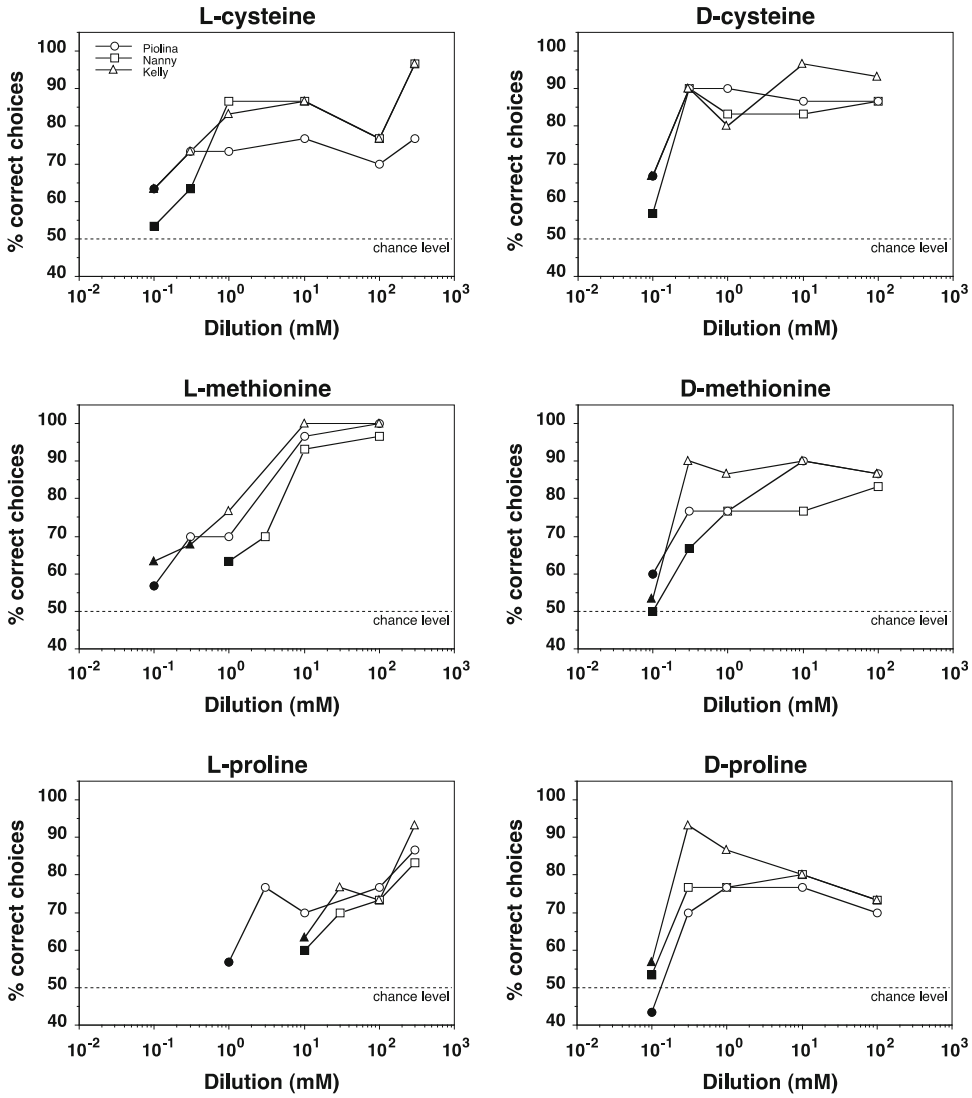

Fig. 3 Performance of spider monkeys in discriminating between various dilutions of an amino acid and demineralized water used as odorless solvent. Each *data point* represents the percentage of correct choices from a total of 30 decisions per individual animal. The *three different symbols* represent data from each of the three individual animals tested per stimulus. *Filled symbols* indicate dilutions that were not discriminated significantly above chance level (binomial test, $p > 0.05$)

Table 2 Olfactory detection threshold values for the six amino acids in spider monkeys, expressed in various measures of gas phase concentrations

	Liquid dilution		Gas phase concentration				
	n	mM	molec./cm^3	ppm	log ppm	mol/l	log mol/l
L-cysteine	1	1.0	1.2×10^{11}	0.0044	−2.35	2.0×10^{-10}	−9.70
	2	0.3	3.6×10^{10}	0.0013	−2.88	6.0×10^{-11}	−10.22
D-cysteine	3	0.3	3.6×10^{10}	0.0013	−2.88	6.0×10^{-11}	−10.22
L-methionine	1	3.0	3.0×10^{11}	0.011	−1.95	5.0×10^{-10}	−9.30
	1	1.0	9.8×10^{10}	0.0037	−2.43	1.7×10^{-10}	−9.78
	1	0.3	3.0×10^{10}	0.0011	−2.95	5.0×10^{-11}	−10.30
D-methionine	1	1.0	9.8×10^{10}	0.0037	−2.43	1.7×10^{-10}	−9.78
	2	0.3	3.0×10^{10}	0.0011	−2.95	5.0×10^{-11}	−10.30
L-proline	2	30	5.5×10^{12}	0.20	−0.69	9.1×10^{-9}	−8.04
	1	3	5.5×10^{11}	0.020	−1.69	9.1×10^{-10}	−9.04
D-proline	3	0.3	5.5×10^{10}	0.0020	−2.69	9.1×10^{-11}	−10.04

n indicates the number of animals

the stimuli tested here to those obtained in an earlier study with human subjects (Laska 2010). Such across-species comparisons should take into consideration that different methods may lead to widely differing results (Hastings 2003). In the present study, for example, the olfactometer used with the mice presented the odor

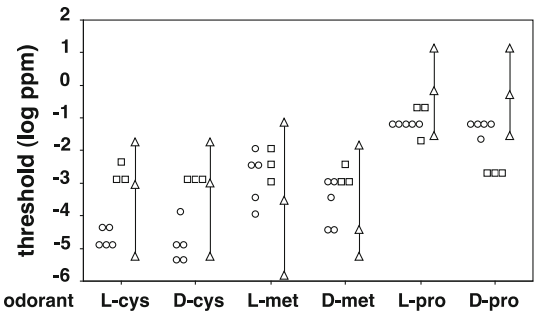

Fig. 4 Comparison of the olfactory detection threshold values (expressed as gas phase concentrations) of the CD-1 mice (*circles*) and the spider monkeys (*squares*) for the six stimuli tested here and those of human subjects (*triangles*). Data points of the two animal species represent threshold values of individual animals. Data points of the human subjects represent the mean threshold values (from $N = 20$) and the highest and lowest individual threshold, respectively (Laska 2010)

stimuli in a constant airstream for a total of 2 s, whereas the method used with the spider monkeys presented the odor stimuli via passive diffusion from a stationary source and, at least theoretically, allowed the animals for much longer olfactory inspection compared with the mice. However, the average odor sampling time observed with the spider monkeys was 2.5 s, including the time needed to switch between the simultaneously presented S+ and S−, and thus in the same range as the odor presentation time with the mice. Although it would have been desirable to use a constant airstream presentation of odor stimuli with both species, this was not feasible as spider monkeys do not tolerate a constant airstream at their face.

As both methods used here are based on instrumental conditioning procedures and allowed for tight control of the animals' motivational status, it is unlikely that differences in motivation might have affected the results. Similarly, both methods used different deprivation schedules (simple overnight fasting with the spider monkeys and strict water deprivation with the mice) and different reinforcers (food with the spider monkeys and water with the mice). However, both the type of deprivation schedule and the type of reinforcer used had to be chosen so that they meet the physiological needs and limitations of the study species in order to achieve successful conditioning. Spider monkeys, for example, are highly frugivorous and meet their water requirements by consuming juicy fruits and thus do not need to drink at all. Using water reinforcement with this species would therefore most likely not lead to success in instrumental conditioning.

Both methods used highly trained animals that had served in previous experiments of the same kind and thus it is unlikely that differences in prior experience with the method might have contributed to differences in performance. Although the number of trials performed for the initial acquisition of the reward value of a given odorant (200 in the case of the mice, and 90 in the case of the spider monkeys) also differed between species, this, too, is unlikely to have affected the results. With both species, these numbers of trials were fully sufficient to achieve stable performance which in all cases was significantly different from chance. We would not know of any study in any species suggesting that overlearning (i.e. prolonged presentation of the S+ at an easily detectable concentration) would affect an animal's olfactory detection threshold with a given odorant. On the contrary, one study which explicitly addressed this question found that repeated preexposure to aliphatic aldehydes had no systematic effect on olfactory detection thresholds in CD-1 mice (Laska et al. 2006b). Similarly, although the number of trials performed per dilution step (40 with the mice, and 30 with the spider monkeys) differed slightly between species, it is unlikely that this affected the results as previous studies employing the same methods and animals varied the number of trials per dilution step but yielded identical threshold values (Laska, unpublished data). Our decision to perform an unequal number of trials with both species was, again, a necessary adaptation of the method to the physiology and behavior of the two study species. The food reward used with the spider monkeys needed to have a certain size for reliable cooperation, probably due to their dietary specialization, and thus limited the number of trials that could be performed per animal and day. The water reward used with the mice, in contrast, could be small enough to allow for a higher number of trials per animal and day. With both species, however, binomial statistics using the same alpha level was employed which allows for valid between-species comparisons of performance despite an unequal number of trials.

To summarize, undeniably, the methods employed here with spider monkeys and mice differ in many aspects and we cannot completely exclude the possibility that one or the other aspect may affect the animals' performance and thus the between-species comparability of our results. However, it should be considered that most of the differences between the two methods employed here are necessary adaptations to meet the physiological, anatomical, and behavioral needs and limitations of our study species to successfully cooperate in an instrumental conditioning paradigm.

With all these caveats in mind it nevertheless seems admissible to state that the mice were not generally more sensitive for the amino acids tested than the spider monkeys and that both species, in turn, were not generally more sensitive than the human subjects. This is of interest as mice possess ≈1,060 functional genes coding for olfactory

receptors (Nei et al. 2008) whereas spider monkeys have only ≈900 such genes (Gilad et al. 2004) and humans even only ≈390 (Niimura and Nei 2006). Several authors have hypothesized that the number of functional olfactory receptor types should be predictive of a species' sensitivity for odorants (e.g. Rouquier et al. 2000; Gilad et al. 2004). The argument behind this hypothesis is that a higher number of olfactory receptor types would increase the statistical probability for an organism to have more than one high-affinity receptor for a given odorant. This, in turn, would aid in detection of the cognate ligand (i.e. the odorant molecule with the best fit to a given receptor) when presented at low concentrations. However, some previous studies that compared olfactory detection thresholds between species for which the number of functional olfactory receptor genes is known have also failed to find correlations between olfactory sensitivity and this genetic property (Joshi et al. 2006; Laska et al. 2009).

Several studies have tried to link between-species differences in olfactory sensitivity to neuroanatomical properties such as the relative or the absolute size of the olfactory bulbs (e.g. Stephan et al. 1988). However, although the relative size of the olfactory bulbs of the mouse (2.0% of total brain volume) is clearly larger than that of the spider monkey (0.09%) and of human subjects (0.01%) (Stephan et al. 1988; Kovacevic et al. 2005) no general superiority in olfactory sensitivity of the mice for the amino acids was found. Similarly, although the three species differ markedly in the absolute size of their olfactory bulbs (mouse 8.3 mm^3, spider monkey 90.4 mm^3, human subjects 114 mm^3; Stephan et al. 1988; Pomeroy et al. 1990), no clear differences in olfactory sensitivity for the amino acids were found between these species. Previous studies also led to ambiguous findings, with some of them supporting a positive correlation between the relative size of the olfactory bulbs and a species' sensitivity and some of them failing to do so (Laska et al. 2005, 2006d, 2007). Further studies, including those addressing the possibility that the degree of neural connectivity rather than absolute or relative numbers of neurons involved in sensory processing may be relevant for the sensitivity of olfactory systems (Keverne 2004), are clearly needed to draw further conclusions.

Comparison of olfactory and gustatory sensitivity for amino acids

The olfactory detection thresholds of the mice for L-methionine (0.03–3.3 mM), D-methionine (0.01–0.3 mM), and L-proline (10 mM) obtained in the present study are clearly lower than their corresponding taste preference thresholds (32, 26, and 55 mM, respectively) obtained in an earlier study (Iwasaki et al. 1985). Unfortunately, no data on taste preference thresholds for the other three amino acids tested here have been published for mice, and no data at all are available for amino acid taste sensitivity in spider monkeys. This finding is in line with earlier reports showing that the olfactory detection thresholds of human subjects for the L- and D-forms of cysteine and methionine (Laska 2010) are clearly lower than their corresponding taste detection thresholds (Haefeli and Glaser 1990). This suggests that in both mice and human subjects amino acids may contribute to the flavor of food via ortho- or retronasal olfaction at concentrations that are not detected by the sense of taste.

Odor structure–activity relationships

Both mice and spider monkeys detected the odors of the sulfur-containing amino acids L- and D-cysteine and L- and D-methionine, respectively, at markedly lower concentrations than the odors of L- and D-proline, which are both lacking sulfur (see Figs. 1, 4). This finding is in line with the pattern of sensitivity found in human subjects (Laska 2010) and also with electrophysiological recordings from the olfactory mucosa in the catfish (Caprio 1977), the zebrafish (Michel and Lubomudrov 1995), and the hammerhead shark (Tricas et al. 2009) which all showed the stimulatory effectiveness of L-cysteine and L-methionine to be much higher compared with L-proline (the D-forms were not tested). Whether the observed difference in detectability is due to the presence or absence of sulfur in the stimulus molecules or due to the fact that the amino group is secondary in proline whereas it is primary in cysteine and methionine remains to be answered. However, low olfactory detection thresholds for sulfur-containing odorants such as thiols and indols are interpreted as an evolutionary adaptation to the perception of putrefaction processes, that is, the microbial degradation of proteins, and thus may help to avoid ingestion of spoiled food (Laska et al. 2007). This idea is also supported by human studies that reported the presence of cysteine and cysteine-S-conjugates to play an important role in olfactory quality perception of fruits and vegetables (Starkenmann et al. 2008a), and food flavors (Starkenmann et al. 2008b).

In the mice, but not in the spider monkeys, the olfactory detection thresholds for L- and D-cysteine were lower than those for the L- and D-forms of methionine. This suggests that not only the presence, but the type of sulfur-containing functional group, a thiol group in the case of cysteine and a thioether group in the case of methionine, may affect the interaction with olfactory receptors and thus detectability in a species-specific manner. This finding, too, is in line with earlier studies in humans (Laska 2004), mice (Laska et al. 2006b), and spider monkeys (Hernandez Salazar et al. 2003) which reported that the type of functional group may systematically affect olfactory detection thresholds.

Electrophysiological studies on the properties of fish olfactory receptors responding to amino acids have found that polarity of the side chain may also affect the ligands' binding affinity (Luu et al. 2004; Nikonov and Caprio 2007a). As all amino acids employed in the present study have a nonpolar side chain, no conclusions as to the possible impact of this molecular feature on detectability can be drawn.

Chirality of the amino acids tested had only little effect on detectability: with the exception of L- and D-proline in the spider monkeys, the olfactory detection thresholds for the L- and D-forms of a given amino acid did not differ systematically from each other in both mice and spider monkeys. This finding is in line with earlier reports on detectability of enantiomeric odor pairs other than amino acids in human subjects (Laska and Teubner 1999; Laska 2004) as well as in mice and spider monkeys (Joshi et al. 2006) which found some optical isomers to differ in detectability whereas others did not.

Taken together, the results of the present study suggest that both mice and spider monkeys have a well-developed olfactory sensitivity for the L- and D-forms of cysteine, methionine, and proline which is comparable to that for other chemical classes of odorants tested previously in these species (Hernandez Salazar et al. 2003; Joshi et al. 2006; Laska et al. 2009). Further, the results suggest an important role of the sulfur-containing functional groups for detectability. A between-species comparison of the olfactory detection thresholds suggests that neither the number of functional olfactory receptor genes nor the absolute or the relative size of the olfactory bulbs reliably predicts a species' olfactory sensitivity for amino acids.

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Conflict of interest The authors declare that they have no conflict of interest.

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