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ORIGINAL ARTICLES

Olfactory Discrimination Ability of Asian Elephants (*Elephas maximus*) for Structurally Related Odorants

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Abstract

Using a food-rewarded two-choice instrumental conditioning paradigm, we assessed the ability of Asian elephants, ***Elephas maximus,*** to discriminate between 2 sets of structurally related odorants. We found that the animals successfully discriminated between all 12 odor pairs involving members of homologous series of aliphatic 1-alcohols, *n*-aldehydes, 2-ketones, and *n*-carboxylic acids even when the stimuli differed from each other by only 1 carbon. With all 4 chemical classes, the elephants displayed a positive correlation between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length. The animals also successfully discriminated between all 12 enantiomeric odor pairs tested. An analysis of odor structure–activity relationships suggests that a combination of molecular structural properties rather than a single molecular feature may be responsible for the discriminability of enantiomers. Compared with other species tested previously on the same sets of odor pairs (or on subsets thereof), the Asian elephants performed at least as well as mice and clearly better than human subjects, squirrel monkeys, pigtail macaques, South African fur seals, and honeybees. Further comparisons suggest that neither the relative nor the absolute size of the olfactory bulbs appear to be reliable predictors of between-species differences in olfactory discrimination capabilities. In contrast, we found a positive correlation between the number of functional olfactory receptor genes and the proportion of discriminable enantiomeric odor pairs. Taken together, the results of the present study support the notion that the sense of smell may play an important role in regulating the behavior of Asian elephants.

Key words: aliphatic odorants, Asian elephants, Elephas maximus, enantiomers, olfactory discrimination

Introduction

Asian elephants possess highly developed olfactory and vomeronasal systems (Koikegami et al. 1941; Rasmussen and Hultgren 1990; Shoshani et al. 2006) and specialized scent glands (Lamps et al. 2001; Rajaram and Krishnamurthy 2003), and observational studies suggest that they strongly rely on olfactory information in a variety of behavioral contexts (Langbauer 2000; Rasmussen and Krishnamurthy 2000). Olfactory social communication by means of body-borne odors has been studied to some extent in *Elephas maximus*, and both male and female sex pheromones have been chemically identified and functionally verified (Greenwood et al. 2005; Rasmussen et al. 2005).

Despite this anatomical and behavioral evidence suggesting the sense of smell to play an important role in regulating the behavior of Asian elephants, experimental studies on their olfactory performance have been sparse, mainly due to the lack of appropriate testing methods. However, in order to fully appreciate the significance of olfaction in Asian elephants, it is necessary to gain knowledge as to the animals' basic perceptual capacities under controlled conditions. The recent development of a food-rewarded two-choice instrumental conditioning paradigm for Asian elephants now enables us to systematically assess the olfactory capabilities of this proboscid species. Arvidsson et al. (2012) demonstrated that *E. maximus* readily learns to cooperate in an olfactory discrimination test and readily masters intramodal stimulus transfer tasks (that is, tasks in which either the rewarded stimulus or the unrewarded stimulus are replaced for a new stimulus). Further, they showed that Asian elephants are capable of discriminating

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between structurally related aliphatic acetic esters, which only differ from each other in the length of their carbon chain. In this study, we continue to explore the olfactory discrimination capabilities of Asian elephants by assessing their ability to distinguish between members of other homologous series of aliphatic odorants and between a set of enantiomeric odor pairs.

Enantiomers are pairs of molecules with mirror image structures that exhibit identical chemical and physical properties except for their optical activity, that is, rotation of polarized electromagnetic waves. They are particularly useful for assessing how molecular structure is encoded by the olfactory system as perceptual differences between enantiomers cannot be caused by differing diffusion rates in the mucus covering the olfactory epithelium or differing air/mucus partition coefficients (Hahn et al. 1994) but must originate from chiral selectivity at the receptor level (Rossiter 1996). Thus, the systematic assessment of the discriminability of enantiomeric odor pairs may contribute to our understanding of odor quality perception and coding.

It was therefore the aim of this study to assess the discrimination ability of Asian elephants for aliphatic 1-alcohols, *n*-aldehydes, 2-ketones, and *n*-carboxylic acids and for a set of 12 enantiomeric odor pairs. Substances were chosen on the basis of earlier studies that used the same stimuli (or subsets thereof) with other species. This allowed us to additionally address the question whether a species' olfactory discrimination abilities may be correlated with neuroanatomical features such as the absolute or the relative size of the olfactory bulbs, or with genetic features such as the number of functional olfactory receptor genes.

Materials and methods

Subjects

Testing was carried out using 3 adult female Asian elephants (*E. maximus*) maintained at Kolmården Wildlife Park, Sweden. Bua, Saonoi, and Saba were 14, 15, and 43 years old at the start of the study. The animals were kept as a group in 2 indoor enclosures (approximately 150 and 250 m^2) but were let outside into an outdoor back enclosure (750 m^2) or an outdoor exhibit (3000 m^2) for a larger part of the day or at least once a day and when the weather was appropriate. The elephants were fed pellets in the morning, and roughage and branches were provided *ad libitum*. Environmental enrichment in the form of scattered and hidden fruits and vegetables throughout the enclosure was provided at least once a day, and no food deprivation was required during the study. The elephants were kept in a hands-on system in which the keepers have full access to the animals, and they were therefore accustomed to follow commands and perform certain motor patterns upon demand.

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 were performed according to a protocol approved by Linköping's Animal Care and Use Committee (Linköpings försöksdjuretiska nämnd, protocol #81/07).

Odorants

In the first experiment, a set of 16 aliphatic odorants comprising unbranched 1-alcohols, *n*-aldehydes, 2-ketones, and *n*-carboxylic acids with carbon chain lengths C4–C7 was used (Table 1 and Figure 1).

In the second experiment, a set of 12 enantiomeric odor pairs comprising members of the chemical class monoterpenoids was used (Table 2 and Figure 2). All odorants used in this study have been shown to be components of plant odors or of mammalian body-borne odors (Flood 1985; Knudsen et al. 2006) and thus are likely to be of at least some behavioral relevance for the elephants.

In the third experiment, an additional odor pair (*n*-pentyl acetate as rewarded stimulus, and *n*-butyl acetate as unrewarded stimulus) was used. Both odorants were already familiar to the animals from a previous study (Arvidsson et al. 2012).

Additionally, in both the first and the second experiment, the aromatic odorant anethole (CAS# 104-46-1) was used.

Table 1 Aliphatic odorants and concentrations used

Odorant	CAS#	Carbon chain length	Dilution
1-alcohols			
1-butanol	71-36-3	4	1:10
1-pentanol	71-41-0	5	1:10
1-hexanol	111-27-3	6	1:3
1-heptanol	111-70-6	7	1:3
n-aldehydes			
n-butanal	123-72-8	4	1:10
n-pentanal	110-62-3	5	1:10
n-hexanal	66-25-1	6	1:10
n-heptanal	111-71-7	7	1:5
2-ketones			
2-butanone	78-93-3	4	1:10
2-pentanone	107-87-9	5	1:10
2-hexanone	591-78-6	6	1:3
2-heptanone	110-43-0	7	1:3
n-carboxylic acids			
n-butanoic acid	67-43-6	4	1:100
n-pentanoic acid	109-52-4	5	1:100
n-hexanoic acid	142-62-1	6	1:100
n-heptanoic acid	111-14-8	7	1:20

Chemical classes are given in italics

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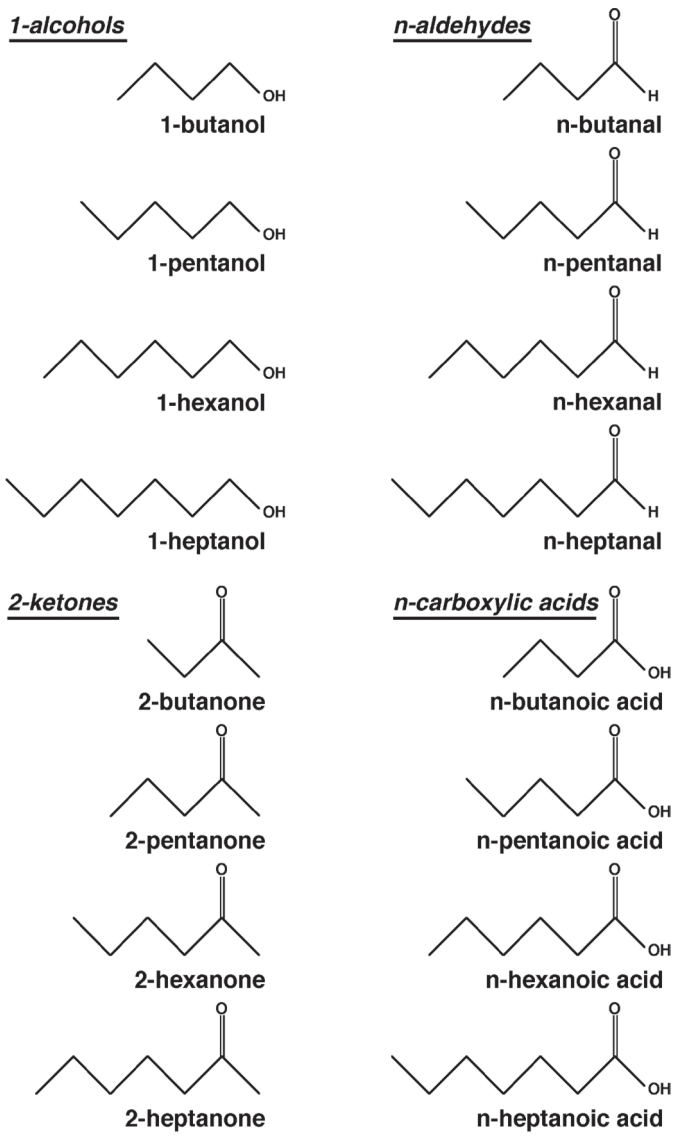

Figure 1 Molecular structures of the 12 aliphatic odorants used.

This odorant was already familiar to the animals as an unrewarded stimulus and was therefore used when introducing them to one of the new odorants as a rewarded stimulus. All odorants were obtained from Sigma–Aldrich (St. Louis, MO) and had a nominal purity of at least 99%. They were diluted using near-odorless diethyl phthalate (CAS# 84-66-2) as the solvent in such a way as to provide clearly detectable stimuli of equal subjective intensity when sniffed at the outlet of the odor boxes by human subjects.

Experimental set-up

For the presentation of odorants 2 high-density polyethylene (HDPE) boxes with removable lids (35 × 35 × 20 cm) were used. The tight-fitting lid of each odor box was equipped with a battery-powered ventilator (6 cm diameter), which provided an ingoing airflow of approximately 0.58 m^3/min. A total of 130 holes of 3 mm diameter drilled in an equidistant pattern and forming a filled circle with a diameter of 7.5 cm were drilled in an exact pattern in the middle of one of the front sides of each odor box, serving as an outlet for the airflow provided by the ventilator. In order to present an odorant, a circular filter paper (9 cm diameter) was placed into an open Petri dish, and 1 ml of the odorant was pipetted onto it. The Petri dish, in turn, was placed into an open white plastic HDPE box (12 × 20 × 12 cm) inside the odor box to avoid contamination. The odor boxes were cleaned with warm water and perfume-free detergent after the completion of each session.

Testing was carried out in a separate indoor enclosure in which the animals could be tested individually. The experimenter was positioned in a room on the second floor where an opening in the wall was fitted with a service door (106 × 90 × 5 cm) made of steel. The door was modified to hold a window (45 × 75 cm) in its upper part, which was covered with a steel grid (with a mesh width of 4 × 4 cm) that physically separated the experimenter from the animals while allowing her/him to observe and interact with the animals and to present the food reward. The service door was located approximately 3 m above the ground of the testing enclosure and was divided by a vertical bar (105 × 20 × 5 cm) in the middle into a left and a right section. Each section contained an odor port, that is, a round opening with a diameter of 21 cm, at the lower half part of the door. Both odor ports were covered with a steel grid (with a mesh width of 4 × 4 cm) on the experimenter's side allowing the animals to sample the odorized airstreams provided by the odor boxes but preventing them from making physical contact with the boxes. Above the odor ports was the rectangular grid-covered window where the animals had to place their trunk in order to indicate their decision and to receive their food reward. A wooden platform on the floor inside the experimenter's room ensured that the odor boxes were placed with their outlets congruent with the odor ports of the experimental set-up (Arvidsson et al. 2012).

Behavioral test

The behavioral test was based on a food-rewarded two-choice instrumental conditioning procedure. In a previous study (Arvidsson et al., 2012), the animals had learned to sniff at 2 odor sampling ports and then to indicate which of them held the rewarded odor stimulus by placing the tip of their trunk onto a defined position above the corresponding odor port.

At the beginning of each trial, the 2 odor boxes were placed with their outlets toward the odor ports, and after a verbal command, the animal selected for testing was allowed to sample the 2 stimuli as often as it liked. Immediately following the animal's decision, the 2 odor boxes were removed, and in the case of a correct response (placing the tip of the trunk onto a defined position above the odor port

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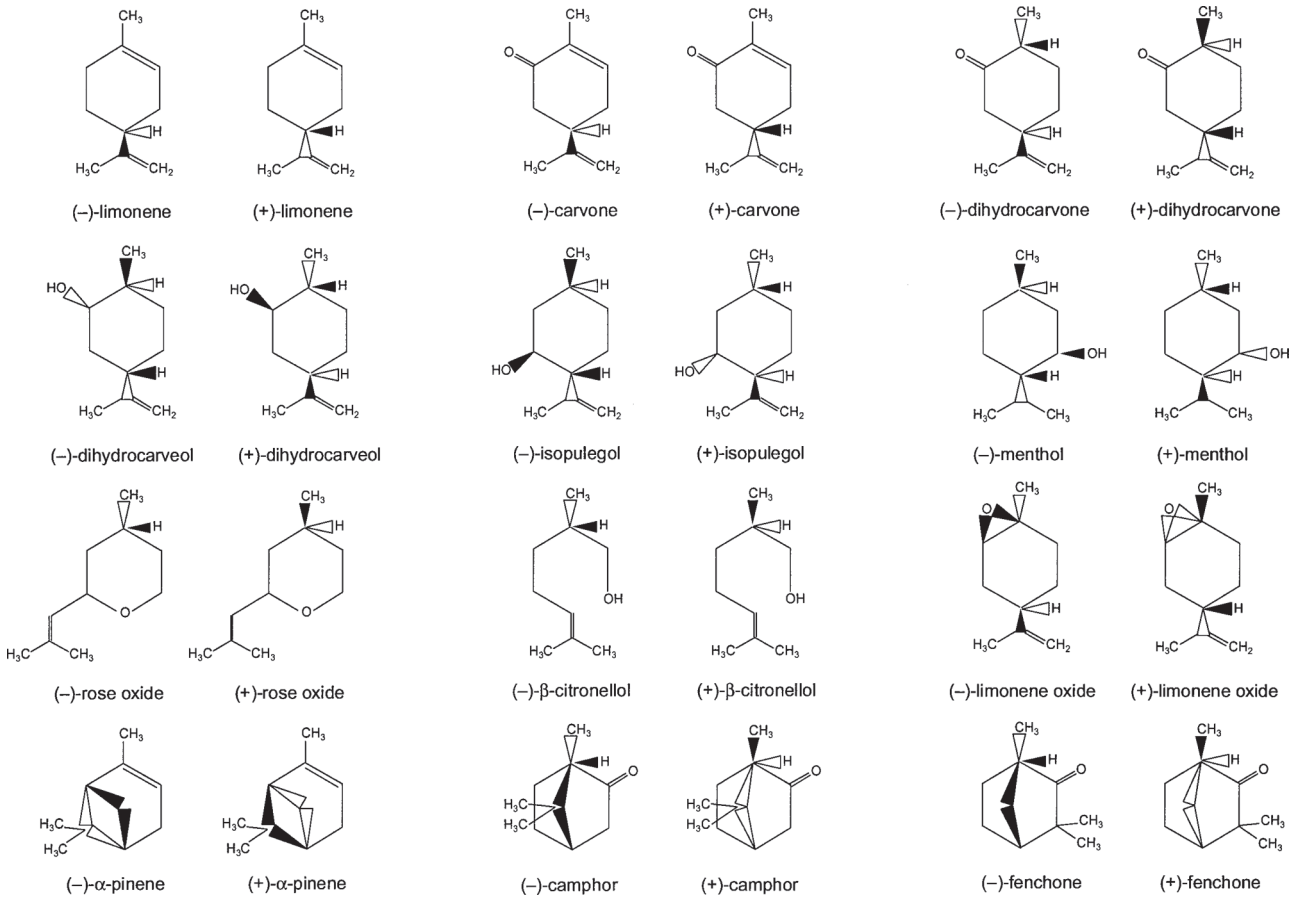

Figure 2 Molecular structures of the 12 enantiomeric odor pairs used.

bearing the rewarded stimulus), the animal was rewarded with a carrot presented through the grid at the position where the animal had placed the tip of its trunk. In the case of an incorrect response (placing the tip of the trunk onto a defined position above the odor port bearing the unrewarded stimulus) no reward was given to the animal. Thirty such trials were performed per session and usually 2 sessions were performed per animal and day. Each session took approximately 30 min. Care was taken to present the rewarded stimulus to the right or the left odor port adopting a pseudorandomized sequence with the limitation that the same option was not used more than 3 times in a row.

For assessing the elephants' ability to discriminate between structurally related aliphatic odorants, the following scheme was adopted: for each of the 4 chemical classes, 1 odorant (the one with 7 carbons, from here on named C7) was assigned as the rewarded stimulus, and in an initial phase, each animal was allowed to become familiar with it. This was done using anethole as unrewarded stimulus for 4–8 sessions. Once familiarized with the rewarded stimulus, the anethole was exchanged for one of the other odorants of the same chemical class (see Table 1), and each of the critical stimulus combinations (C7 vs. C4, C7 vs. C5, and C7 vs. C6) was presented for 2 sessions. To control for possible order effects, one elephant was assigned the C4 odorant as the first unrewarded stimulus, another elephant the C5 odorant, and the third elephant the C6 odorant as the first unrewarded stimulus. Between the different critical stimulus combinations, up to 2 sessions with anethole as the unrewarded stimulus were interspersed if needed to boost the animal's confidence.

For assessing the elephants' ability to discriminate between enantiomeric odor pairs, the following scheme was adopted: with each of the 12 enantiomeric odor pairs the (+)-form was assigned as the rewarded stimulus (see Table 2). Here too, each animal was allowed to become familiar with its rewarded stimulus by using anethole as the already familiar unrewarded stimulus for 4–8 sessions. Then, the anethole was exchanged for the (−)-form of the enantiomer in question, and each of the critical stimulus combinations was presented for 2 sessions. Here too, up to 2 sessions with anethole as the unrewarded stimulus were interspersed if needed to boost the animal's confidence.

For assessing the impact of stimulus concentration on the elephants' ability to discriminate between 2 structurally related odorants, the following scheme was adopted: *n*-pentyl acetate was assigned as the rewarded stimulus and *n*-butyl acetate was

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Table 2 Enantiomeric odorants and concentrations used

Enantiomer	CAS#	Chemical description	Dilution
(S)-(+)-carvone	2244-16-8	Monocyclic terpene-ketone	1:3
(R)-(−)-carvone	6485-40-1	Monocyclic terpene-ketone	1:3
(+)-dihydrocarvone	5524-05-0	Monocyclic terpene-ketone	1:2
(−)-dihydrocarvone	7764-50-3	Monocyclic terpene-ketone	1:2
(+)-dihydrocarveol	22567-21-1	Monocyclic terpene-alcohol	1:2
(−)-dihydrocarveol	20549-47-7	Monocyclic terpene-alcohol	1:2
(R)-(+)-limonene	5989-27-5	Monocyclic terpene-hydrocarbon	1:3
(S)-(-)-limonene	5989-54-8	Monocyclic terpene-hydrocarbon	1:3
(+)-limonene oxide	2485-30-59	Monocyclic terpene-epoxide	1:3
(−)-limonene oxide	2485-06-0	Monocyclic terpene-epoxide	1:3
(+)-isopulegol	104870-56-6	Monocyclic terpene-alcohol	1:5
(−)-isopulegol	89-79-2	Monocyclic terpene-alcohol	1:5
(1S,2R,5S)-(+)-menthol	15356-60-2	Monocyclic terpene-alcohol	1:10
(1R,2S,5R)-(−)-menthol	2216-51-5	Monocyclic terpene-alcohol	1:10
(R)-(+)-β-citronellol	1117-61-9	Acyclic terpene-alcohol	1:3
(S)-(−)-β-citronellol	7540-51-4	Acyclic terpene-alcohol	1:3
(+)-rose oxide	16409-43-1	Monocyclic terpene-oxide	1:3
(−)-rose oxide	5258-11-7	Monocyclic terpene-oxide	1:3
(+)-fenchone	4695-62-9	Bicyclic terpene-ketone	1:3
(−)-fenchone	7787-20-4	Bicyclic terpene-ketone	1:3
(+)-α-pinene	7785-70-8	Bicyclic terpene-hydrocarbon	1:5
(−)-α-pinene	7785-26-4	Bicyclic terpene-hydrocarbon	1:5
(1R)-(+)-camphor	464-49-3	Bicyclic terpene-ketone	1:10
(1S)-(−)-camphor	464-48-2	Bicyclic terpene-ketone	1:10

assigned as the unrewarded stimulus. The animals were already familiar with this odor pair from an earlier study (Arvidsson et al. 2012). Without further pretraining, the elephants were therefore presented with this odor pair at 5 different conditions: with both stimuli presented at a dilution of 1:50, and with one of the stimuli again presented at a dilution of 1:50, whereas the other stimulus was presented at a 10-fold higher concentration (that is: a 1:5 dilution) or at a 10-fold lower concentration (that is: a 1:500 dilution). Each of these 5 stimulus combinations were presented for 2 sessions each.

Data analysis

In the method described here, the animal had 2 options: 1) to correctly respond to the rewarded stimulus (hit) and 2) to falsely respond to the unrewarded stimulus (false alarm). The percentage of hits was taken as the measure of performance. In all tasks, the criterion was set at 70% hits in 2 consecutive sessions of 30 decisions each (corresponding to $P < 0.01$, two-tailed binomial test). The rationale for choosing this criterion was that similar standards have been used in previous olfactory studies allowing for direct comparisons of performance across species.

Correlations between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length or between discrimination performance and molecular complexity were evaluated using the Spearman rank correlation coefficient. Comparisons of performance across individuals and tasks were made using the Mann–Whitney U test for independent samples.

Results

Discrimination of structurally related aliphatic odorants

Figure 3 shows the performance of the elephants in discriminating between aliphatic 1-alcohols, *n*-aldehydes, 2-ketones, and *n*-carboxylic acids, respectively. All 3 animals performed significantly above chance level in all 12 tasks and thus were clearly able to discriminate between all odor pairs presented (two-tailed binomial test, $P < 0.01$ for all tasks and individuals). Interindividual variability with a given task was low, and the mean range between the best- and the poorest-performing animal with a given odor pair was only 6.3%. A comparison of performance across individuals showed that none of the animals performed significantly better or significantly poorer than any of the other animals (Mann–Whitney, $P > 0.05$). Accordingly, Saba scored an average of 93.1% (±4.4%) correct choices across all tasks, and Saonoi and Bua scored an average of 94.2% (±7.0%) and 93.3% (±8.2%) correct choices, respectively.

A comparison of performance between the different chemical classes showed that the *n*-carboxylic acids were significantly more difficult to discriminate than the 1-alcohols, *n*-aldehydes, and 2-ketones, respectively

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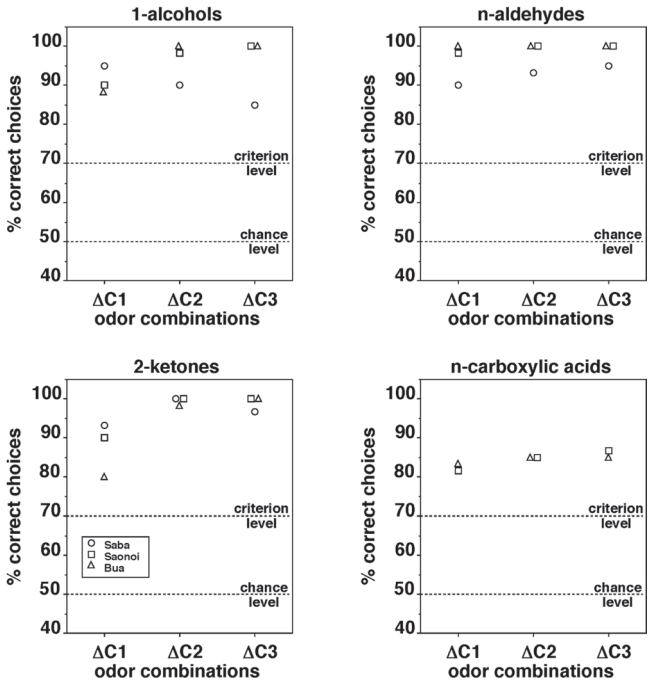

Figure 3 Olfactory discrimination performance of 3 Asian elephants with structurally related aliphatic odorants sharing the same oxygen-containing functional group but differing in carbon chain length. Each data point represents the percentage of correct choices from a total of 60 decisions per task and animal (circle: Saba; square: Saonoi; triangle: Bua). ΔC1 corresponds to the discrimination of odorants that differ by only carbon atom, and ΔC2 and ΔC3 to the discrimination of odorants that differ by 2 and 3 carbon atoms, respectively. The horizontal dashed lines represent chance level (at 50%) and the criterion level (at 70%, two-tailed binomial test, $P < 0.01$), respectively.

(Mann–Whitney, $P < 0.01$). No significant differences in discrimination performance were found between the other 3 chemical classes (Mann–Whitney, $P > 0.05$). With all 4 chemical classes a positive correlation between discrimination performance and structural similarity of the odorants in terms of differences in carbon chain length was found. This correlation was statistically significant with the 2-ketones and the *n*-carboxylic acids (Spearman $r_s = +0.72$ and +0.89, $P < 0.05$, respectively) but fell short of statistical significance with the 1-alcohols and *n*-aldehydes (Spearman $r_s = +0.35$ and +0.29, $P > 0.05$, respectively).

Discrimination of enantiomeric odor pairs

Figure 4 shows the performance of the elephants in discriminating between the enantiomeric odor pairs. All 3 animals performed significantly above chance level in all 12 tasks and thus were clearly able to discriminate between all odor pairs presented (two-tailed binomial test, $P < 0.01$ for all tasks and individuals). Interindividual variability with a given task was higher compared with that found with the discrimination of aliphatic odorants, and the mean range between the best- and the poorest-performing animal with

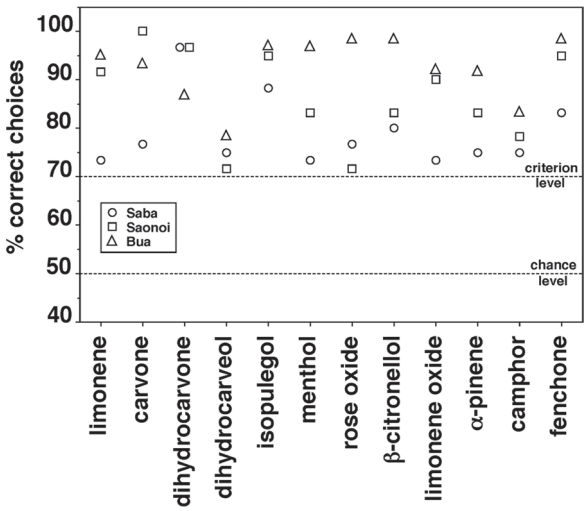

Figure 4 Olfactory discrimination performance of 3 Asian elephants with enantiomeric odor pairs. Each data point represents the percentage of correct choices from a total of 60 decisions per task and animal (circle: Saba; square: Saonoi; triangle: Bua). The horizontal-dashed lines represent chance level (at 50%) and the criterion level (at 70%, two-tailed binomial test, $P < 0.01$), respectively.

a given odor pair was 16.4%. A comparison of performance across individuals showed that both Bua and Saonoi performed significantly better than Saba (Mann–Whitney, $P < 0.01$), whereas no significant difference in performance was found between Bua and Saonoi (Mann–Whitney, $P > 0.05$). Accordingly, Saba scored an average of 78.9% (±7.2%) correct choices across all tasks, and Saonoi and Bua scored an average of 86.9% (±9.6%) and 92.4% (±6.5%) correct choices, respectively.

Although all 12 enantiomeric odor pairs were discriminated above chance level, marked differences in discriminability between the different odor pairs were apparent. Accordingly, the average score across all animals in a given task ranged from 93.3% (±4.4%) correct choices with isopulegol to 75.0% (±3.3%) correct choices with dihydrocarveol.

Table 3 summarizes the discrimination peformance of the elephants and some of the molecular structural properties that may have affected the discriminability of the enantiomeric odor pairs tested. No significant differences in discriminability were found between enantiomeric odor pairs with (87.2 ± 9.7% correct choices) or without (84.7 ± 9.3% correct choices) an isopropenyl group at the chiral center ($P > 0.05$, Mann–Whitney *U* test). The presence (86.2 ± 9.7% correct choices) or absence (85.0 ± 9.3% correct choices) of an oxygen-containing functional group also failed to systematically affect discriminability of the enantiomers ($P > 0.05$, Mann–Whitney *U* test). Similarly, neither the position (*ortho*-, 87.2 ± 9.0%; *meta*-, 86.4 ± 10.2%; *para*-position, 83.6 ± 11.1% correct choices) nor the type of oxygen-containing functional group (alcohol group, 85.0 ± 9.8%; keto group, 88.6 ± 9.1% correct choices) had a systematic effect on discriminability (in all cases, $P > 0.05$, Mann–Whitney *U* test). Discriminability

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Table 3 Discrimination performance with and molecular structural features of the 12 enantiomeric odor pairs

Enantiomeric odor pair	mean (±SD) discrimination performance (%)	Isopropenyl group	oxygen-containing functional group at			methyl group at para-position	Monocyclic structure	Additional chiral center(s)	Molecular complexity[a]
			Ortho	*Meta*	*Para*				
Isopulegol	93.3 ± 4.4	+	+	–	–	+	+	+	151
Dihydrocarvone	93.2 ± 5.8	+	–	+	–	+	+	+	181
Fenchone	92.2 ± 7.9	–	+	–	–	–	–	+	217
Carvone	90.0 ± 12.0	+	–	+	–	+	+	–	223
β-citronellol	87.2 ± 9.7	–	–	+	–	–	–	–	112
Limonene	86.7 ± 11.7	+	–	–	–	+	+	–	163
Limonene oxide	85.0 ± 10.2	+	–	–	+	+	+	+	197
Menthol	84.4 ± 11.7	–	+	–	–	+	+	+	120
α-pinene	83.3 ± 8.3	–	–	–	–	–	–	–	186
Rose oxide	82.2 ± 14.1	–	–	–	+	–	+	–	145
Camphor	78.9 ± 4.2	–	+	–	–	–	–	+	217
Dihydrocarveol	75.0 ± 3.3	+	–	+	–	+	+	+	151

"+" symbol indicates the presence, and "–" symbol the absence of the corresponding structural feature
[a]Molecular complexity values were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/). The structural complexity of odorant molecules can be described by a molecular complexity index initially developed by chemists to predict the probability of interaction between ligands and receptors. This index, which takes into account both the elements composing the molecule and structural features, ranges from 0 (for simple ions) to several thousand (for complex natural products)

also did not differ significantly between enantiomers with (86.8 ± 9.8% correct choices) or without (84.8 ± 9.3% correct choices) a methyl group at *para*-position, between monocyclic (86.2 ± 10.1% correct choices) and bi- or acyclic (85.4 ± 9.1% correct choices) enantiomers, or between enantiomers with (86.0 ± 9.3% correct choices) or without additional chiral center(s) (85.9 ± 10.0% correct choices) (in all cases, $P > 0.05$, Mann–Whitney U test). Finally, no significant correlation between molecular complexity of the enantiomers used here and the olfactory discrimination scores of the elephants was found (Spearman, $r_s = +0.14$, $P > 0.05$).

Discrimination performance as a function of stimulus concentration

Figure 5 shows the performance of the elephants in discriminating between *n*-pentyl acetate and *n*-butyl acetate presented at different concentrations. All 3 animals performed significantly above chance level irrespective of the concentration used for the rewarded or the unrewarded stimulus. More importantly, increasing or decreasing the stimulus concentration of either the rewarded or the unrewarded stimulus by a factor of 10 had very little effect on performance.

Discussion

The results of this study demonstrate that Asian elephants have an excellent olfactory discrimination ability both with homologous series of aliphatic odorants and with enantiomeric odor pairs. Further, they show a positive correlation between discrimination performance and structural similarity of aliphatic odorants in terms of differences in carbon chain length.

Our finding that the elephants successfully discriminated between all odor pairs tested raises the question whether perceived differences in stimulus intensity rather than stimulus quality might have contributed to the excellent discrimination performance that the animals displayed. Although this possibility cannot be ruled out completely, it appears unlikely as first, the elephants failed to learn to discriminate between different concentrations of a given stimulus in the conditioning paradigm used here (Laska, unpublished data), and second, increasing or decreasing the concentration of either the rewarded stimulus or the unrewarded stimulus by a factor of 10 had no significant effect on discrimination performance (see Figure 5). Similarly, the possibility that the nasal trigeminal system might have contributed to the discrimination of the odor pairs appears unlikely as all of the aliphatic odorants used in this study have been shown to have little, if any, trigeminal-stimulating properties at the concentrations used here when tested with human subjects (Cometto-Muñiz and Cain 1994; Cometto-Muñiz et al. 1998). Further, with all enantiomers tested so far with human subjects the (+)- and the (-)-form of a given substance did not differ in their degree of trigeminality (Laska and Teubner 1999a; Laska 2004). A recent study reported that the ability to detect differences in odor intensity is positively correlated with the trigeminal potency of the odor stimuli (Jacquot et al. 2010). Considering the low trigeminal potency of the odor stimuli

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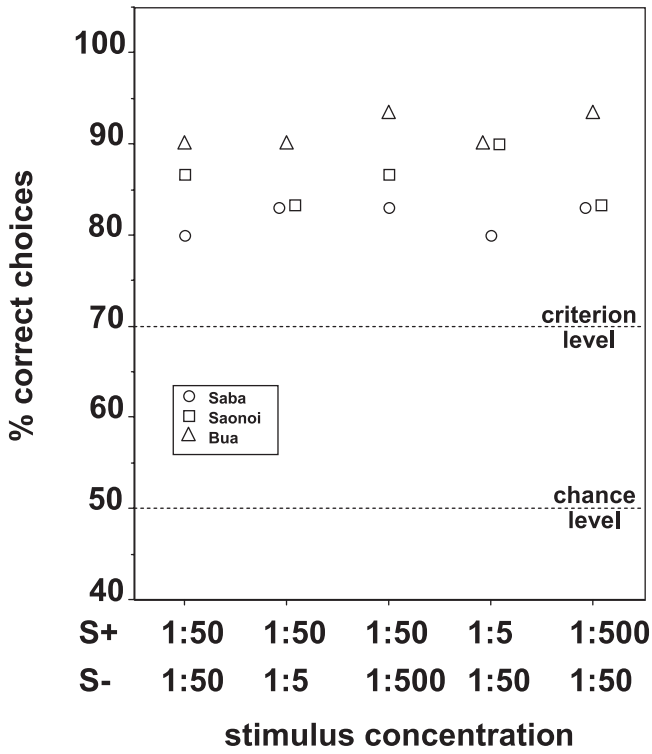

Figure 5 Olfactory discrimination performance of 3 Asian elephants with *n*-pentyl acetate used as rewarded stimulus (S+) and *n*-butyl acetate used as unrewarded stimulus (S–) at different concentrations. Each data point represents the percentage of correct choices from a total of 60 decisions per task and animal (circle: Saba; square: Saonoi; triangle: Bua). The horizontal dashed lines represent chance level (at 50%) and the criterion level (at 70%, two-tailed binomial test, $P < 0.01$), respectively.

used in the present study and considering the inability of our elephants to learn to discriminate between different concentrations of a given odor, we therefore believe the discrimination scores reported here to reflect the ability of the elephants to discriminate between stimulus qualities.

Although only 3 elephants were tested, we have no reason to believe that the results of this study should not be representative for the species *E. maximus*. In addition to the fact that interindividual variability among these genetically nonrelated elephants was low (see Figures 3 and 4), all 3 animals were raised and live in a chemical environment that is likely to be as complex as that of wild Asian elephants.

Understanding how structural properties of odor molecules are encoded by olfactory systems leading to distinguishable odor qualities is arguably one of the central problems in olfaction. Carbon chain length has repeatedly been identified as an important molecular structural property of aliphatic odorants systematically affecting their detectability in terms of olfactory detection thresholds (Cometto-Muñiz and Abraham 2010; Güven and Laska 2012), as well as odor quality perception (Johnson and Leon 2000; Boesveldt et al. 2010) in human subjects and animal models. Our finding that an increasing difference in carbon chain length of 1-alcohols, *n*-aldehydes, 2-ketones, and *n*-carboxylic acids, is positively correlated with discriminability and thus with perceptual dissimilarity in Asian elephants is in line with corresponding findings in human subjects (Laska and Teubner 1998, 1999b; Laska and Hübener 2001), squirrel monkeys (Laska and Teubner 1998; Laska et al. 1999c), and honeybees (Laska et al. 1999a). However, other species tested on the same aliphatic odor pairs such as South African fur seals (Laska et al. 2010) fail to show such a correlation between structural and perceptual similarity or show them only with some, but not all, of these chemical classes as is the case in mice (Laska et al. 2008). This suggests that correlations between discrimination performance and carbon chain length of aliphatic odorants are both species- and substance class-specific rather than a general phenomenon.

Whereas the Asian elephants of this study successfully discriminated between all aliphatic odor pairs, other species tested with the same stimulus combinations were found to fail with at least some of these, typically when presented with direct neighbors in a given homologous series: human subjects, for example, failed to discriminate between 1-hexanol and 1-heptanol (Laska and Teubner 1999b). Similarly, honeybees failed in discriminating between 2-hexanone and 2-heptanone and between 1-hexanol and 1-heptanol (Laska et al. 1999a). Mice, squirrel monkeys, and South African fur seals, in contrast, succeeded with all aliphatic odor pairs tested in this study (Laska and Teubner 1998; Laska et al. 1999c, 2008, 2010).

More pronounced between-species differences in olfactory discrimination capabilities can be found with the enantiomers tested here. Table 4 compares the discrimination performance of the Asian elephants with that of other species tested on the same enantiomeric odor pairs or on subsets thereof. Human subjects were only able to significantly discriminate between 5 of the 12 enantiomeric odor pairs tested (Laska and Teubner 1999a; Laska 2004), and squirrel monkeys distinguished between 6 of the 12 pairs of optical isomers (Laska et al. 1999b; 2005). Pigtail macaques were able to score above chance level with 5 out of 6 enantiomeric odor pairs (Laska et al. 2005), and honeybees discriminated the (+)- and (−)-forms of 5 out of 8 of the enantiomers tested here (Laska and Galizia 2001). South African fur seals discriminated between 7 out of 12 optical isomers above chance level (Kim et al. 2012). The Asian elephants, CD-1 mice, and Sprague–Dawley/Long Evans rats, in contrast, succeeded in discriminating between 12 out of 12, 11 out of 11, and 3 out of 3 enantiomeric odor pairs tested, respectively (Rubin and Katz 2001; Laska and Shepherd 2007; Clarin et al. 2010).

Several studies have shown that the amount of pretraining with or repeated exposure to odor stimuli can affect olfactory discrimination performance (Wilson and Stevenson 2006). Although we cannot rule out the possibility that this phenomenon might at least in part explain the above-mentioned between-species differences in olfactory discrimination performance, we would like to point out that the number of training trials performed with these animal species prior to

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Table 4 Between-species comparison of olfactory discrimination performance with enantiomers

Enantiomeric odor pair	Asian elephants	CD-1 mice	human subjects	squirrel monkeys	pigtail macaques	honey bees	SD/LE rats	Fur seals
Carvone	+ (3/3)	+ (8/8)	+ (17/20)	+ (5/6)	+ (3/3)	+ (5/6)	+ (6/6)	+ (3/4)
Dihydrocarvone	+ (3/3)	+ (8/8)	+ (16/20)	+ (4/4)	+ (3/3)	–	–	+ (3/4)
Dihydrocarveol	+ (3/3)	+ (8/8)	+ (18/20)	+ (4/4)	+ (3/3)	–	–	+ (4/4)
Limonene	+ (3/3)	+ (8/8)	+ (18/20)	+ (6/6)	+ (3/3)	+ (6/6)	+ (11/11)	– (0/3)
Limonene oxide	+ (3/3)	+ (8/8)	– (4/20)	– (0/4)	– (0/3)	–	–	+ (3/3)
Isopulegol	+ (3/3)	+ (8/8)	– (3/20)	– (0/4)	+ (3/3)	–	–	– (1/3)
Menthol	+ (3/3)	+ (8/8)	– (5/20)	– (3/6)	–	+ (6/6)	–	+ (3/3)
β-citronellol	+ (3/3)	+ (8/8)	– (8/20)	– (3/6)	–	+ (6/6)	–	– (2/4)
Rose oxide	+ (3/3)	+(8/8)	– (2/20)	– (0/6)	–	– (0/6)	–	– (0/4)
Fenchone	+ (3/3)	+ (8/8)	– (5/20)	+ (5/6)	–	– (0/6)	+ (6/6)	+ (4/4)
α-pinene	+ (3/3)	–	+ (18/20)	+ (6/6)	–	+ (6/6)	–	+ (3/3)
Camphor	+ (3/3)	+ (8/8)	– (4/20)	– (0/6)	–	– (1/6)	–	– (1/4)

The numbers in brackets indicate how many out of the total number of animals or subjects succeeded in discriminating between a given enantiomeric odor pair. A "+" symbol indicates that the group of animals or subjects succeeded in discriminating a given enantiomeric odor pair, and a "–" symbol indicates failure to do so. mouse data: Laska and Shepherd (2007); human data: Laska (2004) and Laska and Teubner (1999a); squirrel monkey data: Laska et al. (1999b) and Laska et al. (2005); pigtail macaque data: Laska et al. (2005); honey bee data: Laska and Galizia (2001); rat data: Clarin et al. (2010) and Rubin and Katz (2001); fur seal data: Kim et al. (2012)

the critical tests was quite comparable and in all cases within the same order of magnitude.

Some authors have argued that the size of the olfactory bulbs, the first relay station of the olfactory pathway, would be indicative of a species' olfactory capabilities and have, for example, taken the available data as an argument for a "greatly reduced sense of smell in primates" relative to other mammals (Smith and Bhatnagar 2004).

Unfortunately, the absolute size of the olfactory bulbs in the Asian elephant has not been reported so far. However, given the striking similarities in the absolute and relative sizes of brain structures between African and Asian elephants (Shoshani et al. 2006), it seems reasonable to assume that the (unknown) size of the olfactory bulb in the Asian elephant should be similar to the (known) size of this brain structure in the African elephant. The absolute size of the olfactory bulbs in the African elephant is 59.9–62.4 cm^3 (Manger, personal communication) and thus indeed dramatically larger than that of all nonproboscid mammals. However, although the absolute size of the olfactory bulbs of mice (20.5 mm^3, Williams et al. 2001) and rats (28 mm^3, Hinds and McNelly 1977) is more than a 1000-fold smaller than that of the Asian elephant, their olfactory discrimination performance with both structurally related aliphatic odorants and enantiomers is not inferior to that of *E. maximus*. Similarly, the absolute size of the olfactory bulbs of human subjects (114 mm^3, Stephan et al. 1988) is more than 4-fold larger than that of mice and rats, but the olfactory discrimination capabilities of *Homo sapiens* with the odor pairs tested here is clearly inferior to that of both rodent species. Thus, the results of this study do not support the notion of a correlation between absolute size of the olfactory bulbs and olfactory discrimination capabilities.

Other authors have argued that the relative size rather than the absolute size of the olfactory bulbs would allow for conclusions as to the olfactory capabilities of different species (Zelenitsky et al. 2011). However, the reasoning that an increase in the size of nonolfactory brain structures would necessarily lead to a decrease in olfactory capabilities is neither logical from a theoretical point of view nor supported by physiological data including those of this study.

Recent genetic studies have shown that species may differ markedly in their number of functional genes coding for olfactory receptors, and some authors have argued that the size of the olfactory receptor repertoire would be predictive of a species' olfactory capabilities (Rouquier et al. 2000). Whereas rats and mice have been shown to have approximately 1260 and 1060 such genes, respectively (Nei et al. 2008), squirrel monkeys (~900), pigtail macaques (~700), human subjects (~390), and honeybees (~160) have considerably lower numbers of functional olfactory receptor genes (Gilad et al. 2004; Niimura and Nei 2006; Robertson and Wanner 2006). Although several studies have failed to find a significant correlation between the size of the olfactory receptor repertoire and olfactory sensitivity in terms of detection thresholds (e.g., Laska et al. 2007), at least some studies support the notion that the number of functional genes coding for olfactory receptors may positively correlate with a species' ability to discriminate between structurally related odorants (e.g., Laska and Shepherd 2007). Unfortunately, the exact number of functional

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olfactory receptor genes in the Asian elephant is not known. However, its closest living relative, the African elephant, has recently been shown to have 1489 functional genes coding for olfactory receptors (Suwa et al. 2011). As closely related species tend to share large proportions of their olfactory receptor repertoires, particularly when also sharing similar chemical environments (Nei et al. 2008), it seems reasonable to assume that the number of functional olfactory receptor genes of the Asian elephant should be similar to that of the African elephant. Based on this assumption, and using the number of functional olfactory receptor genes reported for the African elephant, the data shown in Table 4 suggest a positive correlation between the number of functional olfactory receptor genes and the proportion of discriminable enantiomeric odor pairs that falls just short of statistical significance (Spearman, $r_s = +0.78$, $P = 0.057$). However, to further corroborate the hypothesis that the number of functional olfactory receptor genes determines a species' discriminative abilities with olfactory stimuli, future studies should aim at identifying the total number of such genes in a larger variety of species and testing those species with sets of the same olfactory discrimination tasks.

Although the Asian elephants succeeded in discriminating between all enantiomeric odor pairs tested here, clear differences in the degree of their discriminability and thus in their perceptual similarity were apparent and raise the question as to possible explanations underlying this finding. A recent study demonstrated that the complexity of odorant molecules systematically affects the number of qualitative notes as perceived by humans (Kermen et al. 2011). Thus, it is reasonable to assume that more complex odorant molecules, which evoke more qualitative notes than more simple odorant molecules, should also be easier to discriminate than more simple odorant molecules. However, we failed to find a significant correlation between molecular complexity of the enantiomers used here and the olfactory discrimination scores of the elephants. Similarly, we failed to find single molecular structural properties that would allow for predictions with regard to the discriminability of a given enantiomeric odor pair. This is consistent with the multipoint attachment theory (Ohloff et al. 2011), which predicts that the interaction of an odor molecule with an olfactory receptor is a process that involves at least 2, and probably more, dipole–dipole interactions or hydrogen bonds. In order for a given olfactory receptor to be enantioselective, the theory predicts a minimum of 3 such points of interaction between odor molecule and olfactory receptor.

A more biological explanation for both the observed between-species differences in discrimination performance with enantiomers and the observed between-odorant differences in discriminability of enantiomers is that these odorants may either differ in their behavioral relevance for a given species or in the frequency of their occurrence in the chemical environment of a given species. In honeybees, for example, a significant positive correlation between discrimination scores for enantiomers and their frequency of occurrence in flower odors has been reported (Laska and Galizia 2001). Using the same database of floral scent compounds from more than 400 plant taxa (Knudsen et al. 1993), no such correlation can be found with the Asian elephants tested here (Spearman, $r_s = -0.05$, $P > 0.05$). This should not be surprising considering that Asian elephants are generalist herbivores that are not specialized on nectar-bearing flowering plants. However, our finding that the elephants clearly differed in their discrimination scores with the different enantiomers (see Figure 4), and the fact that their chemical environment clearly differs from that of the honeybee still leave the possibility that the frequency of occurrence of volatile substances in the chemical environment of the elephant may correlate with its relative discrimination performance for different odor pairs. Unfortunately, with the exception of body-borne social odors, very little is known about the composition of the chemical environment of *E. maximus*. It is interesting to note, however, that male Asian elephants discharge different ratios of the (+)- and the (−)-form of frontalin from their temporal glands during and outside of musth, respectively, and that conspecifics are able to detect such differences in the ratio of frontalin enantiomers, which enables them to distinguish both the maturity of males and the phase of musth (Greenwood et al. 2005). This clearly suggests that enantioselectivity of odor perception is behaviorally relevant for Asian elephants.

Taken together, the results of this study demonstrate that Asian elephants were able to discriminate between all members of homologous series of aliphatic odorants and all enantiomeric odor pairs tested and thus that their olfactory discrimination capabilities with structurally related odorants is at least as good as that of mice and clearly superior to that of human subjects and nonhuman primates. Further, they support the notion that the sense of smell may play an important and hitherto underestimated role in the control of behavior in Asian elephants.

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