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ORIGINAL PAPER

Olfactory discrimination ability of South African fur seals (*Arctocephalus pusillus*) for enantiomers

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Abstract Using a food-rewarded two-choice instrumental conditioning paradigm we assessed the ability of South African fur seals, *Arctocephalus pusillus,* to discriminate between 12 enantiomeric odor pairs. The results demonstrate that the fur seals as a group were able to discriminate between the optical isomers of carvone, dihydrocarvone, dihydrocarveol, menthol, limonene oxide, α-pinene, fenchone (all $p < 0.01$), and β-citronellol ($p < 0.05$), whereas they failed to distinguish between the (+)- and (−)-forms of limonene, isopulegol, rose oxide, and camphor (all $p > 0.05$). 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 enantiomeric odor pairs. A comparison between the discrimination performance of the fur seals and that of other species tested previously on the same set of enantiomers (or subsets thereof) suggests that the olfactory discrimination capabilities of this marine mammal are surprisingly well developed and not generally inferior to that of terrestrial mammals such as human subjects and non-human primates. 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. Taken together, the results of the present study support the notion that the sense of smell may play an important and hitherto underestimated role in regulating the behavior of fur seals.

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Introduction

Investigations of the sensory capabilities in marine mammals have so far concentrated on acoustic, visual, and somatosensory function (Thomas et al. 1992; Supin et al. 2001; Hoelzel 2002; Thewissen and Nummela 2008). This should not be surprising given that the senses of taste and smell have traditionally been considered of only minor, if any, importance in this diverse group of animals (Brown 1985). However, marine mammals which comprise members of the orders Sirenia, Carnivora, and Cetacea differ markedly in their ecology and behavior (Hoelzel 2002) making generalizing statements as to their sensory capabilities more than arguable. An increasing number of behavioral observations now suggest that pinnipeds such as fur seals, for example, use their sense of smell in a variety of behavioral contexts including social communication, foraging, food selection, and reproduction.

In the social context, South American fur seals (*Arctocephalus australis*) have been reported to use olfactory cues for individual recognition and acceptance of pups by their mothers (Phillips 2003). Similarly, South American sea lion (*Otaria flavescens*) mothers have been found to perform olfactory inspection of their offspring during 97.6 % of all mother–pup reunions that were observed (Trimble and Insley 2010). Olfactory recognition of offspring, often accompanied by nuzzling, that is mutual naso-nasal contact, has also been reported in Antarctic fur seals, *Arctocephalus gazella* (Dobson and Jouventin 2003), South African fur seals, *Arctocephalus pusillus* (Ross 1972), and

harp seals, *Phoca groenlandica* (Kovacs 1995). Female Northern fur seals (*Callorhinus ursinus*) have been described to vigorously sniff at their pups during and immediately after parturition (Bartholomew 1959), a behavior commonly found in non-aquatic mammals which is thought to aid in building a bond between mother and offspring (Wyatt 2003). A recent study even provided experimental evidence that Australian sea lion (*Neophoca cinerea*) mothers are able to discriminate between the odors of their own pup and that of other, age-matched pups (Pitcher et al. 2011)

In the reproductive context, male New Zealand fur seals (*Arctocephalus forsteri*) have been observed to sniff frequently at the perineal and facial regions of females during the breeding season, suggesting an olfactory assessment of the female's reproductive state (Miller 1974). Male South American fur seals (*Arctocephalus australis*) and ringed seals (*Phoca hispida*) have been described to emit a strong odor during the rut which is caused by an enlargement and heightened activity in their sebaceous and apocrine facial glands (Hamilton 1956; Hardy et al. 1991). These secretions are discussed as a means of territorial marking and/or attracting females (Ling 1965; Ryg et al. 1992).

In the context of foraging and food selection, grey seals (*Halichoerus grypus*) have been observed to appear downwind of an oil slick resulting from another seal surfacing and consuming a captured fish (Arne Fjälling, personal communication). Similarly, harbor seals (*Phoca vitulina*) have been found to swim on direct routes to their feeding grounds and some authors believe that they may use olfactory cues for spatial orientation and foraging (Kowalewsky et al. 2006). This assumption is supported by the finding that harbor seals have a high olfactory sensitivity for dimethyl sulfide, an odorant which is thought to be indicative of high marine productivity (Kowalewsky et al. 2006). Interestingly, the same odorant has been found to be used for foraging by marine birds such as petrels (Nevitt and Bonadonna 2005) and, possibly, penguins (Cunningham et al. 2008).

Despite this variety of observational studies suggesting the sense of smell to play an important role in regulating the behavior of fur seals, experimental studies on the olfactory performance of pinnipeds have been sparse. The development of a food-rewarded two-choice instrumental conditioning paradigm for South African fur seals (Laska et al. 2008) now enables us to assess the olfactory capabilities of this pinniped species. In a recent study, Laska et al. (2010) demonstrated that *Arctocephalus pusillus* is capable of discriminating between structurally related aliphatic odorants which only differ from each other in the length of their carbon chain. In the present study, we continue to explore the olfactory discrimination capabilities in South African fur seals by assessing their ability to distinguish 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 because perceptual differences between enantiomers cannot be due to properties such as differing diffusion rates in the mucus covering the olfactory sensory epithelium or differing air/mucus partition coefficients (Hahn et al. 1994), but must originate from chiral selectivity at the peripheral receptor level (Rossiter 1996). Therefore, 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 the present study to assess the discrimination ability of South African fur seals for a set of 12 enantiomeric odor pairs. Substances were chosen based on the 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 with enantiomers 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 four adult female (Flisa, Tinny, Sealia, and Villma) South African fur seals (*Arctocephalus pusillus*) maintained as part of a group of eight animals at Kolmården Wild Animal Park, Sweden. The group was housed under semi-natural conditions in a 800 m^2 outdoor pool with an adjacent house bearing eight cages which allowed temporary separation of animals for individual testing. All animals were trained to enter the individual cages voluntarily and were completely accustomed to the procedure. Animals were born in captivity and fed fish (mackerel, *Scomber scombrus*) and squid (Argentine shortfin squid, *Ommastrephes argentinus*) twice a day. Thus, their daily diet was very similar to the diet reported for free-ranging South African fur seals which comprise up to 70 % mackerel and 20 % squid, respectively (Mecenero et al. 2006). No food deprivation schedule was adopted. Rather, the food reward given to the animals during the training sessions was part of their two feeding bouts per day.

The behavioral test

The behavioral test was based on a food-rewarded two-choice instrumental conditioning paradigm. In a previous

study (Laska et al. 2008), the animals had learned to sniff at two odor sampling ports and then to indicate which of the two options held the rewarded stimulus by poking their nose into the corresponding sampling port. An opaque PVC board (50 cm high × 100 cm wide × 1 cm thick) with two openings (7.5 cm diameter) at equal height and 42 cm apart from each other was mounted at the front side of the test cage in such a way that the openings were 47 cm above the ground. For the presentation of the odor stimuli two HDPE (high-density polyethylene) containers with tight-fitting removable lids (Rubbermaid® Cooling Bags, 35 cm high × 35 cm wide × 20 cm deep) were used. Each lid was equipped with a battery-powered ventilator (6 cm diameter) providing an ingoing airflow. A total of 130 holes of 3 mm diameter placed in intervals of even distance 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 container serving as an outlet for the airflow provided by the ventilator. The outgoing airflow was adjusted to 8 l/min and a platform on the outside of the cage ensured that the containers could be placed with their outlets congruent with the openings of the PVC board. In order to present the odor stimuli, 1 ml of an odorant was pipetted onto a Petri dish which, in turn, was placed into an opaque HDPE box (12 cm high × 20 cm wide × 12 cm deep) without a lid inside the containers. This allowed the airflow provided by the ventilator to reach the surface of the odor stimuli and, at the same time, prevented the animals from using visual cues. A mirror placed on top of the cage allowed the experimenter to observe the animal's behavior without being seen behind the opaque PVC board. A previous study in which the same experimental setup had been used (Laska et al. 2008) and which included double-blind trials gave no indication that the animals might use unintended cues from the experimenter.

At the beginning of each trial, the two containers were placed with their outlets towards the odor sampling ports and an animal was allowed to sniff at them as often as it liked. Immediately following the animal's decision (a nose poke into one of the two odor sampling ports), the two containers were removed and, in the case of a correct response (a nose poke into the odor sampling port bearing the rewarded stimulus), the animal was rewarded with a fish or squid. In the case of an incorrect response (a nose poke into the odor sampling port bearing the unrewarded stimulus), no reward was given to the animal. Twenty such trials were performed per session and usually two sessions were performed per animal and day. Care was taken to present the rewarded stimulus to the right or the left odor sampling port adopting a pseudorandomized sequence with the limitation that the same option was not used more than three times in a row. At the end of each session, the containers and the boxes bearing the odor stimuli were thoroughly cleaned using unperfumed detergent and rinsed with demineralized water. A previous study in which the containers assigned to a given stimulus were occasionally changed between sessions (Laska et al. 2008) gave no indication that potential residues of odorants affected the animals' subsequent choice behavior.

Odor stimuli

A set of 12 enantiomeric odor pairs was used (Table 1; Fig. 1). All substances 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 (Merck, Darmstadt) as the solvent. The level of dilution was chosen to provide stimuli that were easily detectable and of approximately equal subjective intensity for humans when sniffed at the outlet of the stimulus containers. The rationale for choosing these substances was to assess the discrimination performance of the fur seals for odorants that are identical in their chemical and physical properties except for chirality. Furthermore, data on discrimination performance for the same set of stimuli (or subsets thereof) from humans (Laska and Teubner 1999; Laska 2004), squirrel monkeys (Laska et al. 1999; 2005), pigtail macaques (Laska et al. 2005), mice (Laska and Shepherd 2007), honeybees (Laska and Galizia 2001), Asian elephants (Rizvanovic 2012), and rats (Rubin and Katz 2001; Clarin et al. 2010) are at hand allowing across-species comparisons of discrimination performance. In addition, the essential oil of black pepper (*Piper nigrum*) was used. This odorant had been used in a previous study (Laska et al. 2008) as unrewarded stimulus and was thus familiar to the animals.

Experimental design

Two of the four animals were trained to associate the (+)-form of a given enantiomeric odor pair as the rewarded stimulus, and the other two animals were trained to associate the (−)-form of the same enantiomeric odor pair as the rewarded stimulus. To allow an animal to build a robust association between a given odorant and its significance as rewarded stimulus, each fur seal received 5–10 sessions of 20 trials, using pepper oil as unrewarded stimulus. Once familiarized with the rewarded stimulus, the black pepper oil was exchanged for the other form of the same enantiomer and this enantiomeric odor pair was presented for four sessions.

Whenever an animal failed to meet the criterion of 67.5 % correct choices with a given enantiomeric odor pair, two sessions with black pepper oil as unrewarded stimulus were implemented between the different test combinations in order to boost the animal's confidence and to refresh its memory for the reward value of the rewarded stimulus.

Table 1 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

With the enantiomers of limonene, only three of the four animals could be tested as one animal (Sealia) was injured on her right flipper, which did not allow her to lift up her upper body to participate in the test. Furthermore, with four pairs of enantiomers (limonene oxide, isopulegol, menthol, and α-pinene), only three animals were tested as one animal (Flisa) had to be euthanized due to health problems.

Data analysis

In the method described here, the animal had two options: (1) to correctly respond to the positive stimulus, and (2) to falsely respond to the negative stimulus. The percentage of correct decisions was used as the measure of performance and in all tasks two levels of criterion performance were used: a more liberal criterion was set at 67.5 % correct choices in two consecutive sessions (corresponding to $p < 0.05$, two-tailed binomial test), and a more rigorous criterion was set at 72.5 % correct choices in two consecutive sessions (corresponding to $p < 0.01$, two-tailed binomial test).

Comparisons of performance across individuals and tasks were made using the Mann–Whitney U test for independent samples. The number of animals tested in the present study was too low to allow for statistical evaluation of discrimination performance at the group level. Therefore, the following criteria were adopted: with the odor pairs that were tested with four animals, at least three of them had to reach the criterion of $p < 0.05$ so that the fur seals as a group were considered as being capable of discriminating a given odor pair. With the odor pairs that were tested with only three animals, all three had to reach the criterion of $p < 0.05$ so that the fur seals as a group were considered as being capable of discriminating a given odor pair.

Results

General discrimination performance

Figure 2 shows the performance of the four South African fur seals in discriminating between the 12 enantiomeric odor pairs. According to the more strict criterion of 72.5 % correct choices (corresponding to $p < 0.01$, two-tailed binomial test), the animals as a group succeeded in discriminating between 7 out of the 12 enantiomeric odor pairs (carvone, dihydrocarvone, dihydrocarveol, menthol, limonene oxide, α-pinene, and fenchone). According to the more liberal criterion of 67.5 % correct choices (corresponding to $p < 0.05$, two-tailed binomial test), the group

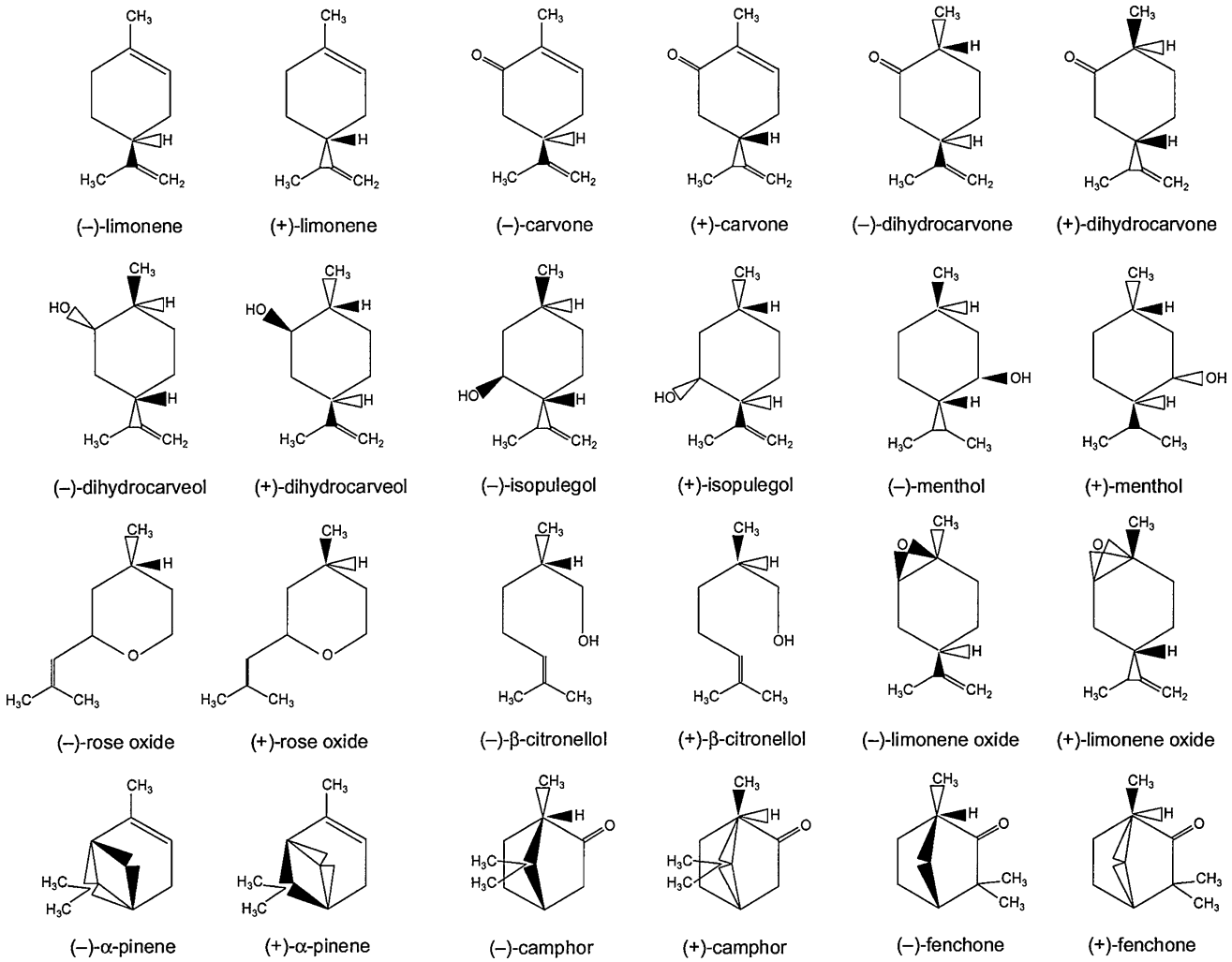

Fig. 1 Molecular structures of the 12 enantiomeric odor pairs used

of animals additionally distinguished between the (+)- and (−)-forms of *β*-citronellol and thus succeeded in discriminating between 8 out of 12 enantiomeric odor pairs (Fig. 2).

Marked differences in discriminability with the different enantiomeric odor pairs were apparent. Accordingly, the average score across all animals in a given task ranged from 81.3 (±4.8) % correct choices with dihydrocarveol to 63.3 (±5.2) % correct choices with limonene.

Interindividual differences in discrimination performance

Interindividual variability in performance with a given odor pair was generally low and the mean range between the best- and the poorest-scoring animal with a given odor pair was only 12.9 %. The largest difference in scores between animals in a given task was 25 % with the enantiomers of carvone. In contrast, all animals tested with the enantiomers of menthol scored the same percentage of correct choices (Fig. 2). Flisa scored an average of 78.4 (±10.4) % correct choices across all tasks in which she participated, and Tinny, Villma, and Sealia scored an average of 76.7 (±7.5), 70.0 (±7.9), and 69.8 (±6.6) % correct choices, respectively. Accordingly, with only one exception (Tinny vs. Sealia), no significant differences in overall performance were found between the animals ($p > 0.05$, Mann–Whitney U test).

Odor structure–activity relationships

Table 2 summarizes the discrimination performance of the fur seals and some of the molecular structural features that may have affected the discriminability of the enantiomeric odor pairs tested. No significant differences in discriminability were found between enantiomeric odor pairs with (74.1 ± 9.1 % correct choices) or without (72.6 ± 8.3 % correct choices) an isopropenyl group at the chiral

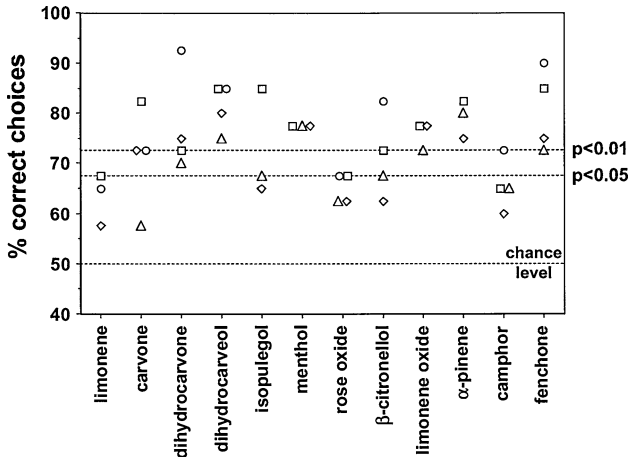

Fig. 2 Performance of four South African fur seals in discriminating between 12 enantiomeric odor pairs. Each *data point* represents the percentage of correct choices from 40 decisions in two consecutive sessions of 20 trials each per animal (*circle* Flisa, *square* Tinny, *triangle* Sealia, *diamond* Villma). The *dashed horizontal lines* indicate chance level (at 50 %), and two levels of criterion (at 67.5 %, corresponding to $p < 0.05$, and at 72.5 %, corresponding to $p < 0.01$), respectively

center ($p > 0.05$, Mann–Whitney U test). The presence (73.7 ± 8.6 % correct choices) or absence (71.3 ± 9.6 % 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*-, 73.8 ± 8.9 %; *meta*-, 75.3 ± 9.0 %; *para*-position, 69.6 ± 6.4 % correct choices, respectively) nor the type of oxygen-containing functional group (alcohol-, 75.7 ± 7.7 %; keto-group, 73.6 ± 9.9 % correct choices, respectively) had a systematic effect on discriminability (in all cases, $p > 0.05$, Mann–Whitney U test). Discriminability did also not differ significantly between monocyclic (73.1 ± 8.7 % correct choices) and bi- or acyclic (73.7 ± 8.9 % correct choices) enantiomers ($p > 0.05$, Mann–Whitney U test). In contrast, enantiomers with additional chiral center(s) (75.8 ± 8.2 % correct choices) were significantly easier to discriminate for the fur seals compared to enantiomers without an additional chiral center (69.9 ± 8.2 % correct choices) ($p < 0.05$, Mann–Whitney U test).

Discussion

General discrimination performance

The results of the present study demonstrate that South African fur seals were capable of discriminating between the majority of the enantiomeric odor pairs tested, and that the enantiomeric odor pairs clearly differed in their degree of discriminability and thus in their perceptual similarity.

Our finding that the fur seals discriminated between the majority of the enantiomeric odor pairs tested raises the question whether perceived differences in stimulus intensity rather than stimulus quality might have contributed to the surprisingly good discrimination performance that the animals displayed. Although this possibility cannot be ruled out completely, it appears rather unlikely as firstly, the fur seals failed to learn to discriminate between different concentrations of a given stimulus in the conditioning paradigm used here (Laska et al. 2008), and secondly, 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 (Laska et al. 2010).

Similarly, the possibility that the nasal trigeminal system might have contributed to the discrimination of the enantiomers of carvone, dihydrocarvone, dihydrocarveol, menthol, limonene oxide, α-pinene, fenchone, and β-citronellol appears quite unlikely as several of these enantiomers have been shown to have little, if any, trigeminal-stimulating properties at the concentrations used here when tested with human subjects (Laska and Teubner 1999; Laska 2004). Further, with all enantiomers tested so far with human subjects the (+)- and (−)-forms of a given substance did not differ in their degree of trigeminality (Laska and Teubner 1999; Laska 2004). Therefore, we believe the discrimination scores reported here to reflect the ability of the fur seals to discriminate between stimulus qualities.

Between-species comparison of discrimination performance

Table 3 compares the discrimination performance of the fur seals to that of other species tested on the same odor pairs or on subsets thereof. Human subjects were only able to significantly discriminate between 5 of the 12 enantiomeric odor pairs tested here (Laska and Teubner 1999; Laska 2004), and squirrel monkeys distinguished between 6 of the 12 pairs of optical isomers (Laska et al. 1999, 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). Asian elephants, CD-1 mice, and SD/LE 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; Rizvanovic 2012).

This raises the question as to possible explanations underlying these between-species differences in olfactory discrimination capabilities with enantiomers. Some authors have argued that the size of the olfactory bulbs, the first

Table 2 Discrimination performance with and structural features of the 12 enantiomeric odor pairs

Enantiomeric odor pair	Discrimination performance	Isopropenyl group	Oxygen-containing functional group at			Methyl group at *para*-position	Monocyclic structure	Additional chiral center(s)
			ortho	*meta*	*para*			
Carvone	+	+	−	+	−	+	+	−
Dihydrocarvone	+	+	−	+	−	+	+	+
Dihydrocarveol	+	+	−	+	−	+	+	+
Menthol	+	−	+	−	−	+	+	+
β-Citronellol	+	−	−	+	−	−	−	−
Fenchone	+	−	+	−	−	−	−	+
α-Pinene	+	−	−	−	−	−	−	−
Limonene oxide	+	+	−	−	+	+	+	+
Isopulegol	−	+	+	−	−	+	+	+
Limonene	−	+	−	−	−	+	+	−
Rose oxide	−	−	−	−	+	−	+	−
Camphor	−	−	+	−	−	−	−	+

With regard to discrimination performance, a "+" indicates that the corresponding odor pair was discriminated at the group level, and a "−" indicates failure to do so

With regard to the other columns, a "+" indicates the presence, and a "−" the absence of the corresponding structural feature

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). However, 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 comparable to that of squirrel monkeys (26.8 mm^3, Stephan et al. 1988) and markedly smaller than that of human subjects (114 mm^3, Stephan et al. 1988). Although the absolute size of the olfactory bulbs of the South African fur seal is not known, closely related pinniped species of comparable body weight and total brain volume such as the Northern fur seal (478 mm^3), the California sea lion (526 mm^3), the Steller sea lion (668 mm^3), and the harbor seal (230 mm^3) all have olfactory bulbs that are markedly larger than those of mice and rats (Reep et al. 2007). Thus, assuming that the brain proportions of the South African fur seals do not differ fundamentally from those of their close relatives, the data of the present study do not support the notion of a correlation between absolute size of the olfactory bulbs and olfactory discrimination capabilities (Table 3).

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 non-olfactory brain structures would necessarily lead to a decrease in the efficiency of olfactory brain structures and thus to a decrease in olfactory capabilities is neither logical from a theoretical point of view nor supported by physiological data including those of the present study.

Yet other authors have argued that the surface area of the olfactory epithelium would allow us to predict between-species differences with regard to olfactory capabilities (Issel-Tarver and Rine 1997). However, apart from the fact that this idea is based on the unverified assumption that different species would have comparable densities of olfactory sensory neurons in their olfactory epithelium, there is physiological evidence from comparative studies on olfactory sensitivity which does not support the notion of a simple correlation between olfactory epithelium size and olfactory detection thresholds (Smith et al. 2004). Unfortunately, the size of the olfactory epithelium in South African fur seals is not known which prevents us from drawing any conclusions concerning possible connections between this anatomical feature and olfactory discrimination performance.

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 (Gilad et al. 2004). Whereas rats and mice have been shown to have approximately 1,260 and 1,060 such genes, respectively (Nei et al. 2008), squirrel monkeys ($\approx$900), pigtail macaques ($\approx$700), human subjects ($\approx$390), and honeybees ($\approx$160) have considerably lower numbers of functional olfactory receptor genes (Rouquier et al. 2000; Niimura and Nei 2006; Robertson and Wanner 2006). While 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

Table 3 Between-species comparison of olfactory discrimination performance with enantiomers

	Fur seals		CD-1 mice	Human subjects	Squirrel monkeys	Pigtail macaques	Honey bees	SD/LE rats	Asian elephants
	$p < 0.05$	$p < 0.01$							
Carvone	+(3/4)	+(3/4)	+(8/8)	+(17/20)	+(5/6)	+(3/3)	+(5/6)	+(6/6)	+(3/3)
Dihydrocarvone	+(3/4)	+(3/4)	+(8/8)	+(16/20)	+(4/4)	+(3/3)			+(3/3)
Dihydrocarveol	+(4/4)	+(4/4)	+(8/8)	+(18/20)	+(4/4)	+(3/3)			+(3/3)
Limonene	−(1/3)	−(0/3)	+(8/8)	+(18/20)	+(6/6)	+(3/3)	+(6/6)	+(11/11)	+(3/3)
Limonene oxide	+(3/3)	+(3/3)	+(8/8)	−(4/20)	–(0/4)	−(0/3)			+(3/3)
Isopulegol	−(2/3)	−(1/3)	+(8/8)	−(3/20)	−(0/4)	+(3/3)			+(3/3)
Menthol	+(3/3)	+(3/3)	+(8/8)	−(5/20)	−(3/6)		+(6/6)		+(3/3)
β-Citronellol	+(3/4)	−(2/4)	+(8/8)	−(8/20)	−(3/6)		+(6/6)		+(3/3)
Rose oxide	−(2/4)	−(0/4)	+(8/8)	−(2/20)	−(0/6)		−(0/6)		+(3/3)
Fenchone	+(4/4)	+(4/4)	+(8/8)	−(5/20)	+(5/6)		−(0/6)	+(6/6)	+(3/3)
α-Pinene	+(3/3)	+(3/3)		+(18/20)	+(6/6)		+(6/6)		+(3/3)
Camphor	−(1/4)	−(1/4)	+(8/8)	−(4/20)	−(0/6)		−(1/6)		+(3/3)

The numbers in brackets indicate how many out of the total number of animals or subjects succeeded in discriminating between the (+)- and the (−)- form of a given enantiomeric odor pair

Mouse data: Laska and Shepherd (2007); human data: Laska (2004) and Laska and Teubner (1999); squirrel monkey data: Laska et al. (1999) 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); Asian elephant data: Rizvanovic (2012)

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 olfactory receptor genes in the South African fur seal is not known. However, assessing a subset of olfactory receptor genes, Kishida et al. (2007) found that the proportion of olfactory receptor pseudogenes in the Steller sea lion, a close relative of the South African fur seal, is not higher compared to that found in the dog. In contrast, the same study found that more than 75 % of the genes coding for olfactory receptors were non-functional in sperm whales and Dall's porpoises. From this the authors concluded that marine mammals such as seals which rely on a terrestrial habitat for breeding, resting, and social interaction maintained their repertoires of olfactory receptors, whereas whales which exclusively rely on the marine habitat did not. Future studies employing whole-genome sequencing (instead of sequencing of a randomly chosen fraction of olfactory receptor genes) of several species of marine mammals including the South African fur seal are necessary to corroborate this hypothesis and to draw conclusions as to possible correlations between olfactory discrimination performance and this genetic feature.

Odor structure–activity relationships

A final aspect of the present study is our finding that no generalizable conclusions can be drawn from our data as to odor structure–activity relationships which would allow us to predict whether a given pair of enantiomers can be olfactorily discriminated by the fur seals or not. Although several of the substances whose optical isomers were significantly distinguished by the fur seals share an isopropenyl group at the chiral center (carvone, dihydrocarvone, dihydrocarveol, limonene oxide), other substances sharing the same molecular property were not discriminated (isopulegol, limonene). In addition, our finding that the antipodes of menthol, β-citronellol, fenchone, and α-pinene were also discriminated despite their lack of an isopropenyl group illustrates that the presence or absence of a single molecular structural property such as a certain functional group at the chiral center is not a sufficient predictor of enantioselectivity. Similarly, the presence of an oxygen-containing functional group at a certain position within the molecule is not sufficient to predict whether the antipodes of a given substance are discriminable or not as, for example, menthol, fenchone, isopulegol, and camphor all share this molecular property, but differed markedly in their discriminability (Table 2). In this context, it is also interesting to note that fenchone, which was discriminated by the fur seals, and camphor, which was not discriminated, represent non-enantiomeric structural isomers, that is, they share the same molecular formula and functional groups but differ in structure in a non-chiral manner (see Fig. 1).

Our finding that no single molecular structural property allows for predictions with regard to the discriminability of a given enantiomeric odor pair is consistent with the

multipoint attachment theory (Ohloff et al. 2011). This theory predicts that the interaction of an odor molecule with an olfactory receptor is a process that involves at least two, 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 three such points of interaction between odor molecule and olfactory receptor. In agreement with this idea, Buchbauer and Shafii-Tabatabai (2003) reported the qualitative attributes of cyclic enantiomers as perceived by human subjects to depend on both an odorant's chirality and the type of functional group(s) at carbon atoms other than the chiral center. In line with these findings, seven of the eight enantiomeric odor pairs that were discriminated by the fur seals in the present study shared an oxygen-containing functional group at carbon atoms other than the chiral center (see Table 2).

A more biological explanation of why some enantiomeric odor pairs were discriminated by the fur seals, whereas others were not is that enantioselectivity of the olfactory system of *Arctocephalus pusillus* may be restricted to substances for which both optical isomers are widely present in their natural habitat. This notion is supported by a study that demonstrated a significant positive correlation between olfactory discrimination performance of honeybees and the frequency of occurrence of the tested enantiomers in flower odors (Laska and Galizia 2001). Unfortunately, our knowledge about the composition of the marine and terrestrial chemical environments inhabited by fur seals is rather fragmentary (Carr 1988). It is interesting to note, however, that neither studies reporting on the chemical composition of the odors of fish which comprise the natural diet of fur seals (e.g., Josephson 1991; Alasalvar et al. 1997) nor studies reporting on the chemical composition of body-borne odors produced by fur seals (e.g., Cadwallader and Shahidi 2001; Koep et al. 2007) ever reported the presence of any of the enantiomers tested here. This should not be surprising given that the enantiomers used in the present study comprise terpenoids that are mostly found in the essential oils of vascular plants (Ohloff et al. 2011) which are not common in the marine chemical environment. However, the possibility that these enantiomers may be present in the natural habitat of fur seals, or may even be produced by the fur seals themselves, cannot be excluded and warrants further investigation.

Taken together, the results of the present study demonstrate that South African fur seals were able to discriminate between the majority of enantiomeric odor pairs tested and thus that their olfactory discrimination capabilities with structurally related odorants is not generally inferior to that of terrestrial mammals such as human subjects and non-human 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 fur seals.

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