STRUCTURE, FUNCTION AND CONTEXT: THE IMPACT OF MORPHOMETRY AND ECOLOGY ON OLFACTORY SENSITIVITY

By

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B.Sc., Massachusetts Institute of Technology, 1998

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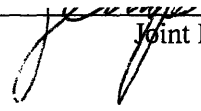

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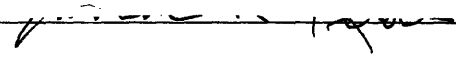

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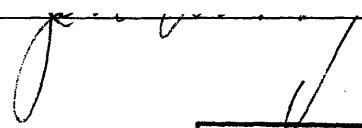

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Structure, Function and Context: the Impact of Morphometry and Ecology on Olfactory Sensitivity

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Abstract

In this thesis, the relationships of olfactory sensitivity to three biological variables were tested. The sensitivity of a marine mammal, the sea otter (*Enhydra lutris*) was measured in order to determine whether a marine lifestyle results in impaired olfaction. The effect of dietary relevance on sensitivity to specific odorants was evaluated. Finally, a new morphometric model of olfactory uptake efficiency was developed and tested against behavioral measurements of olfactory sensitivity in twelve mammalian species from five orders.

Olfactory thresholds were obtained for the first time from two sea otters for seven odorant compounds from various natural sources. Otters were trained using operant conditioning to participate in direct behavioral testing. Sea otter olfactory sensitivity was comparable to that of previously studied terrestrial mammals.

The incidence of an odorant in the diet of the olfactor was found to influence specific sensitivity to that compound but to varying degrees among different mammalian orders.

Nasal cavity specimens were measured using radiologic (CT scan) and histologic (light microscopy) techniques. Surface areas and volumes of the nasal cavity were used to calculate the Olfactory Uptake Efficiency (OUE). OUE is significantly related to olfactory bulb volume. A possible relationship was found between OUE and general olfactory sensitivity.

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Acknowledgements

This document is the product of a great deal of hard work and dedication and was by no means a solo effort. This preface is an inadequate tribute to the contributions made by my colleagues and friends. Without their skill, guidance and support the work described herein would not have been possible.

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manifested itself in the most useful possible way, in demands that I do justice to my research question through careful and thorough analysis. Roger Kamm single-handedly supervised my education in fluid dynamics and sanity-checked countless versions of the olfactory uptake model, always bearing in mind what it needed to accomplish and what it didn't.

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at which they should have beome bored and left in a huff. Judy Tuttle and Ken Lytwyn of the Oregon Coast Aquarium made time for research and their staff cheerfully bent their schedules to accommodate nearly two months of data collection. JoEllen Marshall and Karen Rifenbury of the Oregon Zoo put other projects on hold to devote their time and staff to mine and refused to admit defeat when faced with early setbacks.

A foreign student requires additional paperwork on top of the usual registration, funding, etc. The administrative staff at WHOI have seen it all before and never panic. Marsha Gomes, Aura Holguin and Julia Westwater of the Education office kept track of me through seven years, two advisors and a semester off. Anita Norton and the unflappable Judy McDowell always had time for my questions even amid the Grand Central Station that is the Ketten Group.

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The support of family and friends becomes increasingly important the older a student gets. I'm in the 24th grade, and my parents, Brigitte and David are still as proud of my school projects as they were back in kindergarten. Their love and support has been a constant through my checkered career. My big brother Chris has been my immediate role model throughout my academic career. It will be strange not having his footprints in front

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of Natural History, the Whitehead Institute at the Massachusetts Institute of Technology, the Biology Department of MIT, the California Oiled Wildlife Network, the Harvard Museum of Comparative Zoology, the Institute for Hydrology and Ecology at Monk's Hood, Tufts Veterinary School, the New England Regional Primate Research Center, Lion Country Safari Zoo, and the Cameron Park Zoo. Funding was provided by the Woods Hole Oceanographic Institution's Education Department, Biology Department and Ocean Ventures Fund, the National Science and Engineering Research Council of Canada, the Gen Foundation, the Massachusetts Institute of Technology's Student Assistance Fund, the European Chemoreception Research Organization, the Society for Experimental Biology, the Company of Biologists, and the Office of Naval Research.

Chapter 1: Introduction

Structure, Function and Context: the impact of morphometry and ecology on olfactory sensitivity

Goals

There were three major objectives to this thesis:

1. To test whether a marine lifestyle has a negative impact on general olfactory sensitivity.
2. To determine the influence of nasal anatomy on olfactory sensitivity in mammals.
3. To determine the influence of dietary chemical ecology on specific olfactory sensitivity.

In order to accomplish this, the following hypotheses were tested:

1. A marine mammal will have weak general olfactory sensitivity compared with terrestrial mammals.
2. A calculated olfactory uptake efficiency index based on nasal morphometric measures is related to overall olfactory sensitivity in mammals.
3. Individual mammalian species are more sensitive to compounds with high ecological relevance; eg., characteristic food odour components, than are other mammals for which the same compounds have less relevance.

Olfactory sensitivity is quantitatively represented by the olfactory detection threshold, or lowest detectable concentration. Thresholds vary among individual animals and among odorant compounds. An animal's threshold for a particular compound represents the animal's specific sensitivity to that compound. However, thresholds may also vary with time and context. The range and average of available thresholds for a given mammalian species are currently the best available indicators of general or overall olfactory sensitivity. Despite many recent advances in olfactory genetics and neurophysiology, neither general

nor specific olfactory sensitivity has to date been predicted from any genetic or neuroanatomical trait.

This study used anatomical characters, specifically epithelial surface area and lumen volume in different regions of the nasal cavity, to compare the olfactory system of twelve mammals from five orders:

Rodentia: House mouse (*Mus musculus*); Brown rat (*Rattus norvegicus*)

Carnivora: Domestic dog (*Canis familiaris*); Sea otter (*Enhydra lutris*)

Insectivora: European shrew (*Sorex araneus*)

Chiroptera: Vampire bat (*Desmodus rotundus*); Seba's short-tailed bat (*Carollia perspicillata*); Great fruit bat (*Artibeus literatus*); Pale spear-nosed bat (*Phyllostomus discolor*); mouse-eared bat (*Myotis myotis*)

Primates: Human (*Homo sapiens*); Common squirrel monkey (*Saimiri sciureus*).

Nasal cavities were examined post-mortem by computerized tomography (CT) and light microscopy.

Olfactory function was also evaluated directly in live sea otters, as described in Chapter 2. A behavioral assay was used to determine olfactory sensitivity of these subjects for a set of natural volatile compounds. The animals were trained using operant conditioning to distinguish and report the presence of an odorant in an air stream presented by an air dilution olfactometer. Each compound was presented in different concentrations to determine the lowest concentration that elicits a reliable response: the olfactory detection threshold. Sea otters were selected as an example of both divergent dietary ecology and divergent nasal morphometry, both resulting from their marine lifestyle. These measurements also served to test whether a marine lifestyle decreases olfactory sensitivity compared to that of other mammals.

The nasal anatomical data and sea otter threshold datasets, and published olfactory threshold values were used to test a morphometric model of olfactory sensitivity. Sensitivity

data for the species listed earlier as well as published data for the Pig-tailed macaque (*Macaca nemestrina*) and the European hedgehog (*Erinaceus europaeus)* were also used to evaluate the impact of ecological relevance on specific olfactory sensitivity, by comparing the specific olfactory sensitivities of pairs of species within the same order but with divergent dietary habits.

Background

The evolution of olfactory sensitivity is poorly understood. It is known that olfactory sensitivities vary widely among the Mammalia. Some mammals, such as the Mouse-eared bat have uniformly poor sensitivity relative to other species for compounds available for comparison; i.e., they have poor general sensitivity. In other species, sensitivity to a specific compound can be exceptionally good or poor. The pig-tailed macaque has comparable sensitivity to the other primates for most compounds tested but sensitivity to ethyl acetate fifty times worse than that of the next least sensitive primate; i.e. good general but poor specific sensitivity.

Chemoreception is an extremely important sense for many vertebrates. Its critical role is reflected in the fact that all vertebrate species preserve at least one chemoreceptive sense (smell or taste), while there are numerous known cases of other senses being secondarily lost; e.g., vision in cavefish *(Amblyopsis rosae, A. spelaeas),* European subterranean salamanders *(Proteus anguineus),* and blind snakes (*Ramphotyphlops braminus, Leptotyphlops dulcis, L. humilis*) or hearing in many species of snakes and burrowing lizards (Stoddart, 1980). It is clear that olfaction moderates a wide variety of behaviors, from feeding, territoriality and migration to mate selection, breeding and care of young. Further, in several mammals it has been demonstrated that olfactory experience early in life is responsible for social imprinting, kin recognition and the formation of food preferences (Hepper, 1994; Sun and Mueller-Schwarze, 1997; Vargas and Anderson, 1996).

In mammals, the gene family encoding olfactory receptor proteins is believed to constitute 1% of the genome, the largest known gene family in any species (Buck, 2000). By contrast, primate trichromatic colour-vision, the most sophisticated colour-vision system in the Mammalia, has no more than seven genes coding for three pigment types, and the green gene family's five members are nearly identical (Nathans *et al*, 1986).

Significance

Volatile chemical signals differ from light cues in two important ways. First, while a variety of no-light or extremely low-light habitats exist in subterranean and deep sea environments, there are no odourless or near-odourless habitats in either air or water. If living cells or abiotic chemical sources are present, they may be producing chemical signals of some survival significance. Therefore, an olfactory sense can be useful anywhere, unlike vision, which can be compromise and in some species absent or lost as noted above. Particular ecological constraints that call for the nasal passages to be open infrequently, as in cetaceans, may reduce the importance of nasal chemoreception, but this reduction need not apply to a marine species that spends most of its life at the surface. The persistence of olfactory sensitivity in a marine environment is tested in the sea otter in Chapter 2.

The second relevant way in which chemoreception and light reception differ is that chemical cues carry particular information about their specific sources. If a fish eye has evolved high sensitivity to blue light, this is plausibly explained by the fact that it belongs to a pelagic fish in whose habitat blue light is abundant and therefore useful for detecting a wide variety of objects. If a mammal's olfactory system has evolved high sensitivity to butyric acid, the relative abundance of butyric acid in its habitat is not a sufficient explanation. Butyric acid is only relevant if it is produced by and can aid in the detection of some item of importance.

Rapid advances within the past fourteen years in the molecular biology of olfaction, beginning with the identification of the olfactory receptor protein superfamily by Buck and Axle (1991), suggest that detections of different odorant stimuli are mediated by different sets of genes. This in turn suggests that olfactory sensitivities to particular compounds evolve at least partially independently of one another. Different species can thus be expected to differ in their relative sensitivity to different compounds, depending on the adaptive value of detecting them. Since many volatile chemicals, including aliphatic acids, alcohols, and esters occur with very different frequencies in different organisms, taxa and biomes, it is reasonable to expect sensitivity to different compounds to be related to their usefulness in detecting and identifying objects of importance, such as predators, prey or food items, and conspecifics. This relationship is tested in Chapter 3.

Olfactory sensitivity is extremely difficult to measure directly. Therefore, much of what we know about olfaction is inferred from behavioral, genetic, and anatomical studies. However, the relationships among ethology, genotype, anatomy and olfactory function are not well understood. In particular, no measure has yet been determined that quantitatively relates to olfactory sensitivity across species. Variations in the anatomy of the nasal cavity is tested in Chapter 4 as a predictor of absolute olfactory sensitivity.

What creates selection pressure for increased general or specific olfactory sensitivity? Assuming that such selection pressure exists, what anatomical or physiological traits will affect either general or specific sensitivity? Finally, to what extent is it possible for olfactory selection pressure to alter these anatomical and physiological traits and what are the non-olfactory effects of such alteration? There have been speculations on all three questions, but none has been answered, largely because of a scarcity of data, particularly olfactory threshold data. This project utilized a broad range of mammals to test candidate sources for selection pressure that influence specific sensitivity (dietary chemical ecology)

and general sensitivity (terrestrial versus marine habitat) and a candidate mechanism of increasing general sensitivity (nasal cavity morphometry).

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Chapter 2: Olfactory sensitivity of the sea otter, *Enhydra lutris*

Abstract

Absolute olfactory sensitivity was behaviorally measured in two healthy adult male sea otters. Animals were trained using operant conditioning to distinguish between an odorant and an odorless stimulus. Absolute thresholds were calculated using the staircase method. Thresholds were measured for acetic acid (10^-8.27 mol/L), butyric acid (10^-9.53mol/L), caproic acid (10^-8.98mol/L), octanoic acid (10^-9.38mol/L), amyl acetate (10^-8.81mol/L and 10^-7.85mol/L), benzaldehyde (10^-9.72mol/L) and eugenol (10^-9.75mol/L). Results show otters have sensitivity consistent other mammals. The data do not support the notion that a marine lifestyle leads invariably to reduced olfactory sensitivity.

Introduction

The sea otter, *Enhydra lutris*, is an interesting species for measuring olfactory sensitivity for three reasons. First, it belongs to an order generally believed to possess acute olfactory sensitivity but from which no non-domestic representative has ever been tested. Second, it occurs in a habitat believed to be populated by mammals with poor olfactory sensitivity, but from which no representative has been tested. Finally, for the purpose of assessing the importance of nasal cavity morphology in olfaction, the sea otter

possesses a highly derived nasal cavity structure. This potential difference in anatomy may impact the olfactory function of the animal.

The sea otter belongs to the Mustelid family of the order Carnivora. Olfaction is believed to be a behaviorally significant sense in the Carnivora. The importance of olfaction in carnivores is supported by neuroanatomical data (Gittleman, 1991). Olfactory bulb volumes of most carnivores are large relative to their total brain volumes compared to ratios in other orders of mammals (Stephan *et al*, 1981, Williams *et al*, 2001, Hutcheon *et al*, 2002). However, olfactory sensitivity among Carnivora has been measured in only one species, the domestic dog, *Canis familiaris* (Krestel *et al*, 1984, Marshall *et al*, 1981, Moulton *et al*, 1960).

The sea otter is a mustelid and is both a member of Carnivora and a marine mammal. "Marine mammal" describes a polyphyletic group sharing a suite of environmental adaptations resulting in a number of shared anatomical and physiological traits adaptive for life at sea. Mustelids have a wide range of habitats. There are two marine otter species, *Lutra felina* and *E. lutris*. *Lutra felina* forages in coastal water but dens and spends a good deal of time on land. All other otters inhabit and forage primarily in freshwater systems. Clawless otters and several species of river otters are reported to venture out into coastal water, but this is not their primary nor preferred foraging ground. The seamink, *Mustela macrodon*, is now extinct but was believed to have denned on rocky Atlantic shores and foraged in coastal water. All other extant Mustelidae are either

semi aquatic or fully terrestrial. Despite widespread marine foraging, none of the Mustelidae aside from the true sea otter, *E. lutris*, naturally spend their entire life at sea (Nowak, 1997).

Although olfactory thresholds have not been measured previously in any marine mammal, circumstantial evidence supports a widely held belief that marine mammals have reduced or vestigial olfactory systems and presumably commensurately poor sensitivity. All marine mammals have some respiratory and circulatory adaptations which permit long-duration dives. Cetaceans, for example, spend very little time breathing at the surface. Neuroanatomical data on cetaceans suggest a vestigial or even completely dysfunctional olfactory system. The olfactory bulb is extremely reduced or absent in adult mysticetes (Duffield *et al*, 1992, Oelschlager, 1989, 1992). In odontocetes, it is found usually only in the fetal and neonatal stages and is rarely present in adults (Breathnach, 1960, Breathnach and Goldby, 1954, Kojima, 1951, Kukenthal and Ziehen, 1893, Oelschlager and Kemp, 1998, Schwerdtfeger *et al*, 1984, Oelschlager and Buhl, 1985a, b, Ries and Langworthy, 1937,). Reduction or absence of olfactory bulbs may reflect the extremely limited access of cetacean nasal passages to airborne olfactory stimuli and related retrograde loss from the reduced value of nasal chemoreception.

Ecologically, cetaceans are an extreme case. As a group they have the least surface resident time of any marine mammal taxon. Pinnipeds, by contrast, spend considerable time on land. There is neuroanatomical evidence for reduced importance of olfaction in

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pinnipeds; i.e,. the size of the pinniped olfactory bulb versus total brain size is significantly reduced in several species (Fish, 1898, Harrison and Kooyman, 1968), but a functional brain structure remains.

Both marine otter species differ from other marine mammals in their feeding behaviour. Unlike the carnivorous and piscivorous pinnipeds and the filter-feeding, carnivorous and piscivorous cetaceans which generally consume their prey underwater and often engulf it whole, otters feed primarily at the sea surface, bringing their prey items to the surface, handling them at close range, and chewing them before swallowing, which affords them the opportunity of rejecting prey based on both taste and smell (Kvitek and Bretz, 2004). It has been shown in both captive and wild animals that *E. lutris* reject butter clams (*Saxidomus giganteus*) with high paralytic shellfish poisoning toxin content (Kvitek *et al*, 1991, Kvitek and Bretz, 2004). This discrimination is sufficiently fine that at intermediate toxin concentrations, the more highly toxic tissues are discarded while the rest of the clam is consumed.

Neuroanatomical evidence further supports also a well-developed olfactory sense in sea otters. The relative size of their olfactory bulb is similar to that of the terrestrial mustelids and of the Carnivora in general and is larger than that of freshwater otters (Gittleman, 1991).

Based on phylogeny alone, as a carnivore and a mustelid, the sea otter should have a well-developed olfactory sense. As a marine mammal, the sea otter's olfactory sense

may have degenerated, but if a predominantly submerged lifestyle is the key to inducing degenerate olfaction, we expect the sea otter's sensitivity to be better than most marine mammals and possibly comparable to that of its terrestrial kin.

To test these evolutionarily derived scenarios, olfactory sensitivity was behaviorally measured in live animals for comparison with previously tested terrestrial mammals.

Methods

Two captive animals were tested: one male Northern sea otter at the Oregon Coast Aquarium and one male California sea otter at the Oregon Zoo.

Stimulus selection

Test compounds for this study were selected based upon the availability of threshold data from previous studies in order to compare results with those from terrestrial mammals. All of the compounds have published thresholds for at least two other mammal species including for the closest tested relative, the domestic dog.

Stimulus generation

Clinical olfactometry testing techniques for studies on humans provide useful procedures for testing behavioral thresholds in animals. Air-dilution olfactometry is a standard method for human and nonhuman olfaction studies (Table 1). Pressurized air is filtered and split into multiple clean airstreams. Odorant airstreams are saturated by

passing over or bubbling through a liquid odorant sample and are subsequently diluted with clean air before delivery to the subject. Concentration of odorant in the delivered airstream can be manipulated during the dilution stage. The instrument can be calibrated using chromatographic or other direct in-line methods or by measuring mass change in the liquid sample over time. In this way, airstreams at controlled concentrations of the chosen odorant can be reliably produced.

For this research, a portable air dilution olfactometer, suitable for poolside use, was constructed (Fig.1). The wetted surfaces of the olfactometer were composed entirely of glass or teflon. Compressed air from a scuba tank was used as the carrier gas. Compressed air quality was tested and reported by the participating zoo and aquarium facilities. Contaminants (oil mist + particulate matter) were found to be below the detection limit of 150 ng/L and water was below the detection limit of 2 ppm (vol). Scuba tank air was filtered through commercial Drierite™, activated carbon and Molecular Sieve™, and divided into a diluting flow of 8L/min and a carrier flow. A carrier flow of 25-500ml/min was directed to a sample well into which a pure liquid sample of odorant in a narrow-necked, plastic distillation device was inserted. The sample well was held at 29.4°C (85°F), producing a constant rate of evaporation of odorant through the neck of the distillation device. The evaporation rate was determined by the volatility of the compound and the dimensions of the distillation device. Each device was calibrated by mass measurement over 2-6 days of operation (Fig. 2). The sample well was connected

through a manually operated needle-valve to a diluting air-flow which reached the mixing chamber and to an exhaust air-flow which exited the test area. By directing the appropriate amount of odorant current into the diluting flow the concentration of odorant in the mixing chamber could be varied by a factor of 500, or 2.7 orders of magnitude. The mixing chamber consisted of a sequence of three spherical glass chambers each containing an evagination from the wall which extended approximately halfway across the chamber, perpendicular to the direction of airflow. The mixing chamber was 125 ml in volume and opened directly into the sniff port accessed by the test animal. The carrier flow ran for 1 hour before use to equilibrate the odorant concentration in the sample well. The diluting flow ran blank for at least 2 minutes between trials to flush any odorant from the previous trial.

Behavioral test format

The test animals were trained using operant conditioning with food (their pre-existing diet of crustaceans, mollusks and fish) as a reinforcer. Operant conditioning has been used in a large number of olfactory threshold studies to elicit reliable responses from a variety of mammals as well as with sea turtles (Table 1, Apfelbach *et al*, 1998; Dagg and Windsor, 1971; Dorries *et al*, 1995; Doty and Ferguson-Segall, 1989; Doty *et al*, 1998; Krestel *et al*, 1984; Manton *et al*, 1972). The technique calls for the subject animal to be reinforced with some positive experience, generally a food reward, immediately upon performance of the correct behavior. Incorrect behaviors produce no reinforcement, either

positive or negative. In a behavioral olfactogram, the correct behavior is to sample the stimulus (sniff the airstream) and then touch the negative response target if no odour is present or the positive response target if an odour is present. Although reinforcement of correct negative responses is often omitted in threshold studies for ease of task training, maintaining the same probabilities of occurrence and the same reward for positives and negatives minimizes bias in an animal's responses (Passe and Walker, 1983).

A trial consisted of a single two-alternative discrimination task. The test animal was required to station at the experiment board on which were mounted the scent port and two touch-response objects. At a verbal cue from the trainer, the subject was required to sniff the scent port (Fig. 3) and touch either the 'yes' response object (if an odor was detected) or the 'no' response object (if no odour was detected) (Figs. 4,5). Correct responses (positive and negative) were reinforced with a food reward. A double blind protocol was used, in which the experimenter could not see the animal's response and the trainer did not know in advance which response was correct. After the animal had sniffed and responded, the trainer reported the response to the experimenter, who responded by indicating 'correct' or 'incorrect', on the basis of which the trainer would reinforce the animal if appropriate. A two-minute interval between trials allowed the animal to de-acclimate from the olfactory stimulus as well as purging leftover odorant from the preceding trial.

Sessions were arranged in a descending staircase protocol, as described by Cornsweet (1962): odour and blank trials were interspersed in Ghellerman series of twenty trials with the constraints that each group contained exactly ten odour trials and ten blanks and that no more than three of either occurred in sequence. The odour trials began with a presumed super-threshold concentration slightly above the human threshold for that compound. Each correct response was followed (after any intervening blank trials) by a trial at half the previous concentration until the first incorrect response. Thereafter, the concentration was doubled after each incorrect response and halved after each correct response. The direction of concentration change was allowed to reverse at least six times, and the threshold value for that compound was defined as the mean of the log-transformed concentration values of the final four reversal points.

A variation on this protocol was used at the Oregon Coast Aquarium in 2003, when thresholds for acetic acid, butyric acid, and amyl acetate were collected from subject Aialik. At the beginning of data collection period it became apparent that the distinction between low concentration odour stimuli and blanks was prohibitively difficult. Consequently, 'standard' blanks were introduced, which greatly improved performance: the first trial of every session was a blank stimulus, and the first trial following a smell stimulus was always a blank stimulus. These invariant conditions were quickly acquired by the subject with no cueing and provided a periodic basis for comparison with the intervening data trials. While this protocol was in effect, 50% of the data trials, excluding

the standard blanks, utilized blank stimuli. This modification did not prove to be necessary the following year, when the subject was more familiar with the test protocol, so the standard blanks were not used for the caproic acid, benzaldehyde, or eugenol thresholds for Aialik.

Results

Eight thresholds were collected for seven compounds. Individual thresholds are shown in Table 2 and Fig. 6.

Aialik, Oregon Coast Aquarium, Northern male, 5 and 6 years old

Two experimental series were conducted 10 months apart, in September 2003 and July 2004. In the first series in September, 2003, thresholds were obtained (in chronological order) for amyl acetate (10^-8.8 mol/L), acetic acid (10^-8.3 mol/L), and butyric acid (10^-9.6 mol/L) (Fig. 6a, Table 2). Overall accuracy on all blank or odour trials above threshold was 81%. The incidence of false positives dropped dramatically early in the data collection period, possibly as the subject adjusted to the presence of the standard blanks, which raised the total proportion of blank stimuli presented from 50% during training to 67% during data collection. Only two false positives occurred on standard blanks, both within the first eight sessions. However, a strong "yes" bias persisted; i.e., responses were more often correct for odour trials than for blank trials, for approximately 20 trials during the amyl acetate threshold measurement. Considering this bias, the measured amyl acetate threshold may underestimate the actual threshold.

Aialik's "yes" bias may have led him to respond yes to odour stimuli that he could not detect. However, the last two reversals of that threshold were obtained in the final two days of data collection, after the acetic acid and butyric acid thresholds, when the bias was no longer present (Table 3).

The butyric acid threshold was obtained using a step factor of four rather than two as a concession to time constraints on testing.

In the second series in July, 2004, thresholds were obtained (in chronological order) for caproic acid (10^-9.0 mol/L), eugenol (10^-9.8 mol/L), and benzaldehyde (10^-9.7 mol/L) (Fig. 6b, Table 2). Overall accuracy on all blank or odour trials above threshold was 86%, slightly improved from 2003. In contrast to 2003, a moderate "no" bias was present at the beginning of data collection. Similarly to 2004, however, the bias decreased over the course of the data collection period.

Eddie, Oregon Zoo, California male, 6 years old

One experimental series was conducted in October, 2004. Thresholds were obtained for amyl acetate (10^-7.9 mol/L) and octanoic acid (10^-9.4 mol/L) (Fig. 6c, Table 2). This subject's response accuracy was slightly lower than Aialik's, and so more than six reversals were required (10 for amyl acetate, 7 for octanoic acid). No significant bias was evident.

Discussion

Natural Variation

The chemical trends observed in other species threshold distributions are also found in the sea otter (Fig. 7, 8). For example, threshold decreases with increasing carboxylic acid chain length among the shortest acids (C_2-C_4). Between compound variation in thresholds is similar to that observed in other mammals.

Both animals were tested for one common odorant, amyl acetate. Their amyl acetate thresholds differ by a factor of 13, which is well within the range of variation previously found in other species. Aialik, the more sensitive animal, was one year younger at the time of testing. The animals also belong to different subspecies. The extent of divergence between the Northern and California sea otter populations is subject to debate but is probably very small. Nevertheless, there are slight anatomical and ecological (dietary) differences between sea otter subspecies, and it is possible that there are functional anatomical and responsedifferences that reflect their recent divergent history.

The fact that the younger animal showed greater sensitivity is consistent with previous findings in other mammals. It has been shown in both humans, (Lehrner *et al*, 1999, Stevens and Cain, 1987) and rats (Kramer and Apfelbach, 2004) that measures of olfactory function, including sensitivity, decline throughout adulthood. The presence and strength of this effect in the otters tested is not expected to be large since the animals were so young and similar in age. Differences may also arise from slight variations in

experimental protocol and training technique, individual history, season, hormonal state, and environment.

Potential sources of error

The thresholds measured in this study are reported to one decimal place on a base ten log scale, or approximately ± 25%. The standard deviation of each threshold, measured from the groups of reversal point concentrations from which they were calculated, range from 40% to 80%, or from 0.15 to 0.25 on a log scale. This is not a good measure of the error in the threshold values, because many of the possible sources of error are systemic for individual animals and the magnitude of these effects is unknown.

Slight and unquantified masking effects were present in all cases due to ambient odours in the test areas. Both participating animals were in residence at public zoos and aquaria, and testing was conducted in the animals' home exhibits where rigorous atmospheric control was not practical. Tests were conducted in outdoor facilities which were well-ventilated but subject to natural variations in airborne background odour, humidity and temperature. In most cases the most significant contaminant was most likely the food with which the animals were rewarded, as they were able to handle their food and their noses were in physical contact with it. The presence of a moderate masking effect can lead to calculated thresholds that overestimate actual thresholds (underestimate sensitivity) by up to 1.5 orders of magnitude (Laing *et al*, 1989). Natural background odour in this case was in some aspects more representative of thresholds under natural

conditions than are fully controlled sterile testing conditions common to some olfaction studies. However, it was probably not high enough to produce significant masking, as demonstrated by the following conservative calculation.

According to Laing and colleagues, masking is greatest when the masking odorant is chemically closely related to the target odorant. In their study, acetic acid was the most effective mask for propionic acid, compared with several unrelated compounds. They report a median unmasked threshold of 3.5×10^{-11} mol/L for five rats in a go/no-go task. In the same paradigm, the median threshold for masked propionic acid was elevated by a factor of thirty, a moderate but significant change, in the presence of $1.1 \times {}^{10-6}$ mol/L of acetic acid, just over 30 000 times higher than the unmasked propionic acid threshold and 1000 times higher than the propionic acid concentration that could still be detected in its presence. Assuming that the rat thresholds for acetic and propionic acid are similar, as is the case in mammals for which both are known, the masking agent was present at a factor of close to 30, 000 above threshold. A similarly superthreshold concentration for humans, of any odorant, is generally described as extremely strong or overwhelming. Thus, as long as the masking background in the present study did not appear very strong to the test animal (supporting evidence would include interference with subject animal accuracy on blank trials, detection of the masking smell by human observers, and possible aversive response by both parties) significant threshold changes (a factor of ten or greater) due to masking are unlikely. Nevertheless, sensitivity measurements presented here should be

viewed as conservative. While they represent realistic natural conditions, particularly for an animal feeding in the wild, this difference must be borne in mind for comparisons with other species tested under odorless background conditions.

Repeated exposure to an odorant may also change measured thresholds, in either direction. In the short term, olfactory adaptation may occur in which sensitivity temporarily decreases following exposure. It has been shown in humans repeatedly exposed to the same odorant that detection performance effects of previous exposure is only important if the test is repeated within 60 seconds. Performance, although reduced to 40% accuracy initially, approaches 100% accuracy under nearly all tested conditions after 60 seconds post-exposure (Jacob *et al*, 2003). Similar results were found for several odorants, both pleasant and unpleasant, at near threshold and high superthreshold concentrations, for male and female humans. For these reasons, in this study a two minute interval separated all trials.The between-session interval selected by Jacob and colleagues to allow complete recovery from habituation between test sessions was also two minutes. It is worth noting that the inter-trial intervals used in the threshold studies of other study species vary widely and can be as little as 20 seconds. However, generally in such cases very large numbers of trials are conducted, which most likely mitigates the adaptation effect.

Over days, physiological changes in the nervous system can lead to heightened sensitivity to a familiar odorant (Yee and Wysocki, 2001). However, this possible effect

was unlikely to significantly affect measured thresholds in this study. Yee and Wysocki found in male mice exposed continuously to their test odorant for ten days that the threshold decreased only by a factor of four. Dalton *et al* (2002) found in humans that much larger increases in sensitivity (up to four orders of magnitude) could be induced in reproductive age females, while no significant changes could be induced in nonreproductive age females or males. Significant differences in threshold did not appear even in reproductive females until at least six test sessions, or twelve complete threshold measurements, had been performed. In this study, only one threshold measurement, no more than thirty brief (>20 sec) exposures over the course of 3-10 days, occurred for any odorant. Therefore, significant enhancement of sensitivity is unlikely.

Error in calculated thresholds for individual animals due to bias may also be present. For subject Aialik, food reinforcement for each correct response was approximately constant at 3-4 ounces of mixed shrimp and clam per response throughout the testing period. However, in 2003 reward presentation differed between correct odour trials and correct blank trials. In order to accommodate time constraints and still allow an olfactory deacclimation period, there was a two minute pause following correct odour trials but only a one minute pause (just long enough to deliver reinforcement) following blanks. This may have introduced a response bias: Food was delivered more quickly following a correct no response, which may have added value to that reinforcement. However, the subject was allowed to rest longer in a preferred location (the holding pool)

and ate more slowly while awaiting the trial following a correct yes response. Therefore, the existence of bias in favour of either response is uncertain.

In addition, a last minute protocol change exposed Aialik to an increased proportion of blank stimuli, the standard blanks, when data collection began. During training, odour and blank stimuli were presented equally. Aialik learned very quickly to respond correctly to the standard blank itself and attained 100% response accuracy to standard blanks after eight standard blanks had been presented. However, the standard blanks increased the total number of blank stimuli encountered. The observed yes bias in the first threshold measured (amyl acetate) suggests an expectation on Aialik's part of equal numbers of no and yes responses despite the displacement of the majority of the blank trials to immediately follow session pauses. This bias diminished gradually over the first six days of testing and a marked difference in response frequencies was present only in the early amyl acetate sessions. The final two reversals of the amyl acetate series were obtained in six trials at the end of testing, after the bias had disappeared, and these final reversal values did not raise the calculated threshold, so it appears that the yes bias did not affect the threshold value. The amyl acetate threshold for Aialik is presented here as a preliminary finding, subject to verification in future studies.

It should be noted that while Aialik's initial performance in 2003 reflected a yes bias, in 2004 his initial performance reflected a no bias. In both cases, the bias decreased over the course of data collection. Although the data do not provide a sufficient base to

diagnose the imbalance, it is plausible that slight differences in odour and blank presentation frequency or reward in refresher training immediately prior to data collection (the introduction of the standard blanks in 2003, for instance) introduced a corresponding bias which subsequently diminished due to the balance of presentations during data collection.

The overall response accuracies of blank and odour trials for both years suggest very little total response bias despite all potential sources.

Interspecies comparison-general sensitivity

In order to compare general olfactory sensitivity among species, an Average Threshold (AT) was calculated. All threshold values were log transformed. The Average Threshold was defined as the mean of the log transformed threshold values of seven widely tested odorants, acetic acid (8 species), propionic acid (9 species), butyric acid (12 species), ethanol (7 species), butanol (6 species), ethyl acetate (5 species) and amyl acetate (7 species). These odorants were chosen in order to maximize the size of the dataset while equalizing the representation of the three available chemical groups, straight-chain aliphatic acids, alcohols and acetate esters.

In this study, in toto, fourteen mammal species were compared using these seven compounds, for a total set of 98 thresholds. Of these, 55 were obtained from the literature and from this study. Due to inherent variation in detectability among these seven compounds, it was necessary and plausible to substitute approximations for the missing

values. In all three chemical groups, an approximate logarithmic decrease in threshold with increasing carbon chain length is present in most species. For species with missing values in a chemical group where two or more thresholds were available for related compounds, the missing values were interpolated. If only one threshold value for that species in that chemical group was available, the missing value was extrapolated using the mean of the slope in question for all available species. 17 values were approximated in this way. The remaining 26 were approximated by the following value:

For species Q, odorant Y

Estimated Threshold = (mean [available thresholds(Q)] x mean[available thresholds (Y)])$^{1/2}$

AT served as a general representation of olfactory sensitivity; low AT values indicate high sensitivity, high AT values low sensitivity.

Sea otter thresholds to amyl acetate and all carboxylic acids were near or within the range of previously tested mammals (Fig. 7,8). The sea otter threshold to eugenol can be compared only with the human threshold and the sea otter benzaldehyde threshold only to human and rat (Fig. 9). The sea otter threshold was the lowest for both of these compounds, followed by the human threshold being a factor of 10 higher for benzaldehyde and a factor of 3 higher for eugenol. The sea otter AT ranks 7th lowest of 14 species, approximately midrange (Fig. 10). Since sea otter sensitivity as measured herein should be regarded as conservative (overestimating true thresholds, see above), there is no

reason to regard the sea otter as having poor olfactory sensitivity by terrestrial mammalian standards. Whether the same can be said in comparison to the Carnivora is uncertain. Only one other carnivore (*C. familiaris*) is available for comparison. Compared with the dog, the sea otter AT is elevated by a factor of 5.5. However, absolute difference varies widely among odorants. Thresholds for both species also vary by individual and by breed in the case of the dog. In addition, the domestic dog lineage has been subjected to considerable artificial selection some of which emphasized olfactory ability and is not therefore an ideal comparison species for "natural conditions". Further comparisons amongst the Carnivora will be needed to more precisely evaluate the effect of marine lifestyle on general olfactory sensitivity in Carnivora.

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Table 1: Olfactometry and training methods used in threshold measurement of mammals

Species	Stimulus	Training	Reference
House mouse *(Mus musculus)*	solvent dilution (unspecified solvent)	operant & classical conditioning (food reinforcement and electric shock	Schmidt, 1981
Brown rat *(Rattus norvegicus)*	solvent dilution (propylene glycol)	operant & classical conditioning (water reinforcement and electric shock	Moulton and Eayrs, 1960 Moulton, 1960
Human *(Homo sapiens)*	various	verbal instruction	Devos *et al*, 1990.
Pig-tailed macaque *(Macaca nemestrina)*	solvent dilution (ethyl phthalate)	operant conditioning (food reinforcement)	Laska and Seibt, 2002a,b
Common squirrel monkey *(Saimiri sciureus)*	solvent dilution (ethyl phthalate)	operant conditioning (food reinforcement)	Laska and Seibt, 2002a,b Laska *et al* 2000
Domestic dog *(Canis familiaris)*	air dilution, solvent dilution (water, propylene glycol)	untrained natural responses; operant and classical conditioning, various (water reinforcement, food reinforcement, electric shock, light slap)	Krestel *et al*, 1984 Moulton *et al*, 1960

European hedgehog *(Erinaceus europaeus)*	air dilution	operant conditioning	Bretting, 1972
Common European shrew *(Sorex araneus)*	solvent dilution	operant conditioning (food reinforcement)	Sigmund and Sedlacek, 1985
Seba's short-tailed bat *(Carollia perspicillata)*	solvent dilution	classical conditioning (electric shock, respiration rate monitor)	Laska, 1990
Mouse-eared bat *(Myotis myotis)*	air dilution	classical conditioning (electric shock, heartrate monitor)	Obst and Schmidt, 1976
Vampire bat *(Desmodus rotundus)*	solvent dilution (unspecified), air dilution	operant conditioning (food reinforcement) classical conditioning (electric shock, heartrate monitor)	Schmidt, 1973 Schmidt, 1975
Great fruit bat *(Artibeus lieratus)*	air dilution	classical conditioning (electric shock, heartrate monitor)	Schmidt, 1975
Pale spear-nosed bat *(Phyllostomus discolor)*	air dilution	classical conditioning (electric shock, heartrate monitor)	Schmidt, 1975

Table 2: Sea otter behavioral thresholds. Threshold measured as log mol/L

Animal	acetic acid	butyric acid	caproic acid	octanoic acid	amyl acetate	eugenol	benzaldehyde
Aialik	-8.3	-9.5	-9.0		-8.8	-9.8	-9.7
Eddie				-9.4	-7.9		

Table 3: Olfactogram response accuracy. Thresholds are listed in chronological order. Odour trial accuracy is reported as % of trials at concentrations above calculated threshold. 'Standard blanks' are not included. Totals for year and for blank+odour are calculated from the pooled trials for that row or column (i.e. categories are not weighted). Number of trials are in parentheses.

Aialik

Odorant	Blanks % (n)	Odours % (n)	Total % (n)
amyl acetate	57 (14)	91 (11)	72 (25)
acetic acid	82 (22)	100 (11)	88 (33)
butyric acid	80 (10)	75 (8)	78 (18)
All trials 2003	74 (46)	90 (30)	81 (76)
caproic acid	95 (19)	75 (8)	89 (27)
eugenol	100 (9)	80 (10)	89 (19)
benzaldehyde	83 (18)	80 (15)	82 (33)
All trials 2004	91 (46)	79 (33)	86 (79)
All trials	83 (92)	84 (63)	83 (155)

Eddie

Odorant	Blanks % (n)	Odours % (n)	Total % (n)
amyl acetate	71 (17)	76 (14)	74 (31)
octanoic acid	73 (11)	73 (11)	73 (22)
All trials	72 (28)	75 (25)	74 (53)

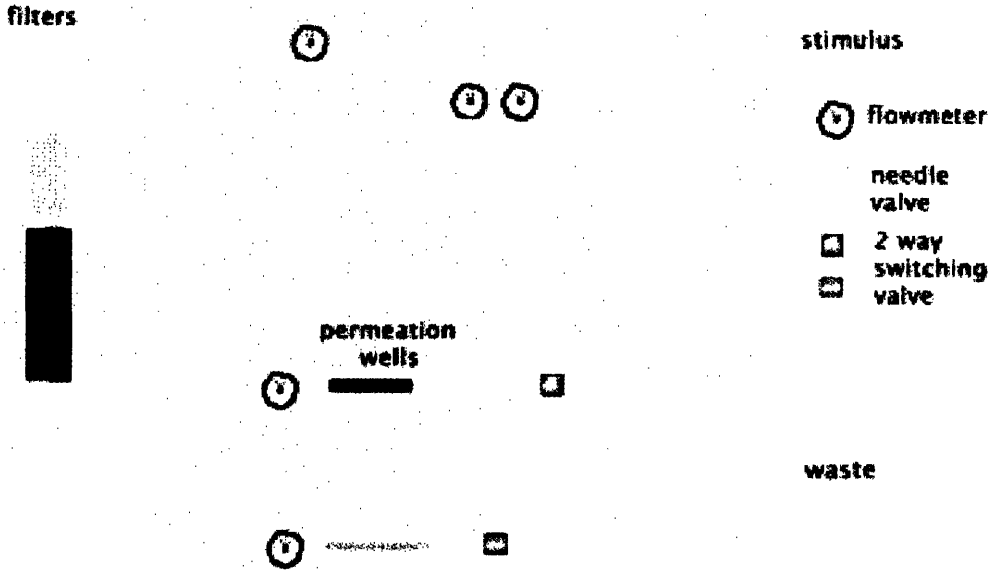

Fig. 1: Air dilution olfactometer schematic

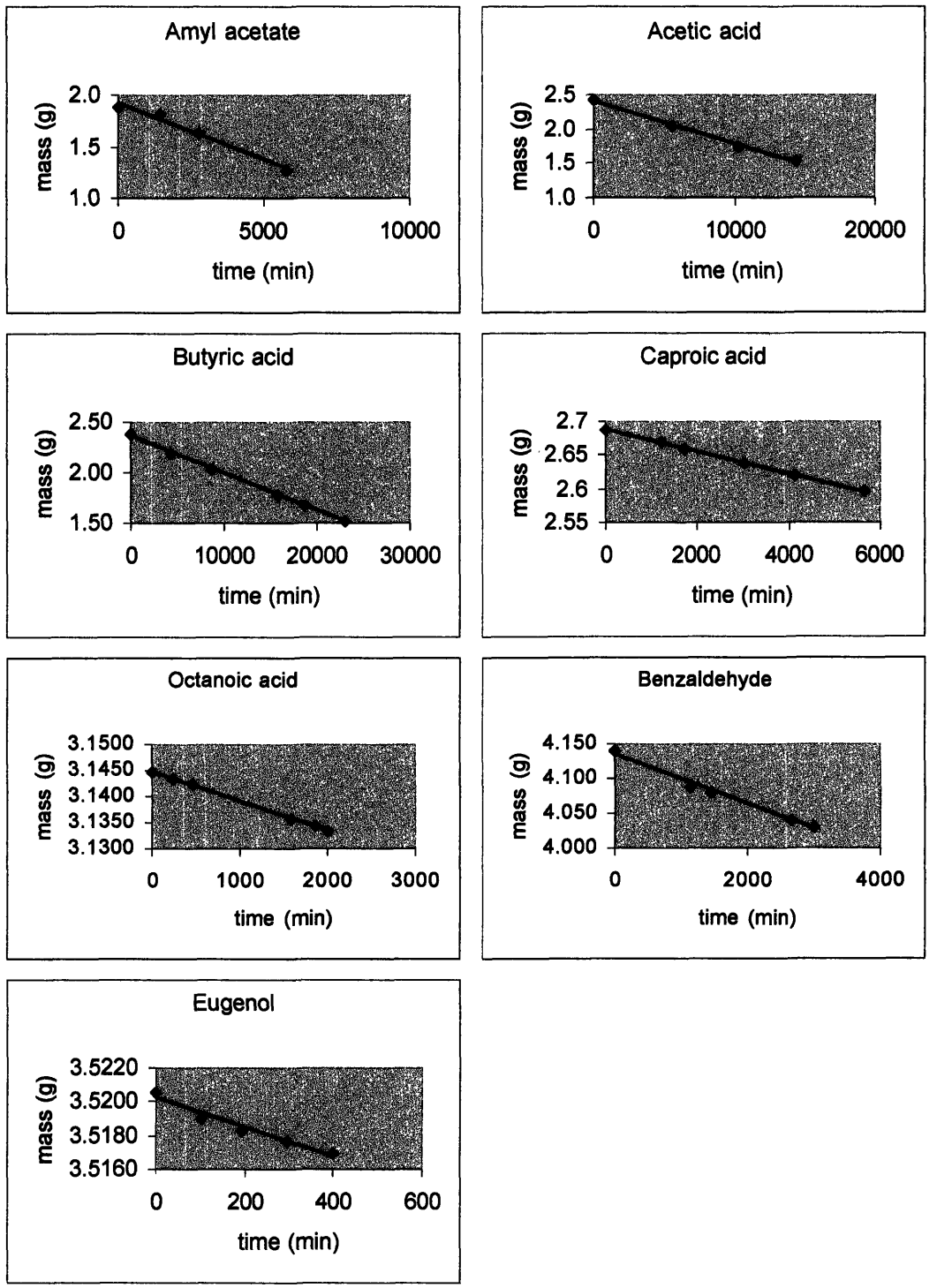

Fig. 2: Olfactometer calibration data: mass lost over time from a sample of liquid odorant under working conditions of temperature and airflow in the olfactometer.

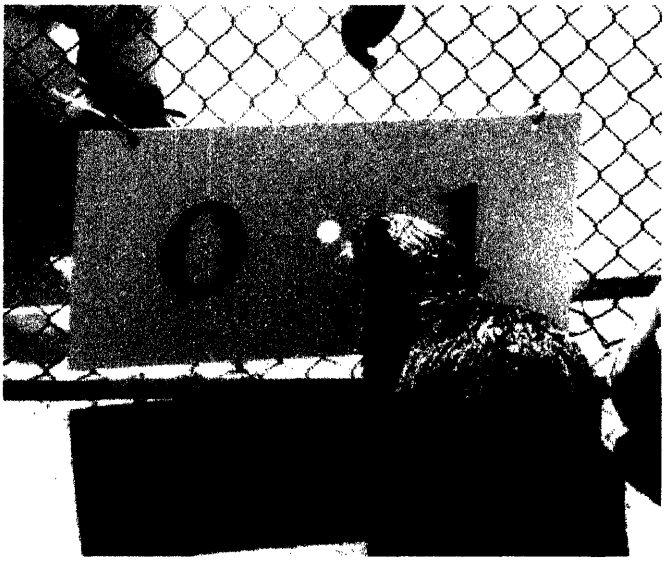

Fig. 3: Subject Aialik performing a sniff, Oregon Coast Aquarium, 2004

Fig. 4: Subject Aialik responds 'yes', Oregon Coast Aquarium, 2003

Fig. 5: Subject Aialik responds 'no', Oregon Coast Aquarium, 2003

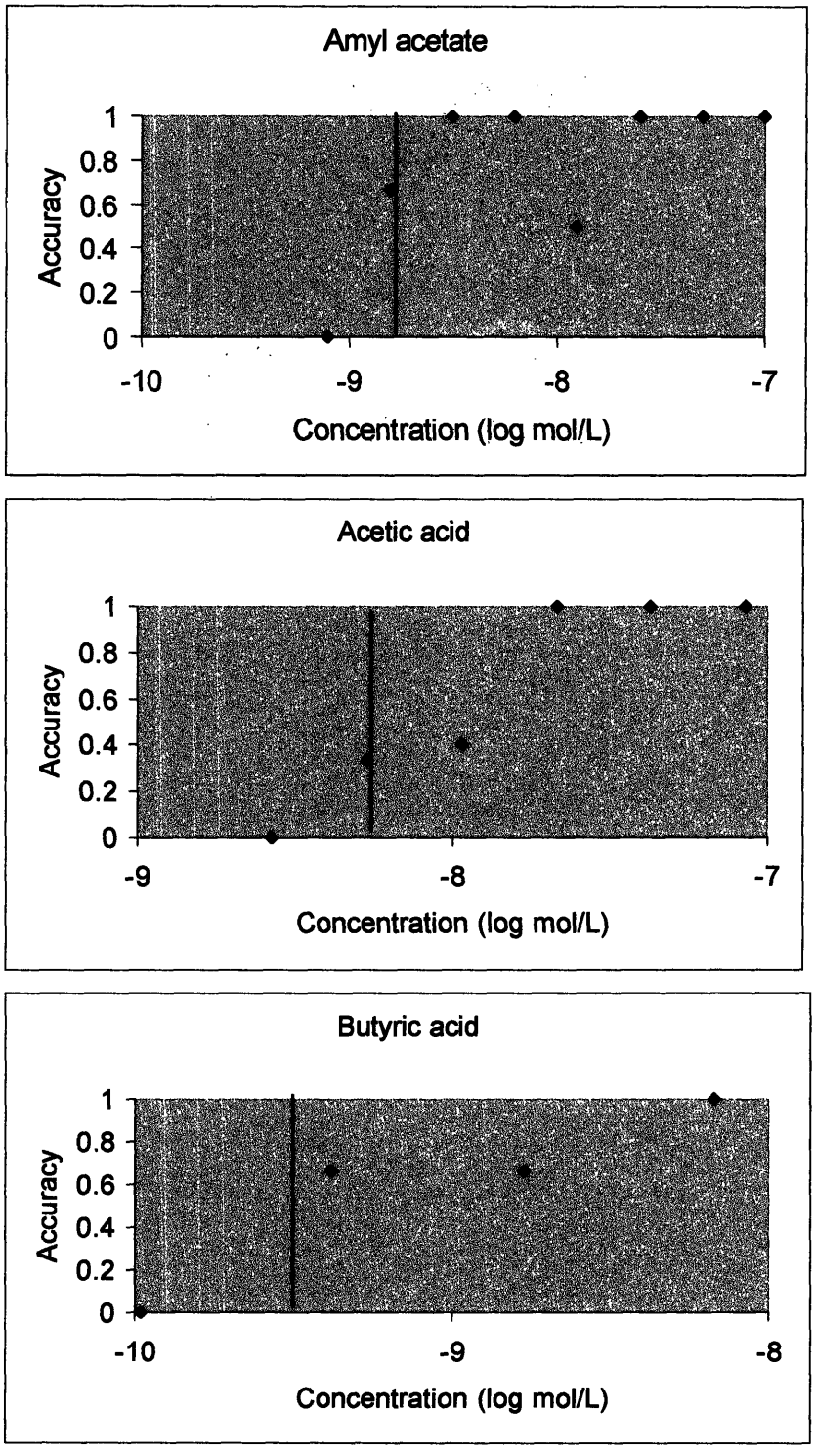

Fig. 6a: Subject Aialik's dose-response data, 2003. Vertical line indicates threshold.

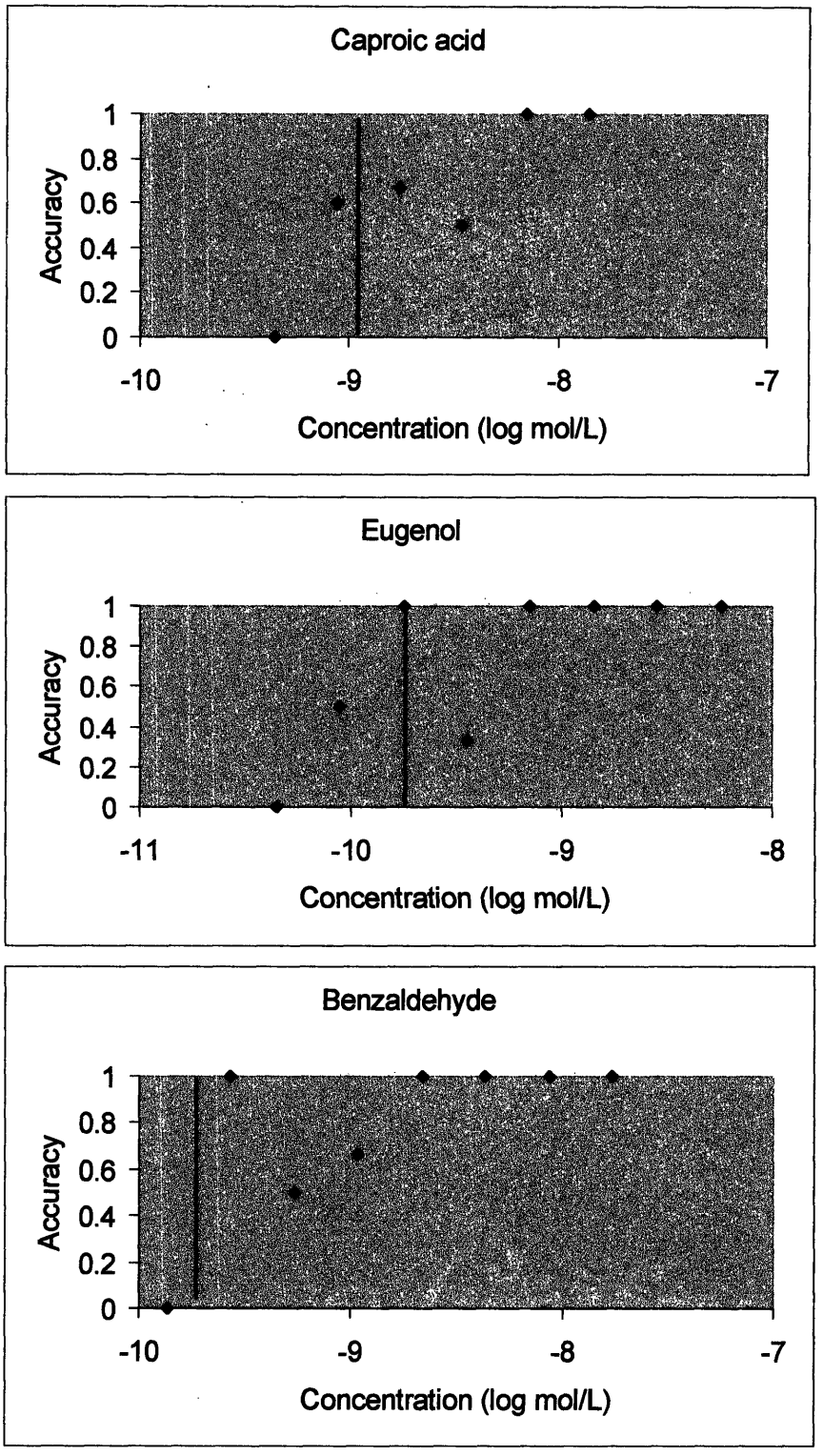

Fig. 6b: Subject Aialik's dose-response data, 2004. Vertical line indicates threshold.

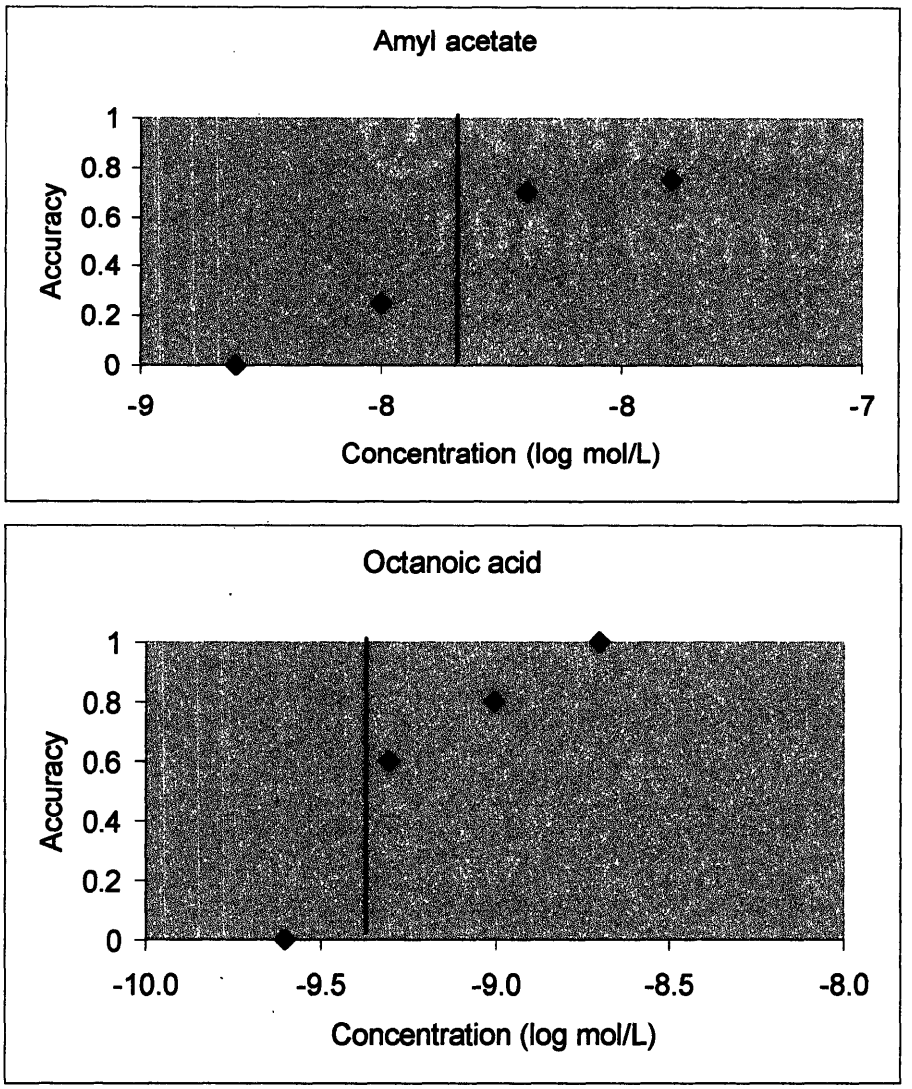

Fig.6c: Subject Eddie's dose-response data, 2004. Vertical line indicates threshold.

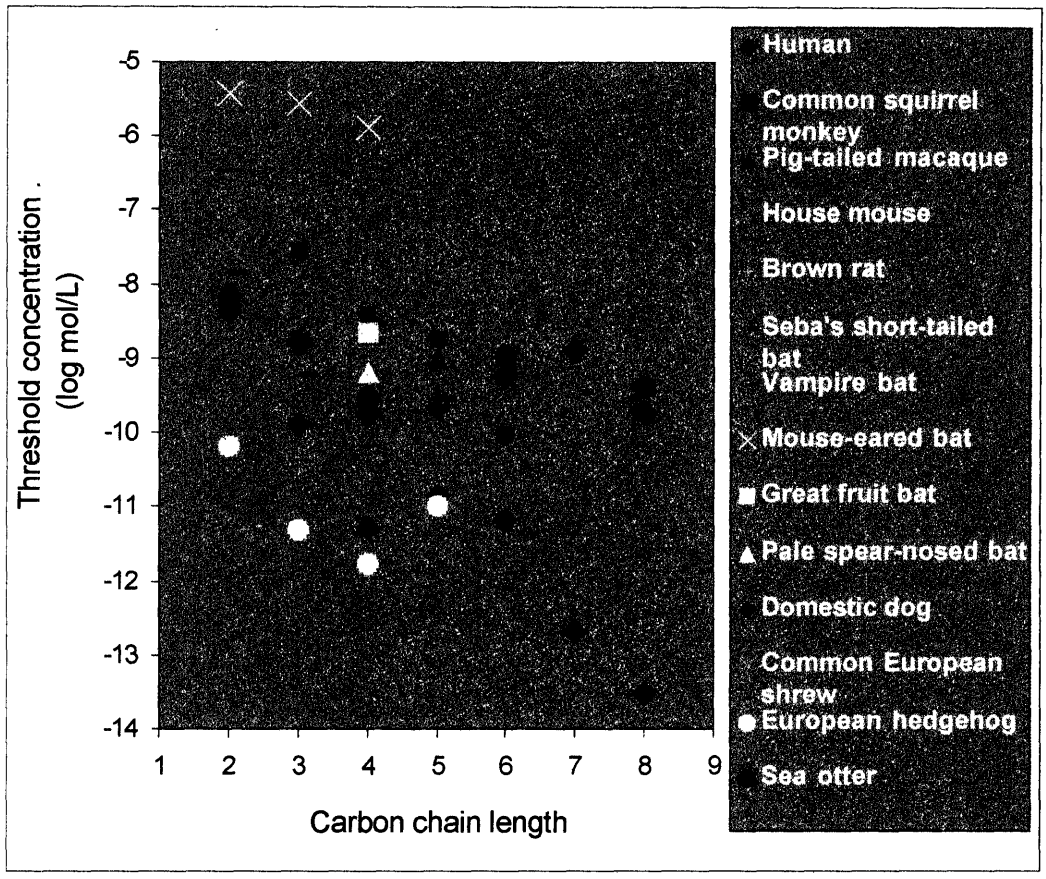

Fig. 7: Olfactory thresholds for short aliphatic acids for all available mammal species. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), Pig-tailed macaque (*Macaca nemestrina*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), European hedgehog (*Erinaceous Europaeus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). From: Bretting, 1972; Devos *et al*, 1990; Hubener & Laska, 2001; Laing *et al*, 1989; Laska, 1990; Laska *et al*, 2000; Moulton *et al*, 1960; Obst *et al*, 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985, this study33

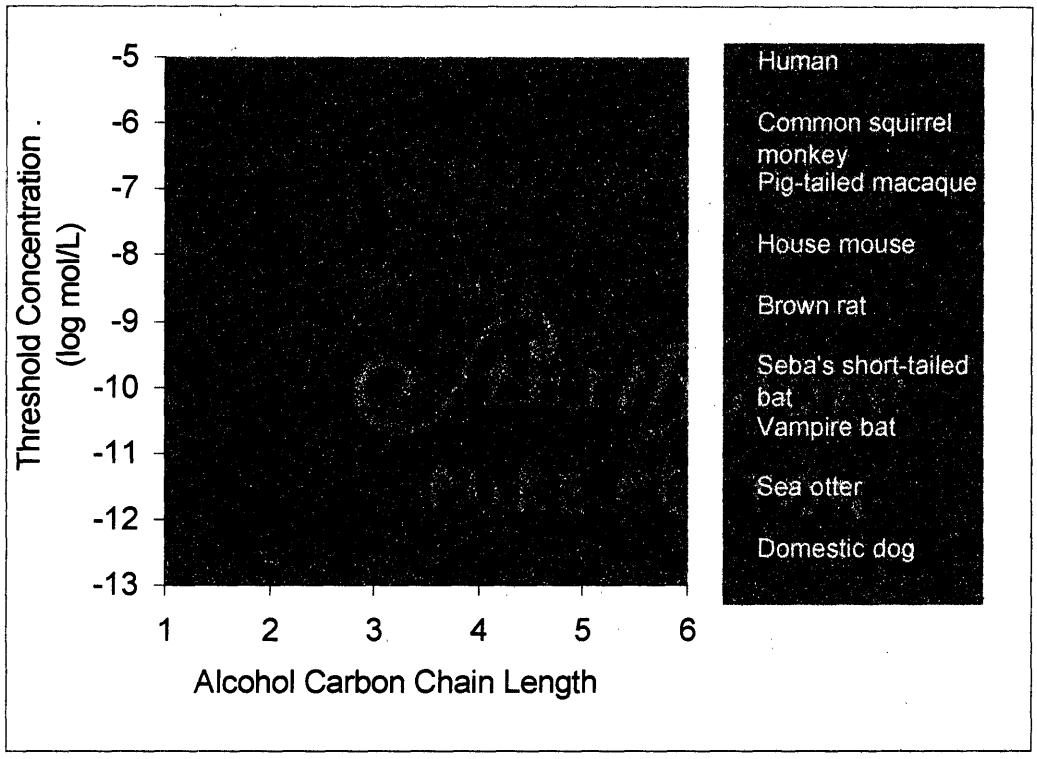

Fig. 8: Olfactory thresholds for acetate esters for all available mammal species. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), Pig-tailed macaque (*Macaca nemestrina*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). From: Devos *et al*, 1990; Krestel *et al*, 1984; Laska, 1990; Laska and Seibt, 2002a; Moulton, 1960; Obst *et al*, 1976; Schmidt, 1975; Yee and Wysocki, 2001; this study

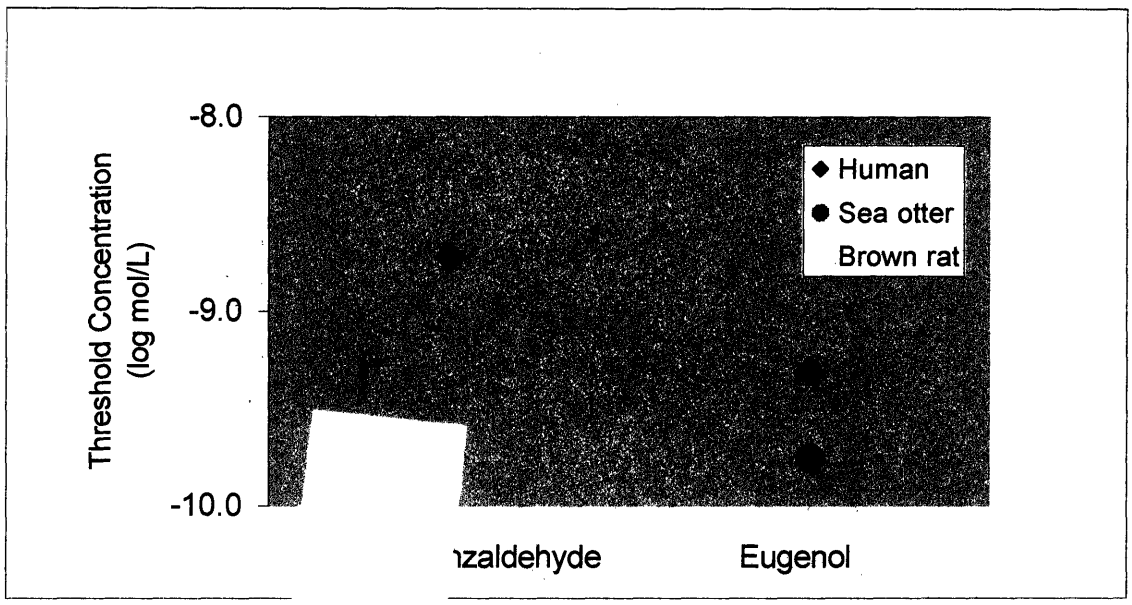

Fig. 9: Benzaldehyde a hresholds for all available species. Data from Devos *et al*, 1990; Laing, 1975;

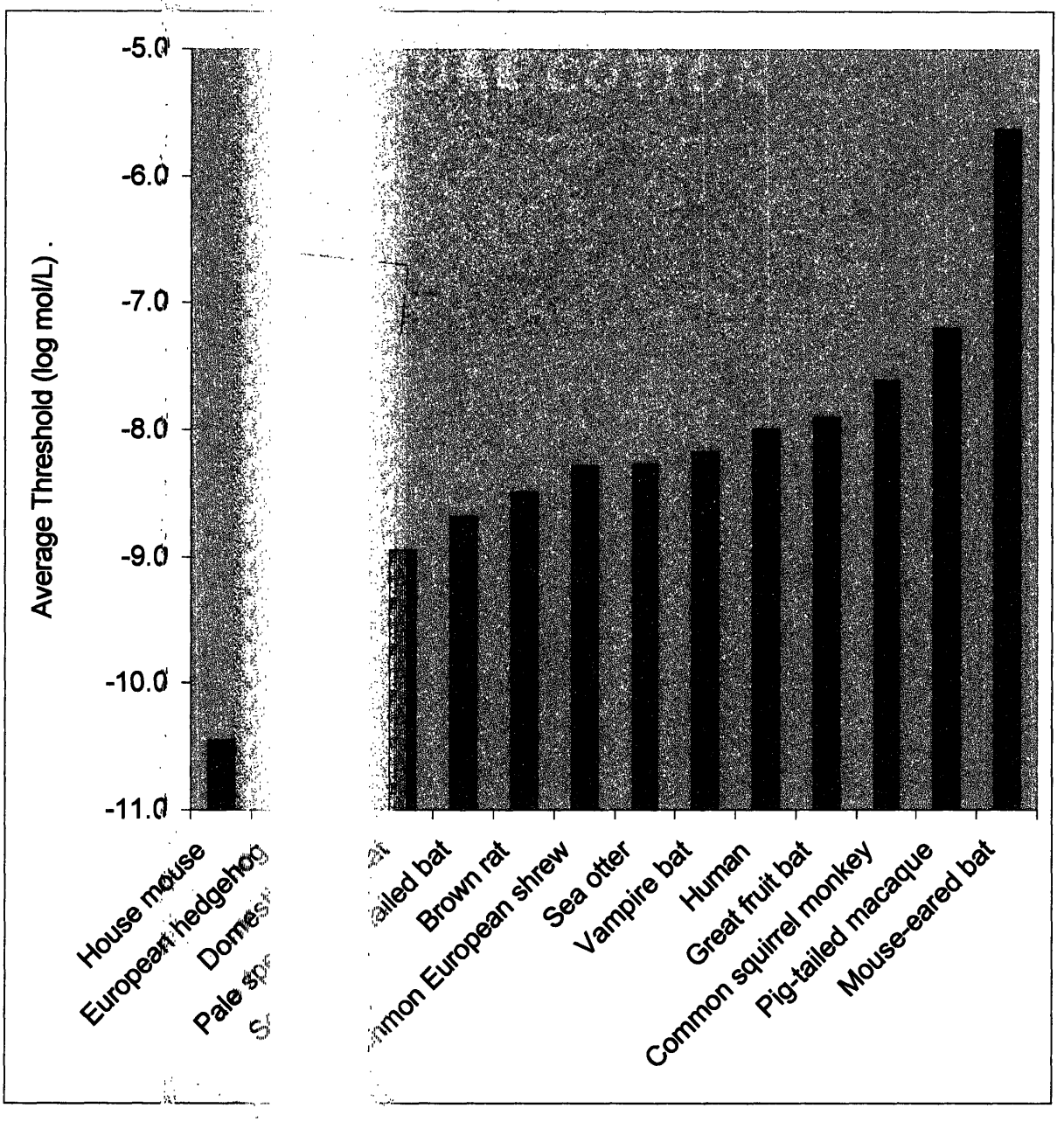

Fig. 10: Average Thresh[illegible] for all available species.

Chapter 3: Chemical ecology and specific olfactory sensitivity

Abstract

Insects are known to navigate and identify important resources using highly specific sets of chemicals and to exhibit specific heightened sensitivity to these stimuli. Little is known about whether important but less specific olfactory stimuli such as food odours for mammals are detected with similar enhanced sensitivity. Specific olfactory sensitivities of eight mammal species for nineteen natural volatile compounds were compared vis-à-vis their ecological relevance to the olfactor, in order to determine whether odorants of greater importance are detected with greater sensitivity. Ecological relevance was estimated from the volatile chemistry literature as the frequency of occurrence of the compound in the dietary category (or categories) of the olfactor (flowers, fruit, grain, foliage, terrestrial vertebrate prey, insect prey, marine prey). The relationship was not supported for the Chiroptera, was strongly suggested to be valid for the primates, and was shown to be significant for a marine vs. terrestrial carnivore. The results suggest that a) chemical ecology plays an important role in determining specific olfactory sensitivity in mammals, b) diet is sometimes but not always an adequate proxy for elucidating differences in chemical ecology, and c) the chemical ecology of species from radically different habitats is easily distinguished Such pairs present a promising model for investigating the influence of ecology on specific olfactory sensitivity.

Background

Recently, there has been considerable progress towards a new understanding of the molecular and cellular basis for olfaction. Since the work of Buck and Axle (1991) identified the gene family that encodes olfactory receptor proteins, catalogues of sequences are accumulating for olfactory receptor proteins in a variety of organisms, including many mammals (Skoufos *et al*, 2000). It has also been shown that each olfactory receptor cell expresses a single receptor protein type (Li *et al*, 2004, Malnic *et al*, 1999, Nef *et al*, 1992, Ressler *et al*, 1994, Serizawa *et al*, 2003, Vassar *et al*, 1994).

The visualization studies of Vassar *et al* (1994), Nagao *et al* (2000) and Ressler *et al* (1994) in mouse (*Mus musculus*) and rat (*Rattus norvegicus*) strongly suggest that all receptors of a specified type synapse in one lateral-medial pair of glomeruli in the olfactory bulb. If a single olfactory receptor gene is associated with a molecular label, all of the labeled neurons will project to a specific pair of loci. The knock-in experiments of Mombaerts *et al* (1996) and Wang *et al* (1998) further support the genetic basis of this organization: substitution of the coding region of one olfactory receptor gene for the coding region of another will cause the axon of the altered neuron to project to the (highly specific) glomerular target of the substitute gene. The ligand-screening work of Katoh *et al* (1993) and Malnic *et al* (1999) suggest that each glomerular response may encode a simple molecular feature of the odorant molecule such as a functional group or carbon chain length. Cell-culture screening studies have begun to identify individual receptor proteins that are sensitive to particular compounds (Zhao *et al*, 1998, Hamana *et al*, 2003).

These discoveries are significant for questions about olfactory specialization. Odorant-specific molecular architecture suggests that olfactory sensitivities to particular compounds evolve at least partially independently of one another. Different species can thus be expected to differ in their relative sensitivity to different compounds, depending on their ecological importance.

Specificity of this kind is well documented in other animal taxa, notably among the insects. The highly specific relationships of insect predators, herbivores, parasites and pollinators provide simple, readily testable models for olfactory specialization, and both behavioral methods and

electro-anetennogram detection have shown that thresholds are lowered for ecologically relevant compounds. This kind of specificity is well known for conspecific pheromone components (Cabrera *et al*, 2001, Francke *et al*, 2002, Gemeno *et al*, 2003, Jintong *et al*, 2001, Kalinova *et al*, 2003, Naka *et al*, 2003, Priesner *et al*, 1975, Yamamoto *et al*, 1999, Yarden *et al*, 1996, Zhang *et al*, 2004, and others) Other important compounds identifying preferred prey, forage plants, oviposition sites and other important resources are also detected with higher sensitivity (Backman *et al*, 2000, Bichao *et al*, 2001, Costantini *et al*, 2001, Rostelian *et al*, 2000, Stensmyr *et al*, 2001, Stranden *et al*, 2003, and others). Antennal detection is highly selective, discriminating very slight changes in odorant compound structure (carbon chain length, functional group, stereochemistry) that in turn reflect prey or host specificity that is in some cases very narrow, famously in the case of the human-specializing malaria vector *Anopheles gambiae* (Costantini *et al*, 2001). Antennal receptors of this mosquito are strongly activated by three complex carboxylic acids specific to human sweat. Where measured in the above listed studies, antennal response to compounds closely related to the ecologically relevant optimal stimuli (isomers or other close analogues) typically drops by a factor of 10-100.

There have been very few studies related to the specificity of olfactory sensitivity in vertebrates. This is not surprising given the logistical difficulties involved in sensitivity measurement in vertebrates and the small number of published thresholds. However, there are a number of behavioral response threshold and taste distinction studies available. Comparisons among insectivorous and omnivorous lizards (Cooper, 1999, Cooper *et al*, 2000) show that tongue-flicking and other investigative responses to prey and plant odours (detected by lingual transfer to the vomeronasal system) correspond to natural dietary habits. Omnivorous lizards are more likely than insectivorous lizards to respond to plant or fruit odours. Unfortunately, these studies do not distinguish between detection sensitivity and feeding preference or interest.

Roe deer (*Capreolus capreolus*) unlike most ungulates, selectively browse tannin-rich plants. Feeding studies show that they are capable of distinguishing tannins added to their feed, and, if offered a choice of tannin-enriched and tannin-free feed, they will regulate their intake precisely at

28g tannin/kg feed pellets. This regulation persisted despite variation in the concentrations of tannins in their tannin-enriched feed (Verheyden-Tixier and Duncan, 2000). While this study does not identify the mechanism of regulation and does not quantify sensitivity to different tannin concentrations presented, it is a striking example of effectiveness of detection for specific dietary elements.

Among primates, taste preference and intensity-difference thresholds for sugars have been found to correspond to the proportion of fruit in the diet of the species (Hladik and Simmen, 1996, Laska *et al*, 1999, Laska, 1994, 1996). Frugivorous new world primates select sugar solutions over water at lower concentrations than omnivorous species do, and they will also successfully discriminate smaller concentration differences between sugar solutions. These findings are particularly important in that they compare several species along an ecological gradient and directly measure sensitivity, providing a vertebrate example of heightened specific chemoreception for ecologically relevant compounds.

Laska and Seibt (2002a) note that three frugivores, the common squirrel monkey (*Saimiri sciureus*), pig-tailed macaque (*Macaca nemestrina*) and Seba's short-tailed bat (*Carollia perspicillata*), have generally higher sensitivity to acetate esters than carnivores or granivores. By contrast, they note also elevated sensitivity to carboxylic acids among carnivores, insectivores and sanguivores (domestic dog, *Canis familiaris*; European hedgehog, *Erinaceous europaeus*; vampire bat, *Desmodus rotundus*) relative to the frugivores. Esters are major components of fruit odours, while carboxylic acids are important in animal body odours. This, the authors suggest, supports a role for diet in determining specific olfactory sensitivity.

There are many non-dietary sources of potentially relevant odour stimuli. The odours of conspecifics, predators, favored shelter foliage, and many other resources are important to the survival and success of any olfactor. However, the availability of volatile chemical data makes diet a logical candidate for testing. The species available for olfactory specialization comparisons are those which have been tested for olfactory sensitivity. Of these, the only volatile chemical profile available is for humans, so comparative analysis of sensitivity to conspecific odours is currently

impossible. Similarly, for most species shelter, predators, etc. volatile chemical profiles are also unknown. Diet is the only ecological variable for which there is sufficient information and sufficient differences among mammal species that comparisons can be made from existing volatile chemistry data.

This study tested the following hypothesis:

> A mammal species will possess elevated sensitivity to compounds of high ecological relevance such as characteristic food odour components, compared with related species for whom the same compounds have little or no relevance.

Since the sea otter (*Enhydra lutris*) inhabits a distinctive olfactory landscape compared to terrestrial mammals, it presents an excellent opportunity to test this hypothesis by comparing sensitivity to marine versus terrestrial odorants. The Carnivora forage on a wide variety of items, including fruit and other plant matter. While sea otters specialize in marine invertebrates and to a lesser extent fish, other otter species take both marine and freshwater prey as well as frogs and occasionally terrestrial prey including birds and rodents. Non-aquatic Mustelidae depend largely on rodents, other small mammals, birds, and eggs. Some diets (particularly in the genus *Martes*) also include fruit, honey and carrion, and many species take insects and worms. Among the semiaquatic species frogs, fish and aquatic invertebrates are also included (Nowak, 1997).

There is no dietary specialization at the level of the Carnivora *per se* for which the collective diet encompasses mammals, birds, reptiles, amphibians, fish, invertebrates and plant material. However, the natural diets of the two carnivore species available for comparison in this study, the sea otter and the domestic dog (*Canis familiaris*), are to a first approximation completely nonoverlapping. Determining the natural or evolutionarily relevant diet of the domestic dog is problematic because of its domesticated status, but taking into account the lifestyle of domesticated

and feral dogs as well as dingoes and congeneric species, it is reasonable to describe the diet as consisting of terrestrial vertebrates, indeed, primarily of mammals and birds, supplemented very occasionally with plant matter.

Methods

Two hundred and twenty literature references (omitted from Ref. section due to space constraints, see Appendix) were used to estimate incidence of odorants in various marine and terrestrial dietary sources.

There are patterns of both consistency and variation in the volatile chemistry of the taxa and other categories that distinguish the most broadly defined dietary habits. For example, all animals give off a wide variety of carboxylic acids. Many are unique at the species or genus level, and many others are given off in different quantities by many species. Olfactory sensitivity studies in insects (see above) have often revealed high specific sensitivity to compounds that are not highly specific to the species' preferred food item and generally conclude that it is the proportion of many fairly common compounds that allows even highly specialized feeders to identify their host plant or prey. Since the mammal species compared herein tend to have broader diets than the insects in the studies listed above, volatile profiles were assigned only to the following broad dietary categories: fruits, grains, flowers, plants (other tissue), terrestrial vertebrates, insects and marine animals (fish and invertebrates).

Aliphatic acids, alcohols, and esters occur with very different frequencies in organisms from these dietary categories (see Fig 1). Relative importance or Incidence (I) was defined as the mean (over all dietary categories consumed by the species) of the fraction of items in each category containing the compound. Dietary categories were assigned to species based on natural diet descriptions in Walker's Mammals of the World (Nowak, 1997) and are listed in table 1. (All categories were arbitrarily assigned equal weight for the calculation of species I value, as

quantitative dietary breakdowns were not available for all species.) This served as an estimate of the proportion of the species diet containing the compound. A sample calculation follows for ethanol for the Pale spear-nosed bat, *Phyllostomus discolor*:

Dietary categories of *P. discolor*: flowers, fruit

Literature available for flowers: 29 species; ethanol is reported in 2. $I_{flowers,\ ethanol}=2/29=0.069$

Literature available for fruit: 35 species; ethanol is reported in 12. $I_{fruit,\ ethanol}=12/35=0.343$

$I_{Spear\text{-}nosed\ bat,\ ethanol}$ = mean ($I_{fruit,\ ethanol}$, $I_{flowers,\ ethanol}$)= (0.069+ 0.343)/2=0.206

Fourteen mammal species which had been previously tested for olfactory threshold on at least one natural odorant were used in the analysis: five bats, Seba's short-tailed bat (*Carollia perspicillata),* the Vampire bat (*Desmodus rotundus),* the Mouse-eared bat *(Myotis myotis),* the Pale spear-nosed bat *(Phyllostomus discolor)* and the Great fruit bat *(Artibeus literatus),* three primates, Human *(Homo sapiens),* the Common squirrel monkey (*Saimiri sciureus),* and the Pig-tailed macaque *(Macaca nemestrina),* two carnivores, the Domestic dog (*Canis familiaris)* and the Sea otter (*Enhydra lutris),* two rodents, the House mouse (*Mus musculus)* and the Brown rat (*Rattus norvegicus),* and one basal and one Soricid insectivore, the European hedgehog (*Erinaceous europaeus)* and the Common European shrew (*Sorex araneus).* For each species, each odorant compound for which a published threshold was available was assigned an I value.

Comparisons among species were confined to simple contrasts between sister lineages where sufficient data were available. Ordinarily, all data would be transformed using Felsenstein's (1985) method of independent contrasts in order to permit comparisons across the entire phylogeny. However, in this case the variable I was so labile (dietary specialization so plastic) that many interordinal or higher comparisons would not be realistic. Thus five comparisons were available: Seba's short-tailed bat vs. Vampire bat, Seba's short-tailed bat vs. Mouse-eared bat, Human vs. Common squirrel monkey, Human vs. Pig-tailed macaque, and Domestic dog vs. Sea otter.

Results

Specific sensitivity comparisons involved 16 odorant compounds; six carboxylic acids: acetic acid, butyric acid, valeric acid, caproic acid, caprylic acid and octanoic acid; five alcohols: ethanol, butanol, hexanol, heptanol and octanol; and five acetate esters; ethyl acetate, propyl acetate, butyl acetate, amyl acetate and hexyl acetate. These compounds vary significantly in their natural sources (Fig. 1). Most of the carboxylic acids are found in terrestrial vertebrate and invertebrate animals and in grains. The shorter carboxylic acids also appear in the marine fish and shellfish categories and honey. Those containing even numbers of carbons are common in fruit. Acetic, butyric and caprylic acids are common in fungi.

The alcohols are less common than the carboxylic acids in general, with the notable exception of hexanol, which is common in both marine and terrestrial plant and animal categories. The other alcohols are most common in fruit, grains and flowers. Ethanol is also common in fungi and honeys, and ethanol and octanol are also common in marine animals.

The acetate esters are rare outside the fruit and flower categories, except for ethyl acetate, which is common in shellfish, fungi and grains.

The most distinctive distributions (occurring in at least 20% of 1-3 natural source categories) of these and closely related compounds are illustrated in Fig. 1.

The species pairs whose olfactory repertoires were contrasted vary in their ecological separation. The Macaque-Human contrast has the least separation because the entire macaque diet is a subset of the human diet and includes more than half (three of five) of the human dietary categories. Therefore, the I values of the odorants in this contrast differ very little between the two species (Fig. 2a). The Squirrel monkey-Human contrast also compares the human diet with a subset, but in this case a specialized one. The squirrel monkey's high degree of frugivory, paired with the importance of the otherwise rare acetate esters in the fruit category result in a very distinctive I profile for the squirrel monkey. A large set of acetate esters have published olfactory thresholds for both humans and squirrel monkeys and are therefore available for this comparison. The result is a more obvious separation of I values between human and squirrel monkey than between human and macaque (fig 2b).

The highly specialized, completely nonoverlapping diets of the three chiroptera examined yielded very distinct I distributions for both species pairs that were contrasted (Fig. 3).

The diets of the domestic dog and sea otter are also entirely nonoverlapping, but dog prey and otter prey are less chemically distinct from each other than are the diets of the Chiroptera. Therefore the carnivore I distributions are only moderately distinctive. Both species have identical values (0) for amyl acetate. The sea otter has highly varied I values for the remaining compounds (all carboxylic acids) while those of the dog vary little (Fig. 4). Incidence values for

benzaldehyde and eugenol are also included, although only a qualitative comparison of thresholds for these compounds will be possible. Incidence for eugenol is zero for both species, and for benzaldehyde, the I value is significant for both species but greater for the sea otter.

By comparing thresholds for each compound among all available species it is easily shown that in most cases, macrosmatic (sensitive) species tend to retain their rank throughout most of this range of odorants. Although thresholds for certain compounds is low for all species, for instance, thresholds for carboxylic acids tend to be uniformly lower than for alcohols, the species rank order of sensitivity is largely preserved among different compounds (Fig. 5).

However, the magnitude of the difference between species varies significantly among compounds. Among the contrast species pairs, one species is often clearly generally more sensitive, with the notable exception of the primates. Still, it is possible to measure the effect of ecology, as represented by I, in the variation of this difference (Fig. 6).

There was no clear rank order of sensitivity among the primates. In the Human-Squirrel monkey contrast, humans showed slightly higher sensitivity among the animal odour compounds (mostly carboxylic acids) while the squirrel monkey was slightly more sensitive to fruit specific compounds (mostly esters). No such ecological influence is visible in the Human-Macaque comparison; this may be due in part to the absence of carboxylic acids available for comparison, which left a range of compounds varying rather little in their relative dietary relevance.

There are relatively few odorants available for comparison for the other species pairs. However, the Seba's bat-Vampire bat comparison showed a marked though erratic trend favoring the frugivorous Seba's bat among the fruit odorants and with variable results among the animal odorants: one for which Seba's bat is more sensitive, one for which the vampire is more sensitive,

and one for which sensitivity is similar for both. The Seba's bat-Mouse-eared bat comparison did not show a trend. The Dog-Sea otter comparison shows the dog to be uniformly more sensitive, but the difference steadily and monotonically decreased for less terrestrial, more marine-based odorants.

Regressions of Threshold vs. Incidence showed a nearly significant relationship for the Squirrel monkey-Human contrast, (Fig. 7a, R^2=0.22, P=0.064). The Macaque-Human, Seba's bat-Vampire and Seba's bat-Mouse-eared bat contrasts were not significant (figs. 7b,c,d). The Dog-Sea otter contrast, despite its very small sample size, was significant (P=0.04) and accounted for most of the observed variation (R^2=0.80).

Discussion

The highly varied results of these five comparisons suggest that the estimate (approximate dietary chemistry) of chemical ecology is sometimes productive but possibly unreliable.

The most obvious shortcoming is the approximation of the diet itself. First, only fresh food items were utilized in the calculation of I values. The chemical profiles of spoiled food differ markedly from the same item live or fresh, and detection of spoilage during feeding is of obvious adaptive importance. However, since the difference in importance of spoilage detection among the study species was not known and spoilage-induced chemical changes specific to the diets of any of the study species were not available, this was not attempted. Second, since volatile chemical profiles of the specific items consumed by each species were unavailable, general categories of taxonomy, geography and plant anatomy were used to distinguish the diets. Between species with nonoverlapping, taxonomically distant diets, this may not have much impact. However, between the human and the pig-tailed macaque, for example, diets that are almost certainly easily distinguishable were necessarily assigned 60% equivalence. Considering that there is substantial overlap in the

chemistry of any two dietary categories, the remaining category difference does not preserve sufficient chemical difference to distinguish the two species. This is very likely to have contributed significantly to the nonsignificance of the Macaque-Human contrast.

The second shortcoming is the use of dietary chemistry to estimate all of chemical ecology. It is more difficult in this case to speculate upon the possible impact of this drawback on the contrast regressions. The estimate will be inappropriate in cases where one or both species being contrasted experience significant selection pressure on their ability to detect non-dietary olfactory signals, and where those signals differ either chemically or quantitatively in importance between the two species. Not only food items but also kin, mates, other conspecifics, predators, and presumably many other odour sources are ecologically important stimuli to most species. Not enough is known about these myriad potential nondietary signals to predict in which cases they will be either important or greatly different between species. However, the variation in one ecological variable in this dataset is suggestive of this effect: habitat.

The primates and chiroptera examined vary in their geographical distributions, but all are terrestrial and tropical. The two carnivores, by contrast, inhabit dramatically different environments. The Dog-Sea otter contrast demonstrates by far the strongest differentiation in the dataset, despite the fact that the dietary comparison showed only moderate chemical differences. A detailed examination of this contrast shows that, relative to the dog, the sea otter encounters an elevated incidence of acetic acid in its diet, and reduced frequencies of the longer carboxylic acids. The incidence of amyl acetate is equal for both species at zero. It must also be borne in mind that the sea otter is, overall, not as sensitive an olfactor as the dog. For the four compounds tested, the dog threshold is lower in every case. However, the sea otter threshold for the ecologically important compound acetic acid is reduced only by a factor of 1.2, while sensitivity for the reduced-incidence butyric caprylic and octanoic acids is reduced by a factor of 60, 180, and 1530, respectively. Amyl acetate sensitivity may be the most representative of the general or background difference in sensitivity. For this odorant, sea otter sensitivity has fallen by an intermediate amount, a factor of

17. The regression of this contrast shows convincingly that in this case, dietary relevance was a major factor in determining evolved differences in specific sensitivity.

In addition, sea otter thresholds for eugenol and benzaldehyde can be compared in a qualitative manner with dog thresholds for the same compounds (Myers and Pugh, 1985). Sea otter thresholds, measured in this study, were nearly equivalent for the two compounds, at 10^-9.8 and 10^-9.7 mol/L, respectively. The dog threshold for eugenol, the significantly more terrestrial compound, was just over 4000 times lower than the benzaldehyde threshold. Unfortunately it is not possible to use these odorants in a quantitative comparison, since the dog thresholds were reported in arbitrary concentration units, precluding the possibility of calculating threshold contrasts between the two species. Still, the trend in this case also supports the role of dietary relevance in sensitivity differences.

The distinction of the Dog-Sea otter contrast may reflect the fact that while diet represents an inadequate proxy for ecological relevance for frugivores or omnivores within a single terrestrial biome, the same approximation between a marine and a terrestrial carnivore are coincidentally an adequate representation thereof. Most of the ecologically relevant organisms with which the dog may interact, prey, predators and conspecifics, are vertebrates and have a similar volatile profile among the odorants in the dataset. (Terrestrial vertebrate taxa are quite distinctive in other chemical groups such as reduced nitrogen and sulfur compounds and species-specific pheromones.) Terrestrial vertebrates are, however, quite distinct from the marine fish, mollusks, echinoderms and crustacea. While the available volatiles data does not include sea otter predators, these, approaching from underwater, are most likely not detected by smell. Therefore, terrestrial vertebrates and marine fish and invertebrates adequately represent major relevant olfactory stimuli for these two carnivores (excluding only sea otter conspecifics). Finally, the separation of these habitats ensures that the food items have no ecological relevance for the nonconsuming species because they are not encountered in natural settings. Dogs are not exposed to marine animals and sea otters are very rarely exposed to vertebrates of any kind (still excepting interactions with conspecifics). This suggests that examining carnivorous mammals native to radically different chemical landscapes may

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be a productive way to further evaluate the effect of chemical ecology on specific olfactory sensitivity in mammals.

An additional factor that may influence whether incidence-sensitivity relationships are evident is evolutionary distance or time since divergence. In this study sufficient phylogenetic branch length data were not available to compare the comparisons made in the Carnivora, Chiroptera and primates, but it is plausible that species pairs which diverged earlier are more likely to have developed divergent olfactory repertoire.

It must be noted that sea otter sensitivity to butyric acid remains higher than to acetic acid (Fig. 6e) despite the estimated greater importance of acetic acid (Fig. 4). There are two intimately related plausible explanations for this. The first is phylogenetic history. The primitive mammalian condition, judging by the otter's seven available relatives, has greater sensitivity to butyric acid. In that case, the change in the sea otter lineage has provided a reduction in that difference. Secondly, it is very likely that molecular constraint is operating, which limits the independence of individual thresholds. The olfactory receptor code is combinatorial, and Malnic *et al* (1999) and Hamana *et al* (2003) have shown in mice that closely related odorants may share most of their repertoire of responsive receptor types. For example, of eight receptor types found to be sensitive to octanoic acid, all but one are also sensitive to nonanoic acid, in a sample of 14 receptor types tested (Malnic *et al*, 1999). Evolved changes in sensitivity to a specific odorant, if they are attributable to differences in the olfactory epithelium, most likely result either from changes in the molecular structure of the responsive odorant receptors (in the ORP genes themselves) or from changes in the expression patterns of those receptors. Optimizing either structure or expression patterns of receptor proteins to detect one compound will very likely have reduced but significant sympathetic effects on sensitivity to related compounds, creating evolutionary inertia in the differences between sensitivity to, for example, acetic and butyric acid. A more detailed comparison using psychophysical, molecular and ecological data from a variety of species will be needed in order to determine the relative importance of odorant-specific selective pressure and molecular constraint for specific olfactory sensitivity.

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Table. 1: Dietary categories assigned to study species.

Species	**Dietary categories**
Human *(Homo sapiens)*	grain, fruit, fungi, terrestrial plants and vertebrates
Common squirrel monkey *(Saimiri sciureus)*	fruit
Pig-tailed macaque *(Macaca nemestrina)*	fruit, grain, terrestrial plants
Seba's short-tailed bat *(Carollia perspicillata)*	fruit
Vampire bat *(Desmodus rotundus)*	terrestrial vertebrates
Mouse-eared bat *(Myotis myotis)*	insects
Domestic dog *(Canis familiaris)*	terrestrial vertebrates
Sea otter *(Enhydra lutris)*	marine animals

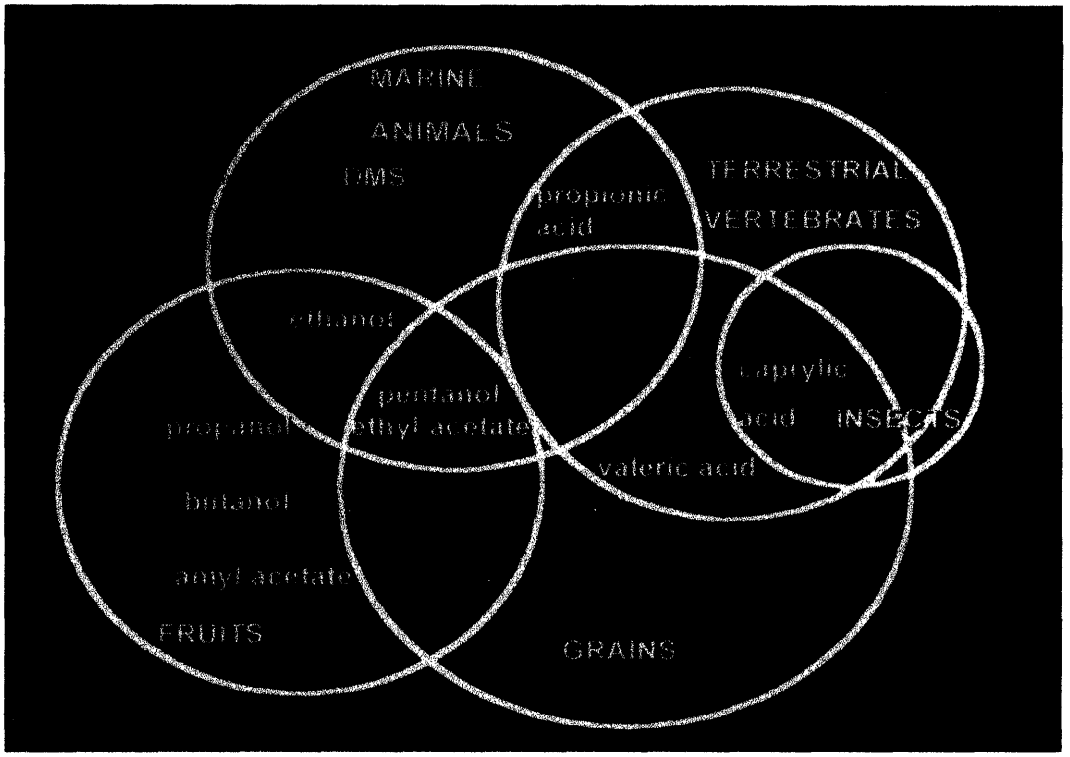


Fig. 1: Sample distributions of volatile compounds in nature. Compounds contained within a circle are found in at least 20% of items in that category reported in the literature, as listed in Appendix A.

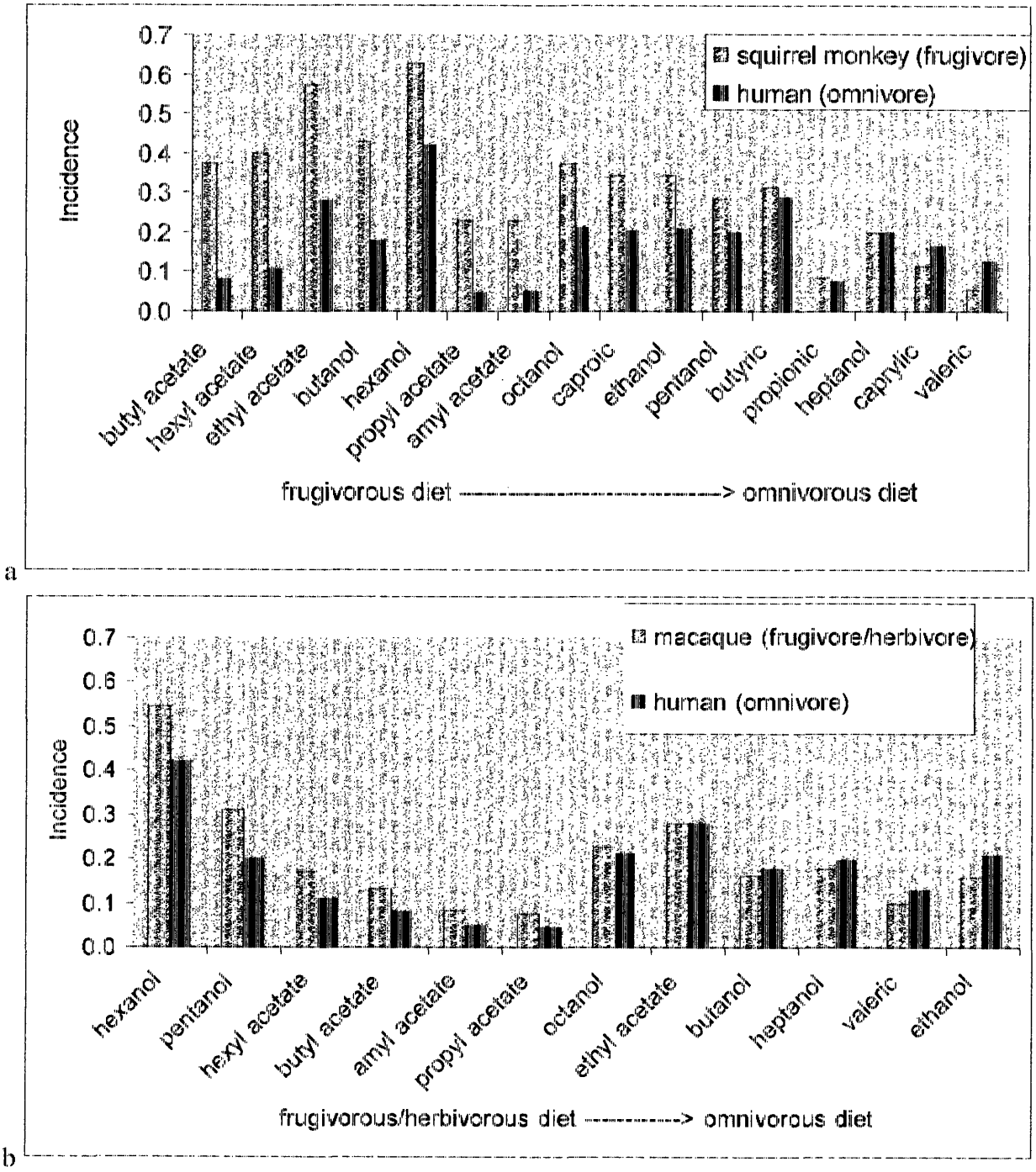
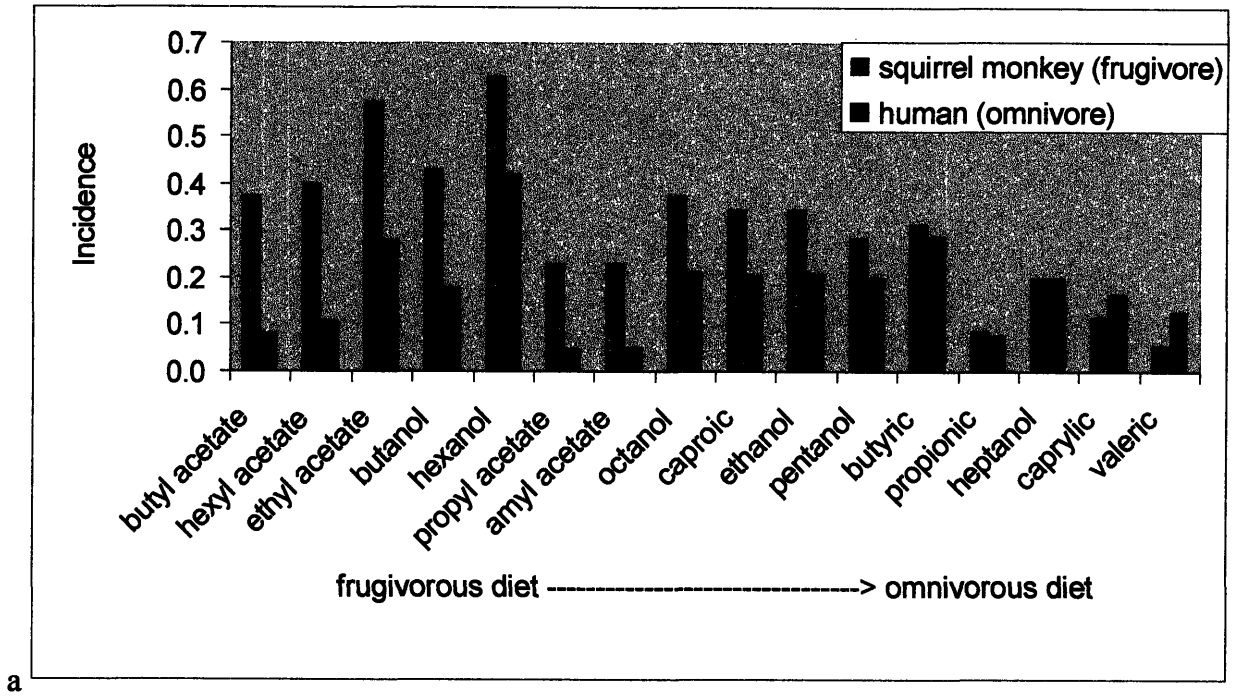


Fig. 2: Incidence values of sensitivity-tested odorants in primate diets. a) Squirrel monkey-Human (*Saimiri sciureus-Homo sapiens*) contrast, b) Macaque-Human (*Macaca nemestrina-Homo sapiens*) contrast



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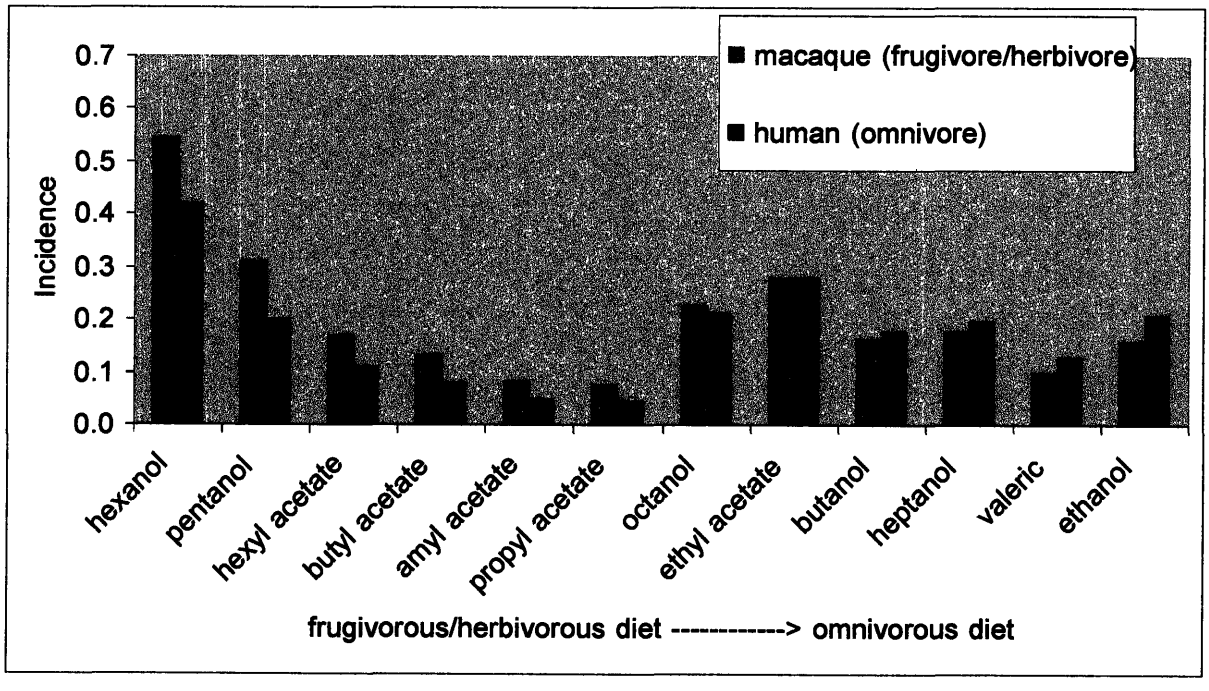

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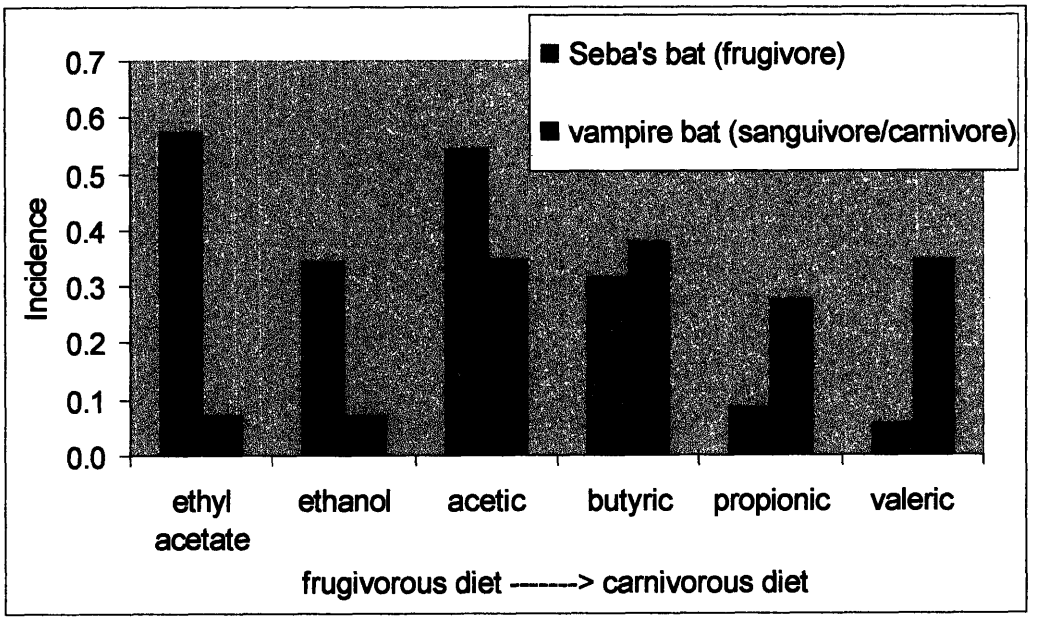
Fig. 2: Incidence values of sensitivity-tested odorants in primate diets. a) Squirrel monkey-Human (*Saimiri sciureus-Homo sapiens*) contrast, b) Macaque-Human (*Macaca nemestrina-Homo sapiens*) contrast



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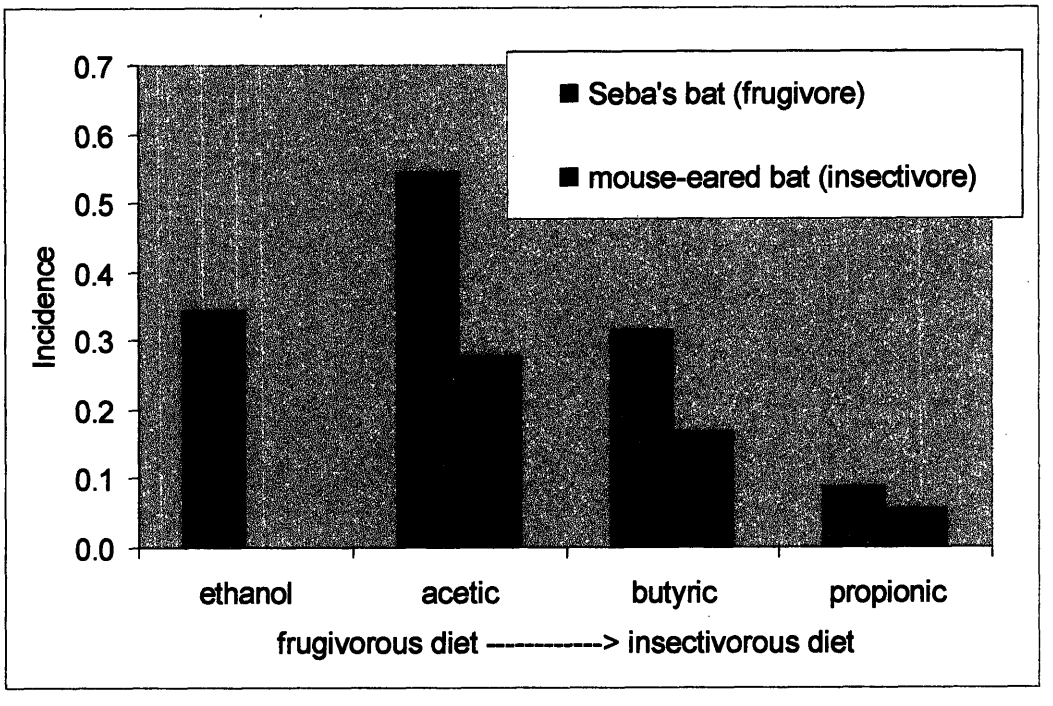

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Fig. 3: Incidence values of sensitivity-tested odorants in bat diets. a) Seba's short-tailed bat-Vampire bat (*Carollia perspicillata-Desmodus rotundus*) contrast, b) Seba's short-tailed bat-Mouse-eared bat (*Carollia perspicillata-Myotis myotis*) contrast

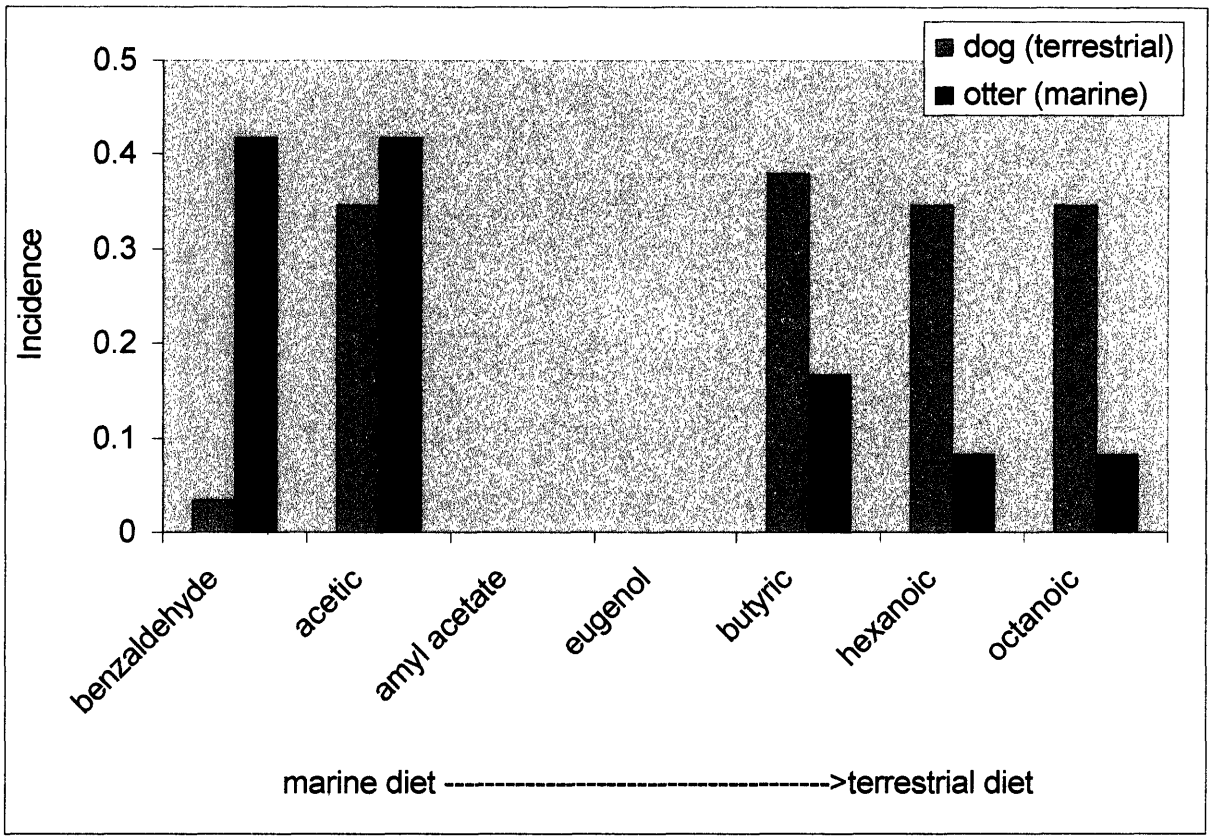


Fig. 4: Incidence values of sensitivity-tested odorants in carnivore diets: Domestic dog- Sea otter (*Canis familiaris-Enhydra lutris*) contrast

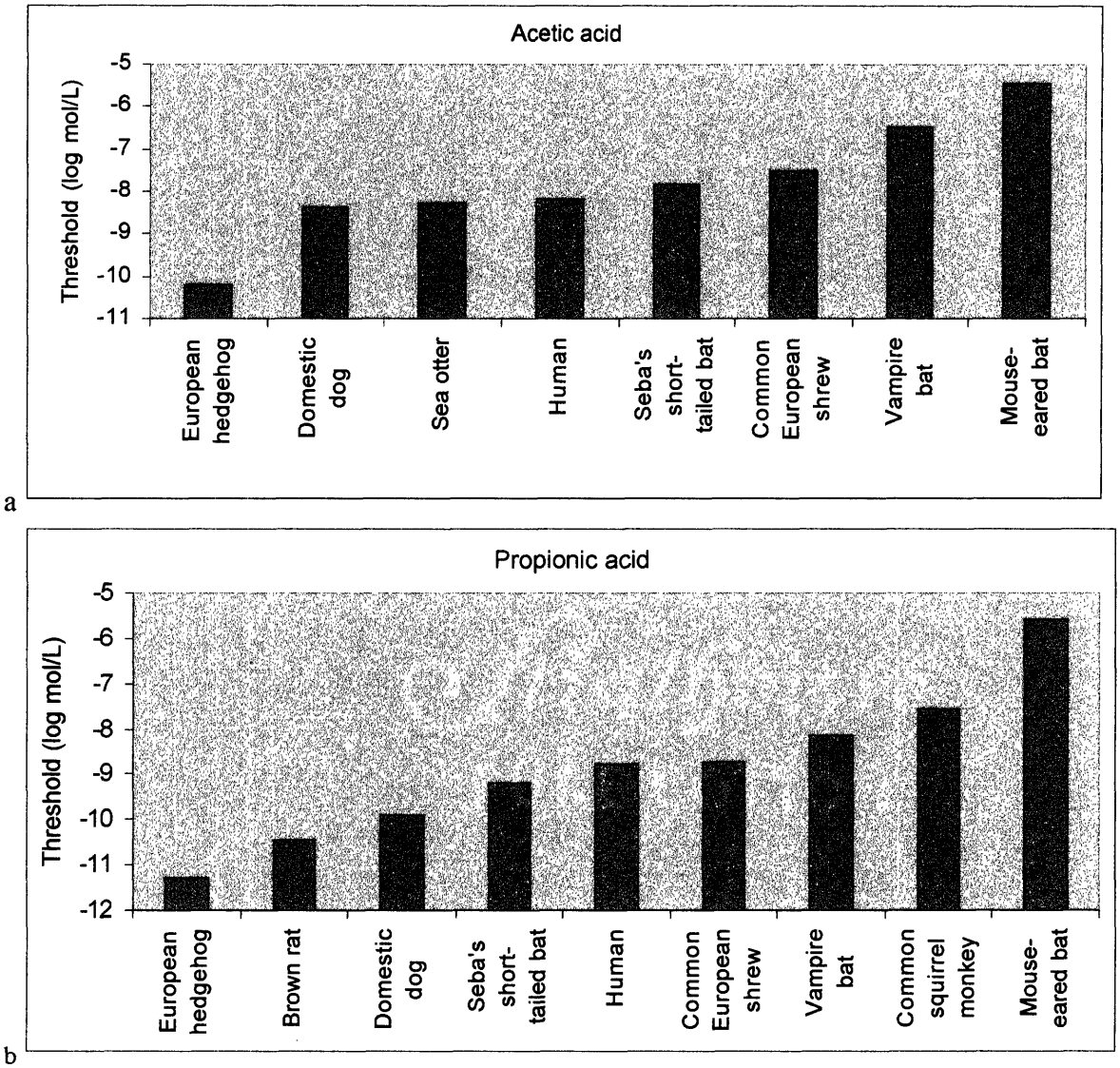
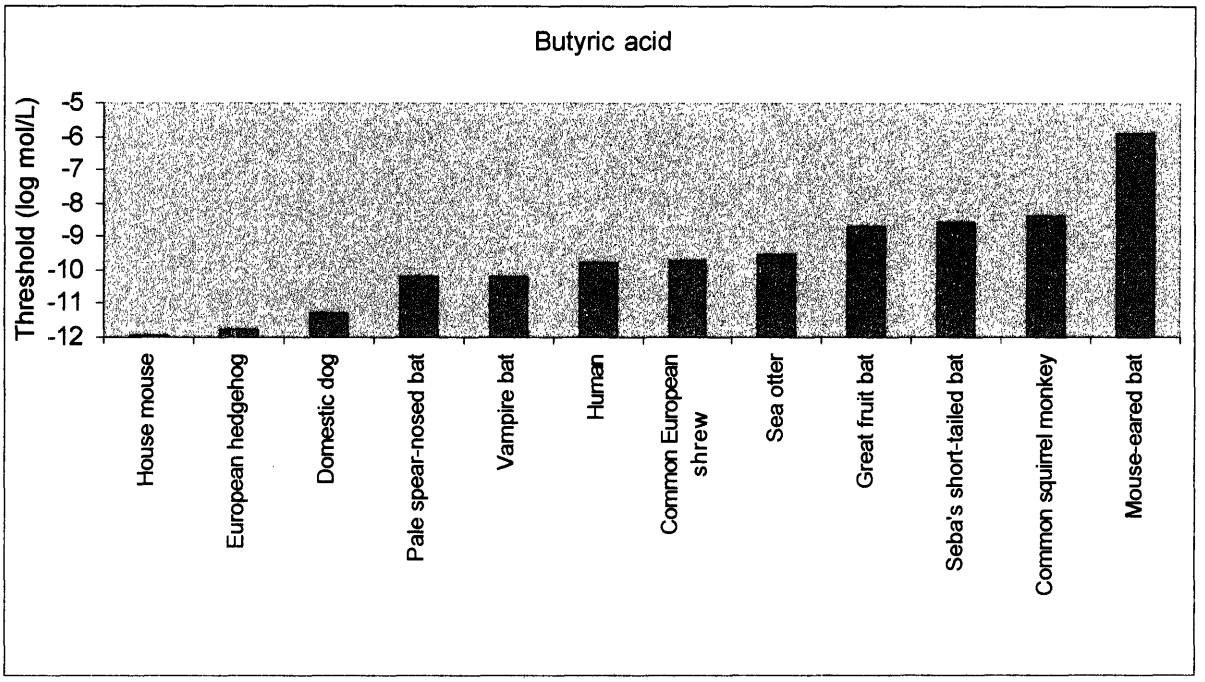


Fig. 5a-b: Thresholds of all study species compared for each individual odorant- carboxylic acids. Seba's short-tailed bat (*Carollia perspicillata),* Vampire bat (*Desmodus rotundus),* Mouse-eared bat *(Myotis myotis),* Pale spear-nosed bat *(Phyllostomus discolor),* Great fruit bat *(Artibeus literatus),* Human *(Homo sapiens),* Common squirrel monkey (*Saimiri sciureus),* Pig-tailed macaque *(Macaca nemestrina),* Domestic dog (*Canis familiaris)* Sea otter (*Enhydra lutris),* House mouse (*Mus musculus),* Brown rat (*Rattus norvegicus),* European hedgehog (*Erinaceous europaeus),* Common European shrew (*Sorex araneus).* Data from: Bretting, 1972; Devos *et al,* 1990; Hubener & Laska, 2001; Laing *et al,* 1989; Laska, 1990; Laska *et al,* 2000; Moulton *et al,* 1960; Obst *et al,* 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985



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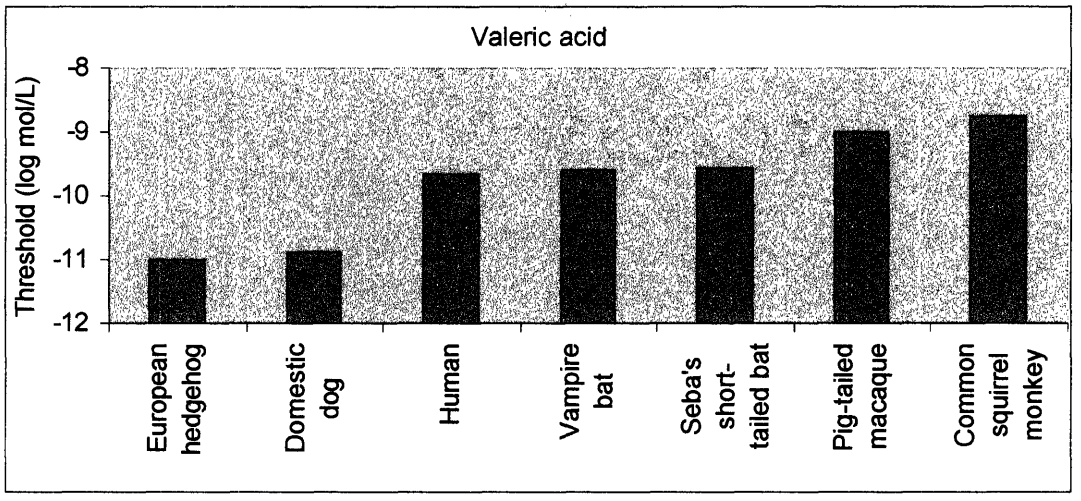

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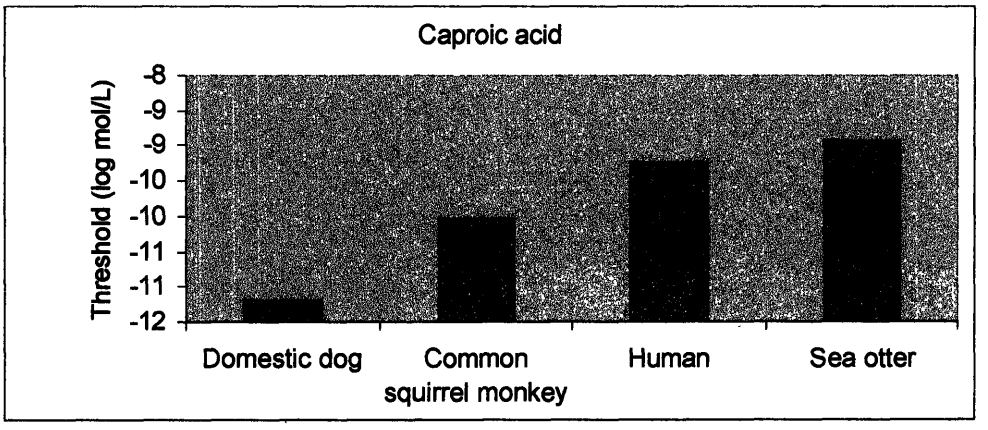
Fig. 5c-d: Thresholds of all study species compared for each individual odorant- carboxylic acids. Seba's short-tailed bat (*Carollia perspicillata),* Vampire bat (*Desmodus rotundus),* Mouse-eared bat *(Myotis myotis),* Pale spear-nosed bat *(Phyllostomus discolor),* Great fruit bat *(Artibeus literatus),* Human *(Homo sapiens),* Common squirrel monkey (*Saimiri sciureus),* Pig-tailed macaque *(Macaca nemestrina),* Domestic dog (*Canis familiaris)* Sea otter (*Enhydra lutris),* House mouse (*Mus musculus),* Brown rat (*Rattus norvegicus),* European hedgehog (*Erinaceous europaeus),* Common European shrew (*Sorex araneus).* Data from: Bretting, 1972; Devos *et al,* 1990; Hubener & Laska, 2001; Laing *et al,* 1989; Laska, 1990; Laska *et al,* 2000; Moulton *et al,* 1960; Obst *et al,* 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985



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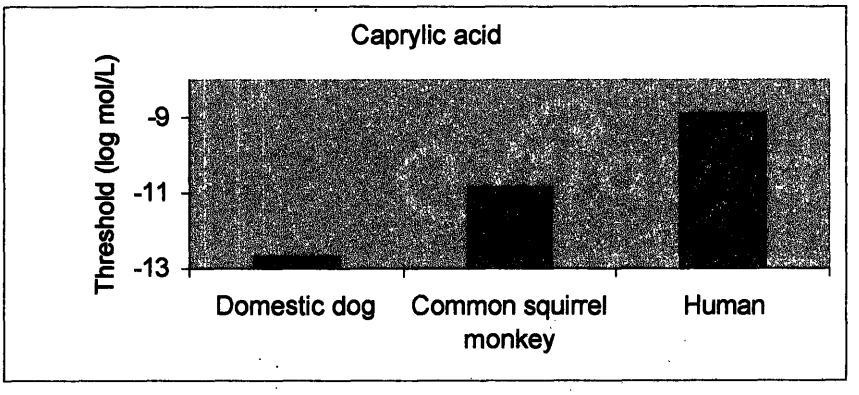

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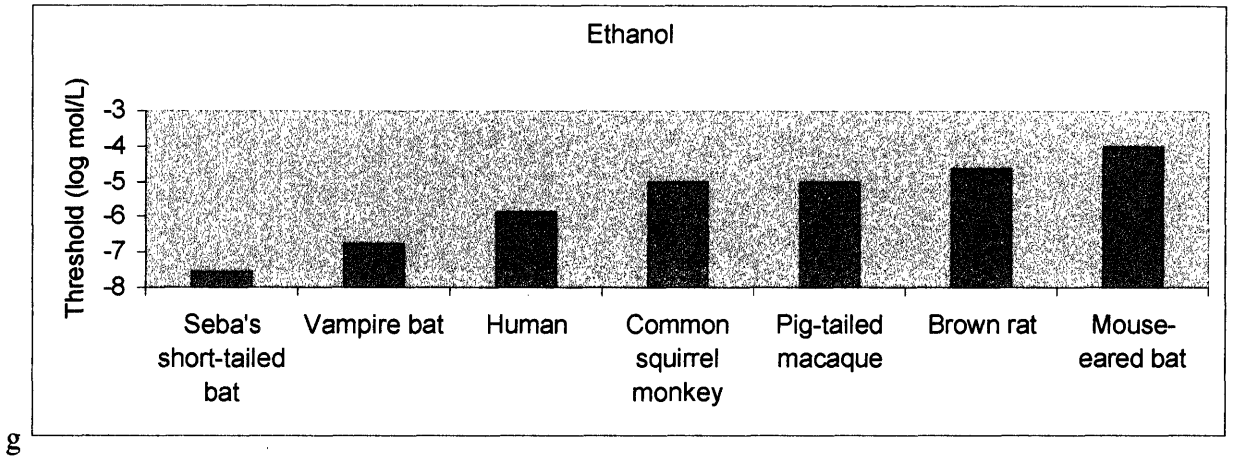
Fig. 5e-f: Thresholds of all study species compared for each individual odorant- carboxylic acids. Seba's short-tailed bat (*Carollia perspicillata),* Vampire bat (*Desmodus rotundus),* Mouse-eared bat *(Myotis myotis),* Pale spear-nosed bat *(Phyllostomus discolor),* Great fruit bat *(Artibeus literatus),* Human *(Homo sapiens),* Common squirrel monkey (*Saimiri sciureus),* Pig-tailed macaque *(Macaca nemestrina),* Domestic dog (*Canis familiaris)* Sea otter (*Enhydra lutris),* House mouse (*Mus musculus),* Brown rat (*Rattus norvegicus),* European hedgehog (*Erinaceous europaeus),* Common European shrew (*Sorex araneus).* Data from: Bretting, 1972; Devos *et al,* 1990; Hubener & Laska, 2001; Laing *et al,* 1989; Laska, 1990; Laska *et al,* 2000; Moulton *et al,* 1960; Obst *et al,* 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985



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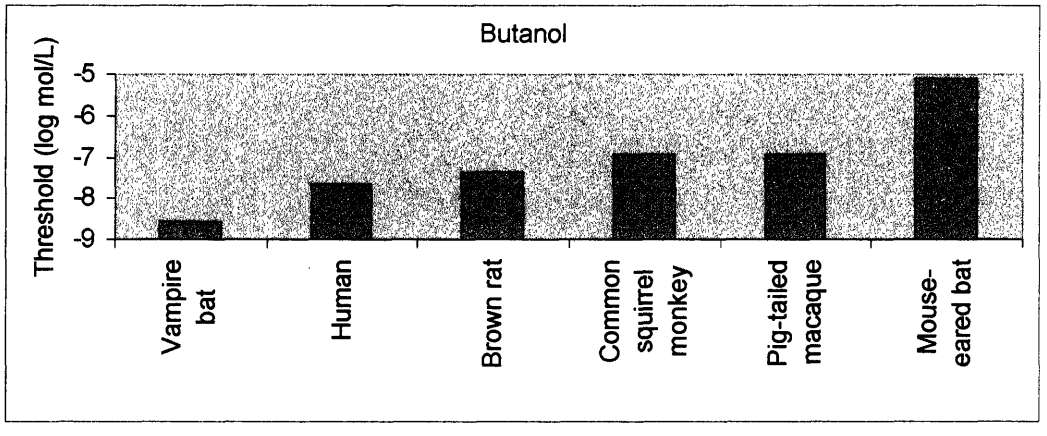

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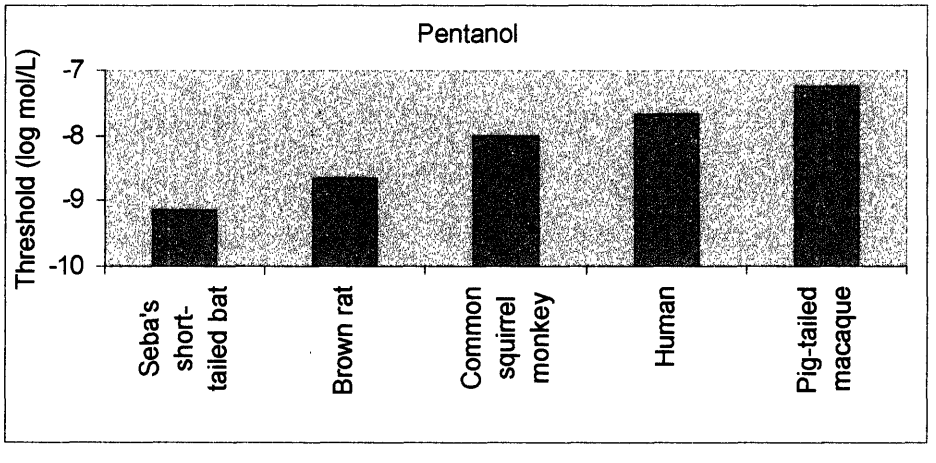

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Fig. 5g-i: Thresholds of all study species compared for each individual odorant- alcohols. Seba's short-tailed bat (*Carollia perspicillata),* Vampire bat (*Desmodus rotundus),* Mouse-eared bat *(Myotis myotis),* Human *(Homo sapiens),* Common squirrel monkey (*Saimiri sciureus),* Pig-tailed macaque *(Macaca nemestrina),* House mouse (*Mus musculus),* Brown rat (*Rattus norvegicus),* Data from: Devos *et al,* 1990; Laska, 1990; Laska *et al,* 2000; Laska, & Seibt, 2002b; Moulton and Eayrs, 1960; Obst *et al,* 1976; Schmidt, 1975

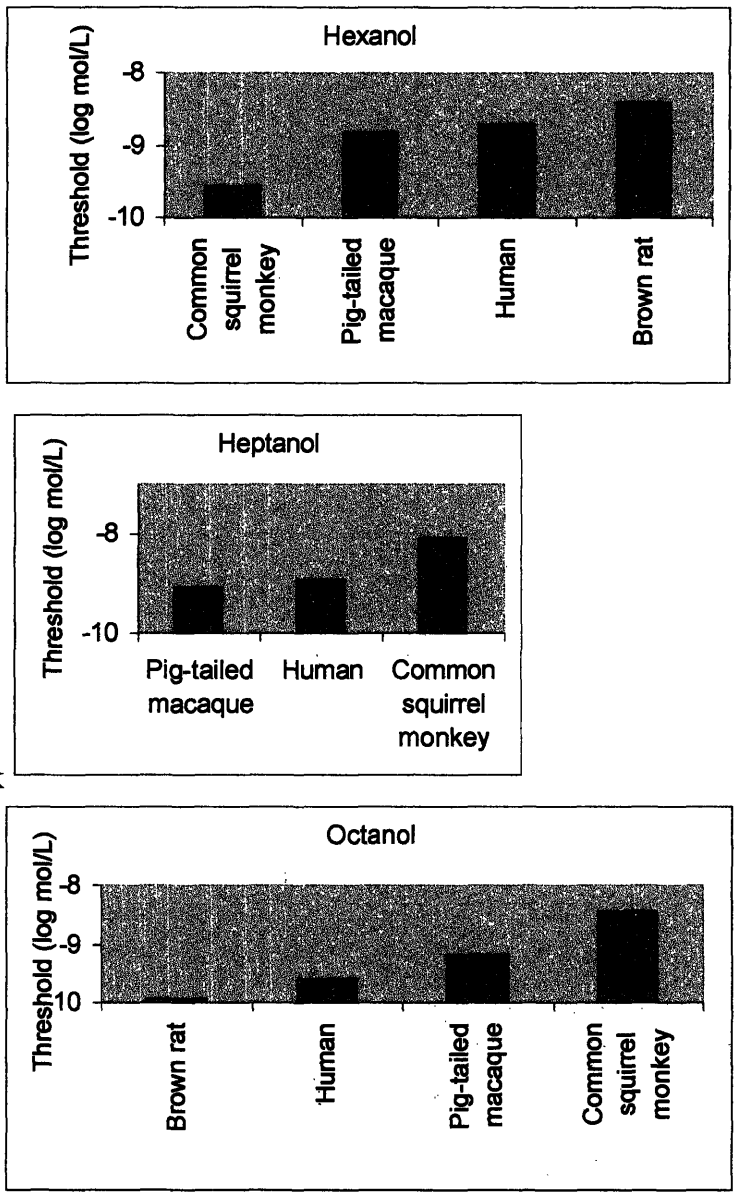
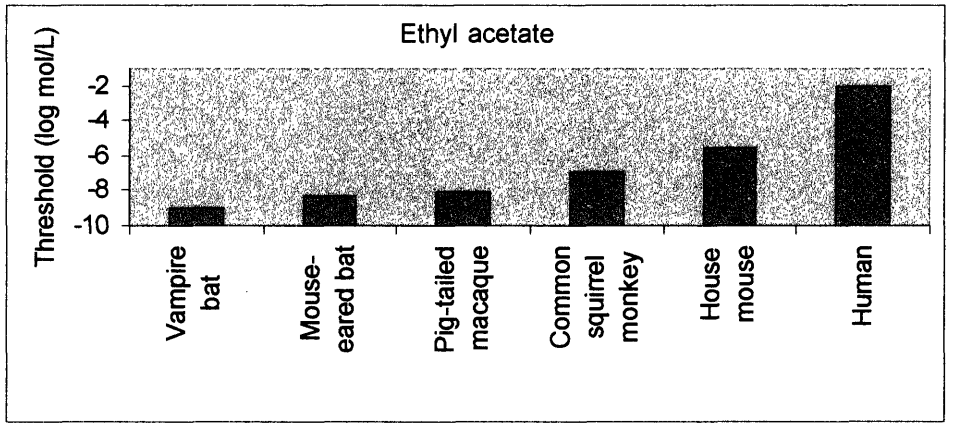


Fig. 5j-l: Thresholds of all study species compared for each individual odorant- alcohols. Seba's short-tailed bat (*Carollia perspicillata)*, Vampire bat (*Desmodus rotundus)*, Mouse-eared bat *(Myotis myotis)*, Human *(Homo sapiens)*, Common squirrel monkey (*Saimiri sciureus)*, Pig-tailed macaque *(Macaca nemestrina)*, House mouse (*Mus musculus)*, Brown rat (*Rattus norvegicus)*, Data from: Devos *et al*, 1990; Laska, 1990; Laska *et al*, 2000; Laska, & Seibt, 2002b; Moulton and Eayrs, 1960; Obst *et al*, 1976; Schmidt, 1975



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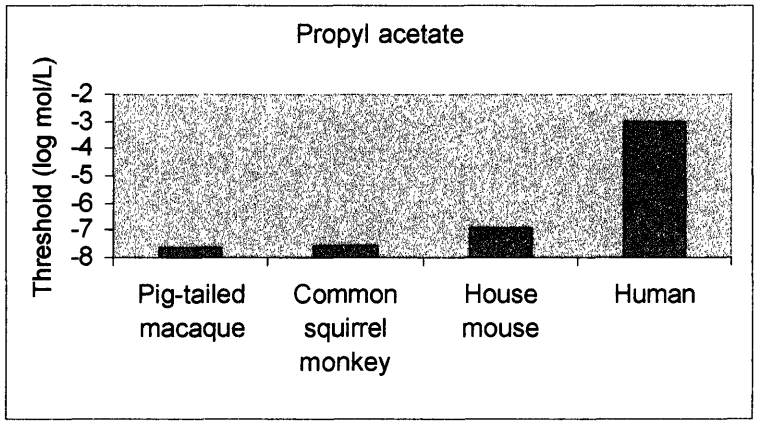

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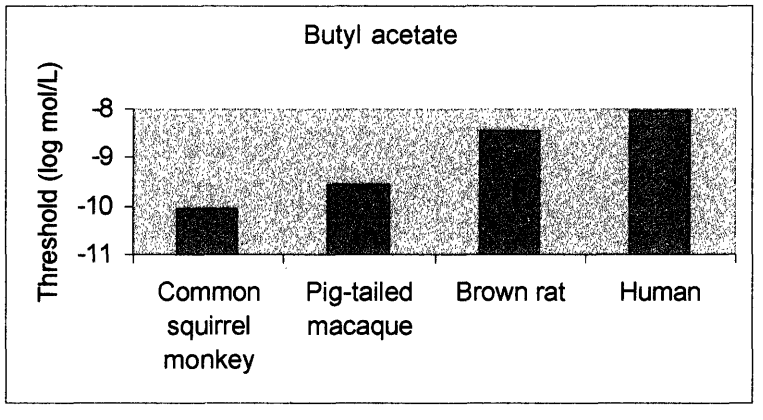
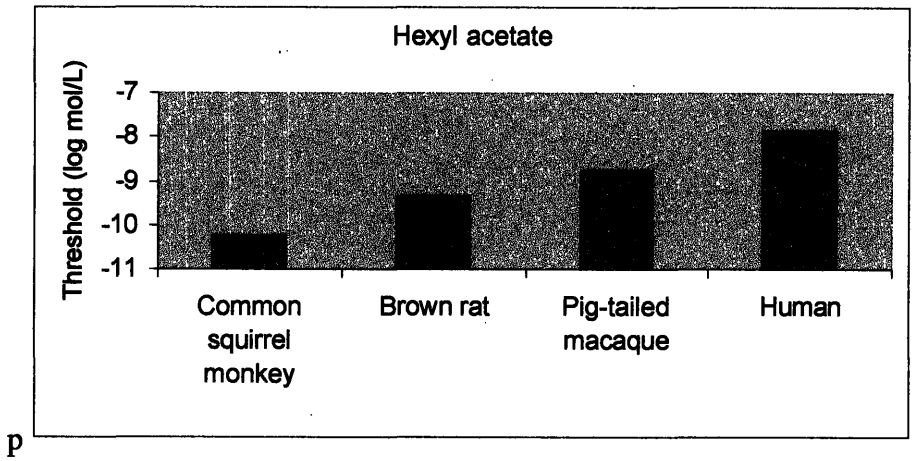


o

Fig. 5m-o: Thresholds of all study species compared for each individual odorant. Seba's short-tailed bat (*Carollia perspicillata),* Vampire bat (*Desmodus rotundus),* Mouse-eared bat *(Myotis myotis),* Human *(Homo sapiens),* Common squirrel monkey (*Saimiri sciureus),* Pig-tailed macaque *(Macaca nemestrina),* Domestic dog (*Canis familiaris)* Sea otter (*Enhydra lutris),* House mouse (*Mus musculus),* Brown rat (*Rattus norvegicus).* Data from: Devos *et al,* 1990; Krestel *et al,* 1984; Laska, 1990; Laska, & Seibt, 2002a; Moulton, 1960; Schmidt, 1975; Yee & Wysocki, 2001



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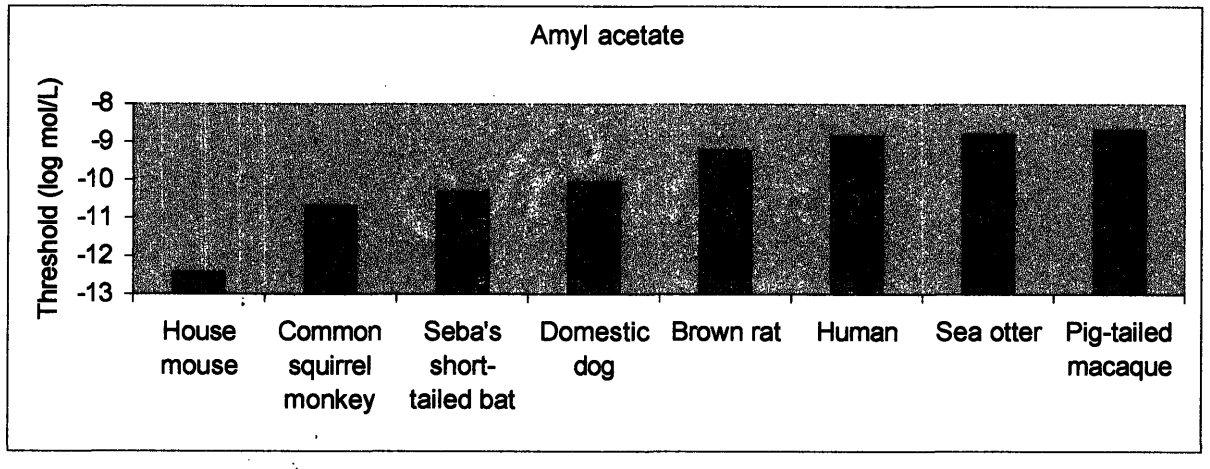
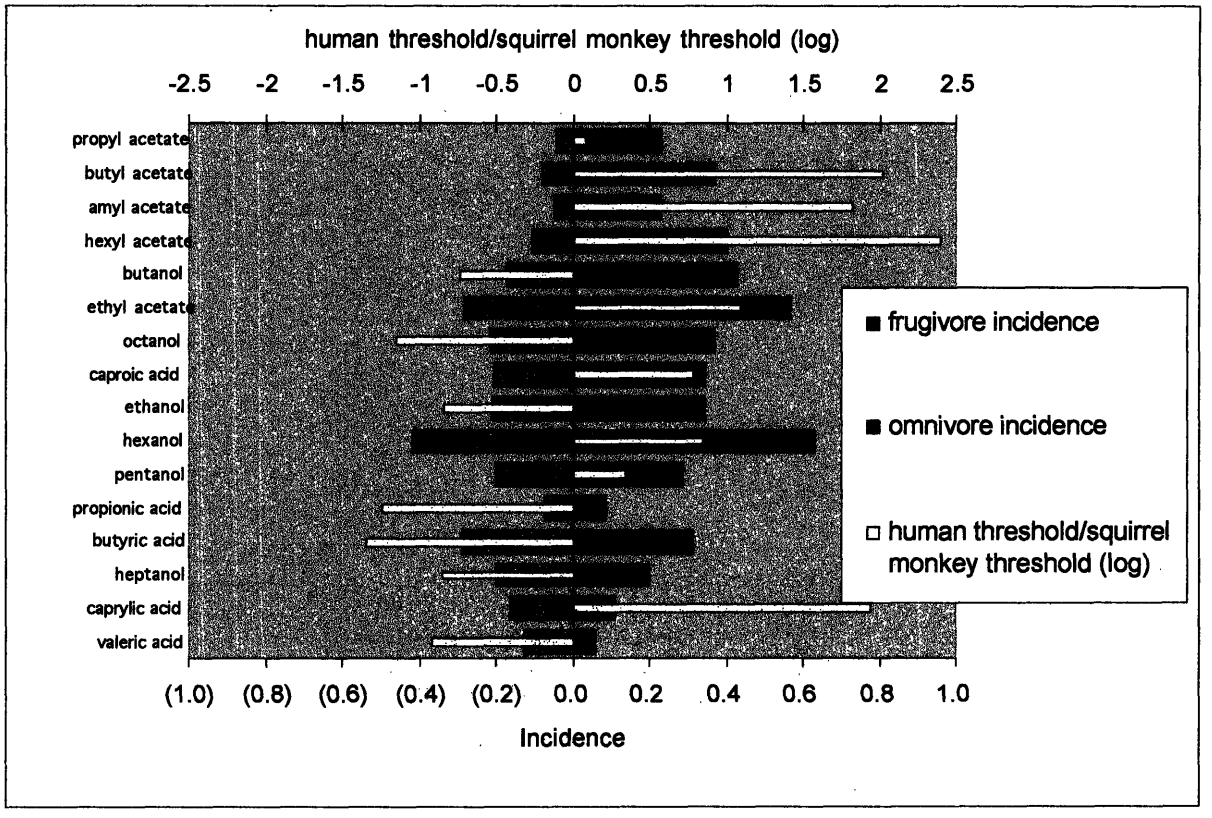
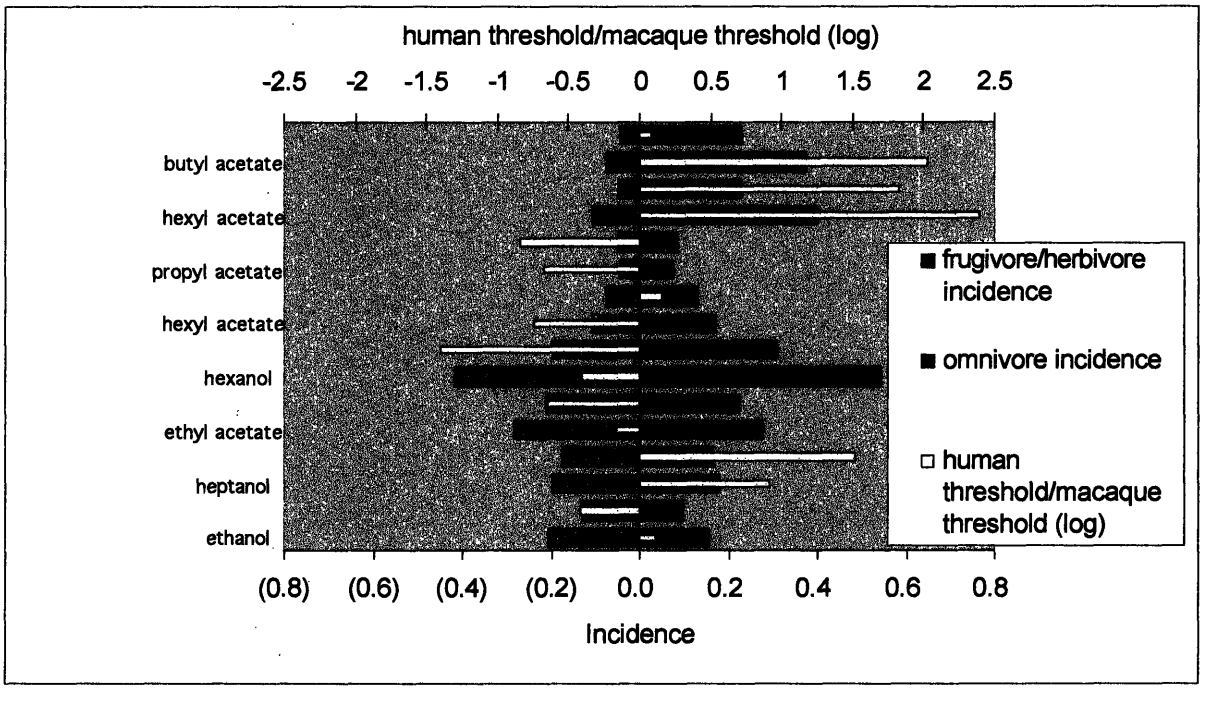


q

Fig. 5p-q: Thresholds of all study species compared for each individual odorant. Seba's short-tailed bat (*Carollia perspicillata)*, Vampire bat (*Desmodus rotundus)*, Mouse-eared bat *(Myotis myotis)*, Human *(Homo sapiens)*, Common squirrel monkey (*Saimiri sciureus)*, Pig-tailed macaque *(Macaca nemestrina)*, Domestic dog (*Canis familiaris)* Sea otter (*Enhydra lutris)*, House mouse (*Mus musculus)*, Brown rat (*Rattus norvegicus)*. Data from: Devos *et al*, 1990; Krestel *et al*, 1984; Laska, 1990; Laska, & Seibt, 2002a; Moulton, 1960; Schmidt, 1975; Yee & Wysocki, 2001

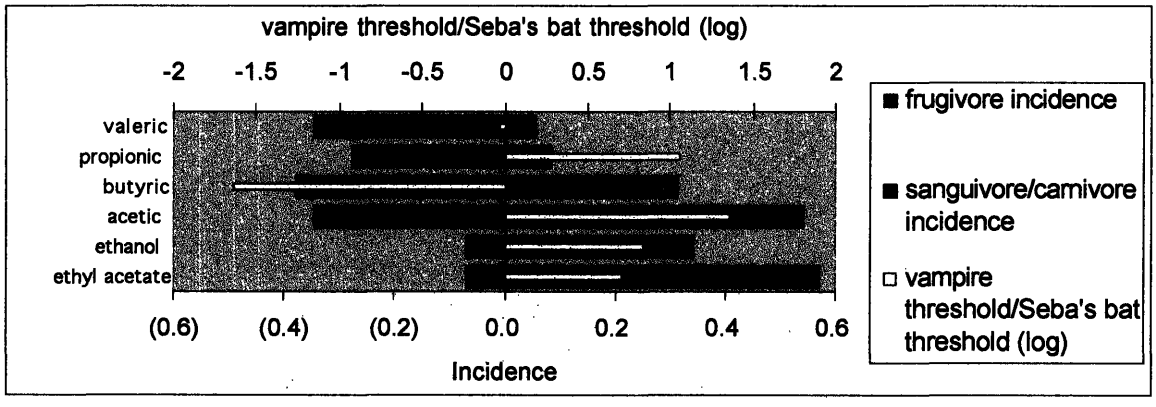


a



b

Fig. 6a,b: Incidence and threshold difference values of primate study species pairs, for all available comparison odorants. Squirrel monkey (*Saimiri sciureus*), Human (*Homo sapiens*), Pig-tailed macaque (*Macaca nemestrina*). Data from: Devos *et al*, 1990; Hubener & Laska, 2001; Laska *et al*, 2000; Laska, & Seibt, 2002a,b



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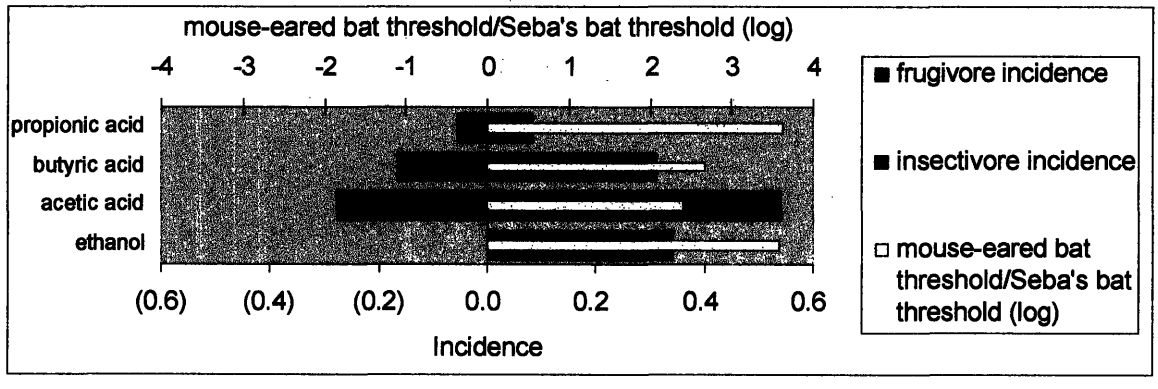
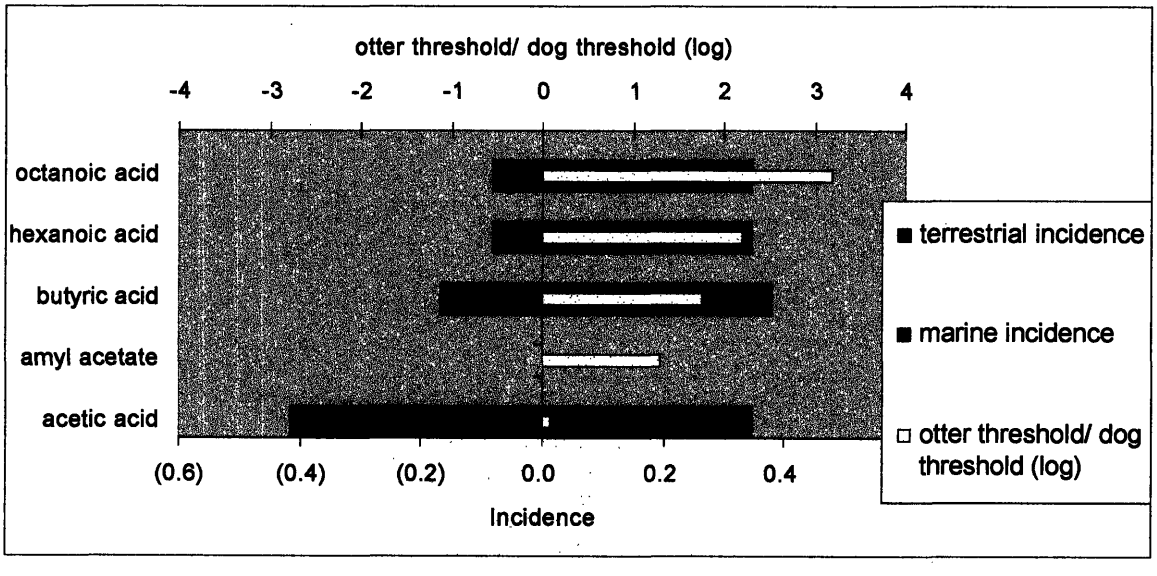
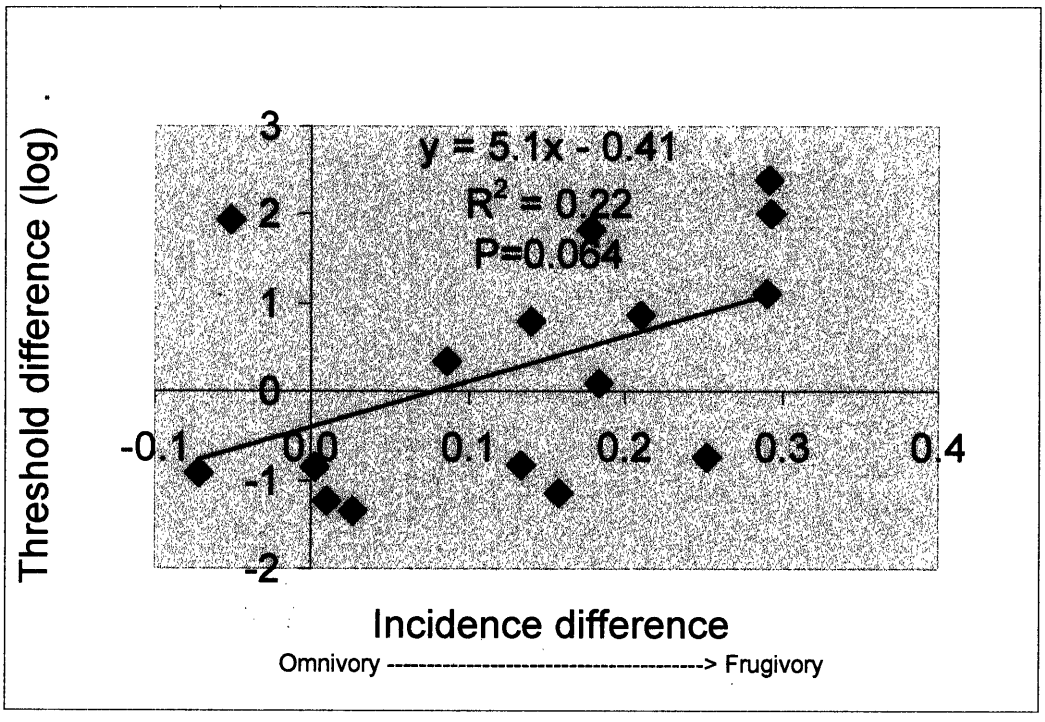


d

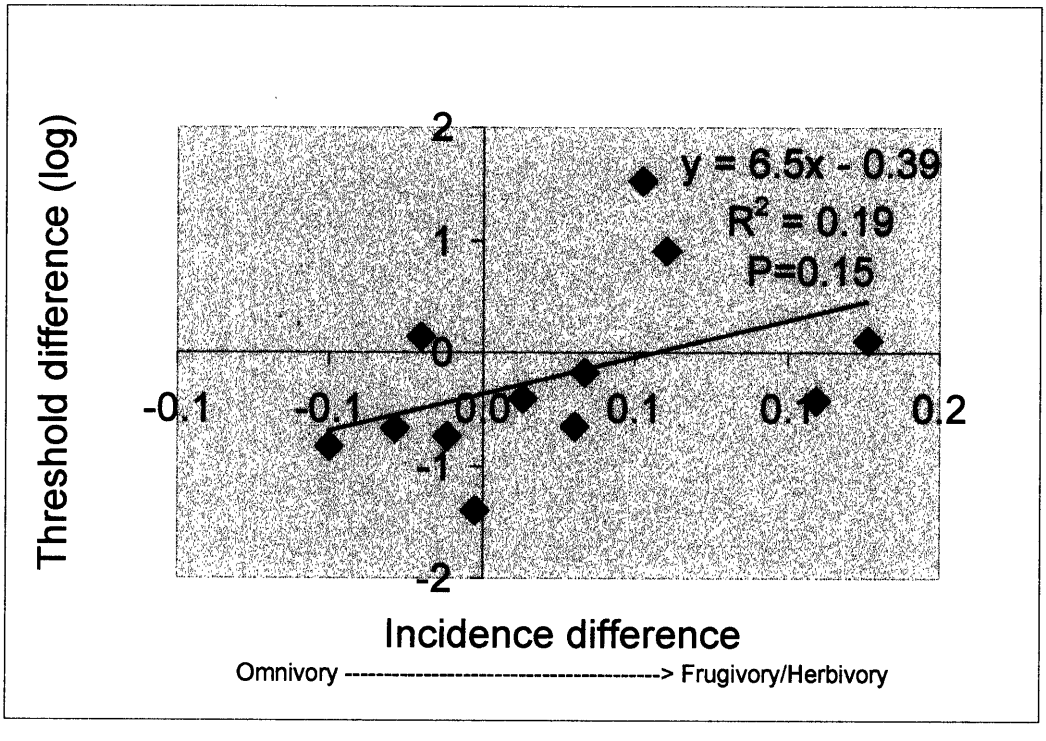


e

Fig. 6c,d,e: Incidence and threshold difference values of chiroptera and carnivore study species pairs, for all available comparison odorants. Seba's short-tailed bat (*Carollia perspicillata*), Mouse-eared bat (*Myotis myotis*) Vampire bat (*Desmodus rotundus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). Data from: Krestel *et al*, 1984; Laska, 1990; Moulton *et al*, 1960; Obst *et al*, 1976; Schmidt, 1975, this study

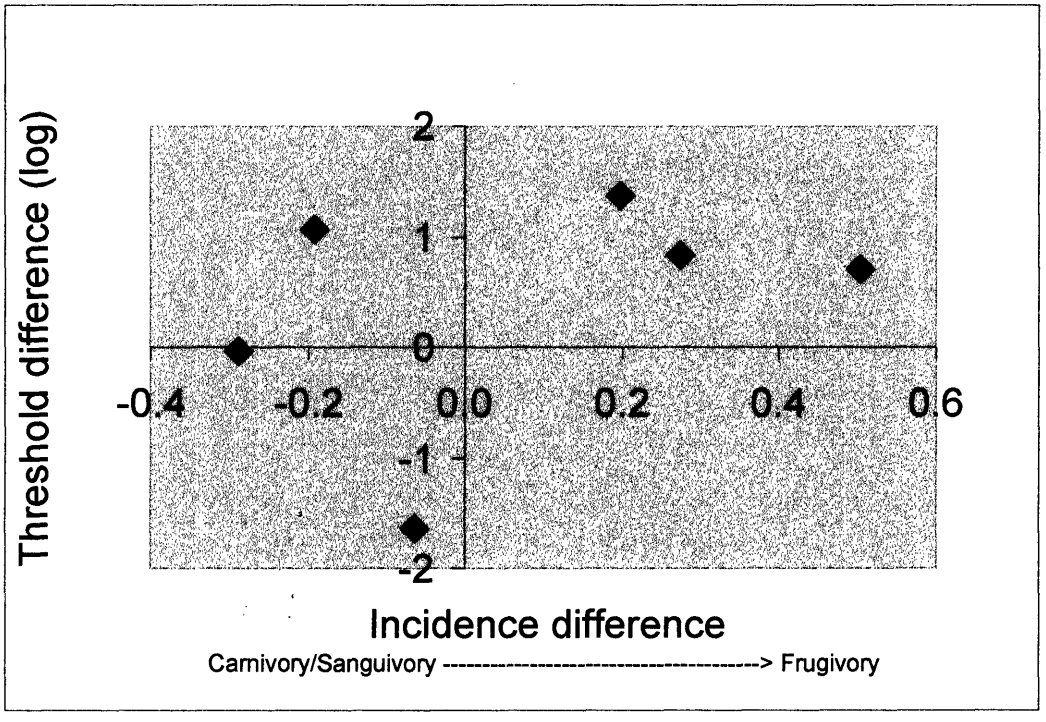


a



b

Fig. 7a,b: Threshold vs. Incidence contrast regressions. a) Squirrel Monkey - Human (*Saimiri sciureus-Homo sapiens*) contrast, b) Macaque- Human (*Macaca nemestrina-Homo sapiens*) contrast



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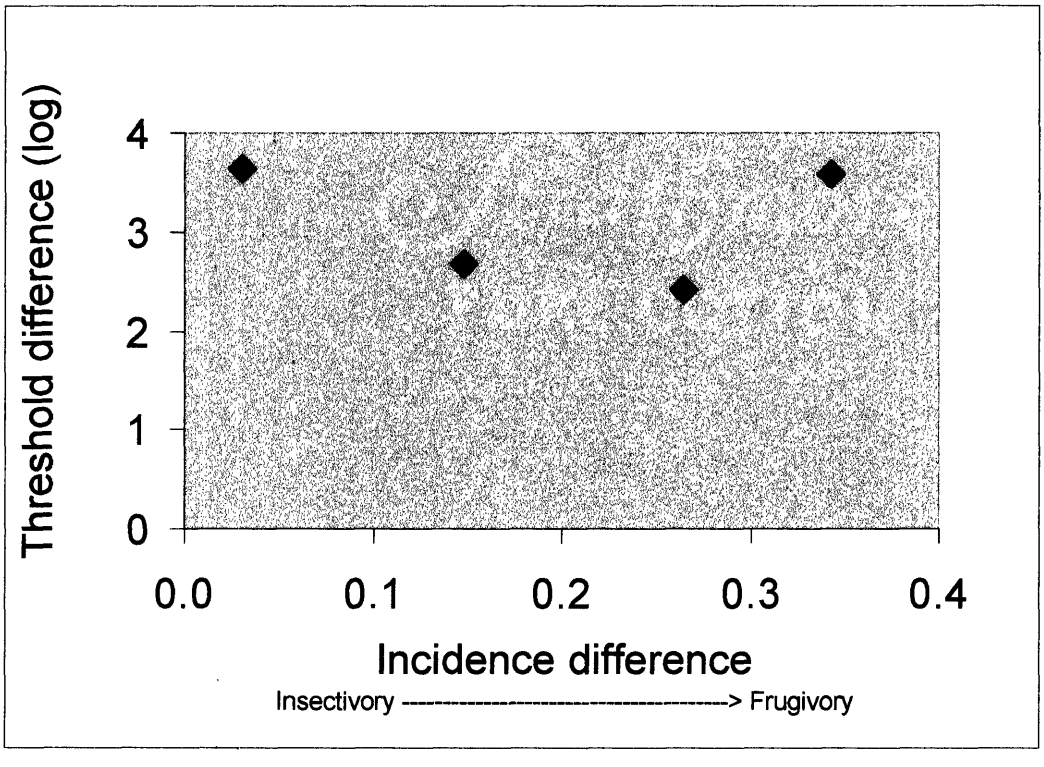

d

Fig. 7c,d: Threshold vs. Incidence contrast regressions. c) Seba's short-tailed bat- Vampire bat (*Carollia perspicillata-Desmodus rotundus*) contrast, d) Seba's short-tailed bat- Mouse-eared bat (*Carollia perspicillata-Myotis myotis*) contrast

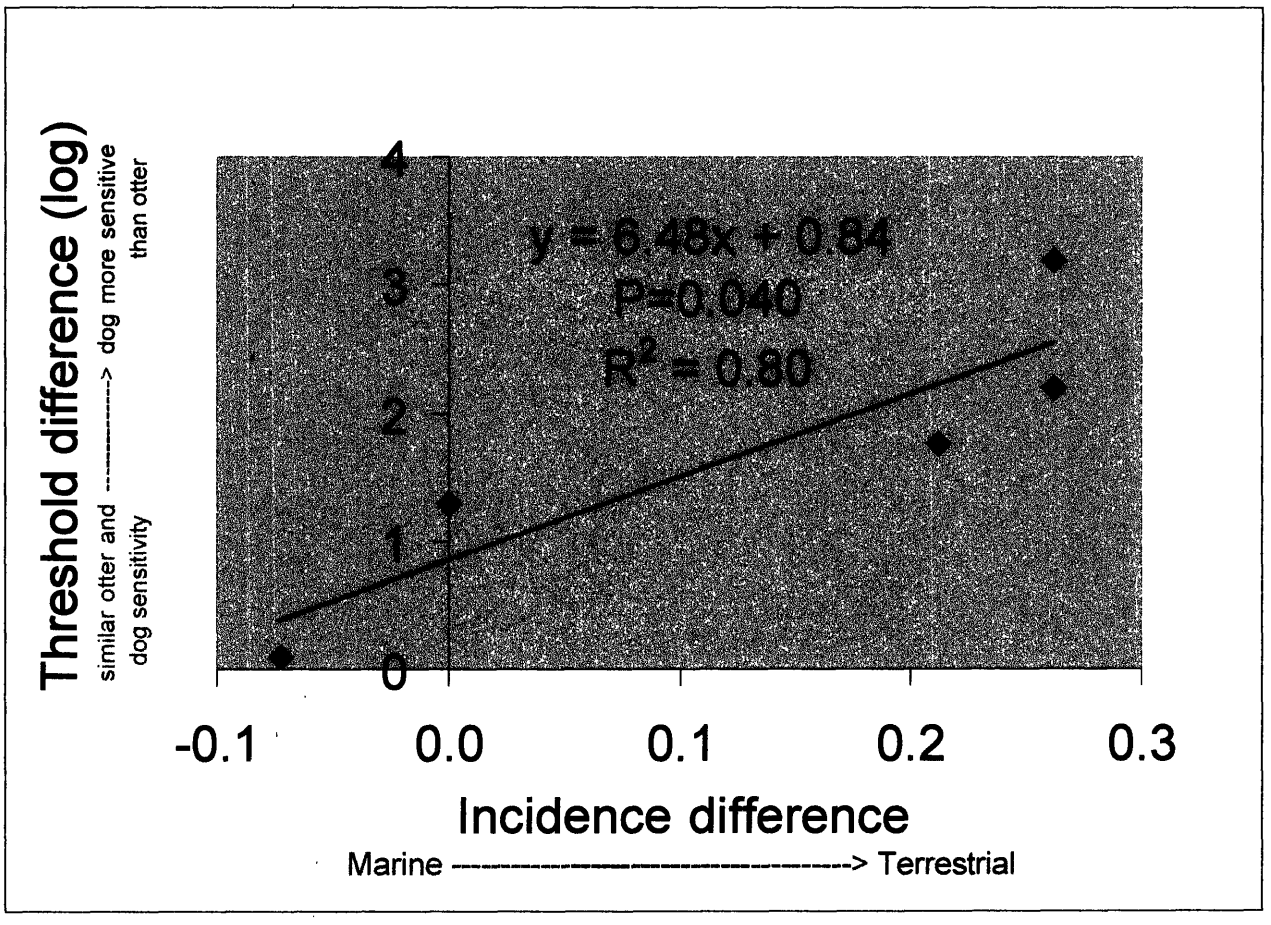

Fig. 7e: Threshold vs. Incidence contrast regression, Domestic dog- Sea otter (*Canis familiaris-Enhydra lutris*) contrast

Chapter 4: Nasal cavity structure and general olfactory sensitivity

Abstract

Absolute olfactory sensitivity (ability to detect very low concentrations of an odorant) is a highly variable trait among mammals, ranging over several orders of magnitude for a single odorant among the limited number of species that have undergone olfactory sensitivity testing. However, it is unknown what biological mechanism causes this variation. A morphometric proxy of odorant uptake in the olfactory region, Olfactory Uptake Efficiency (OUE) was tested against behaviorally measured olfactory sensitivity in twelve species of mammals. Nasal cavities were imaged by computer tomography (CT) and conventional histological methods. Surface areas and lumen volumes in the olfactory region and in the whole nasal cavity were then measured from digitized images. An airflow distribution and mass-transfer model was used to estimate the proportion of inhaled odorant molecules delivered to the olfactory epithelium (OUE) for each mammal species. Model output was tested against known physical and chemical trends in nasal uptake and olfaction, and OUE values were compared with averaged olfactory threshold values and relative olfactory bulb volumes across all species. Model predictions were consistent with several empirically observed phenomena in olfaction. Independent contrasts analysis showed that OUE is significantly related to relative olfactory bulb volume ($P=0.02$), and possibly to behaviorally measured average olfactory threshold (AT) ($P=0.10$). Results strongly suggest that nasal morphometry plays an important role in olfaction, although sensitivity comparisons among species remain problematic because of the inherent difficulty of accurately measuring thresholds and the variation in experimental protocols in the published threshold literature.

Introduction

Olfaction is fundamentally a chemical sampling process that is subject to sampling efficiency which must be related to the design of the sampling apparatus; i.e., the nose. The olfactory epithelium in the nasal cavity is responsible for the translation of chemical input into a neural electrical signal. It is likely that absolute olfactory sensitivity is closely related to the probability of the olfactory epithelium intercepting an inhaled odorant molecule and thus to nasal cavity size and geometry. The model described herein utilizes several measures of the nasal cavity to estimate olfactory uptake efficiency (OUE), defined as the ratio of molecules that make physical contact with the olfactory tissue to molecules that were inhaled. In addition, an Average Threshold (AT) will be calculated for each species based on widely tested odorant thresholds, in order to test the following hypothesis:

A calculated olfactory uptake efficiency index based on morphological measures will be significantly related to general olfactory sensitivity in mammals.

Background

Olfactory Morphology

The most peripheral olfactory neurons in the Mammalia are found in the olfactory epithelium in the upper region of the nasal cavity (Fig. 1). The olfactory epithelium is generally coextensive with the ethmoid bone, which in the rear nasal cavity comprises the cribriform plate, the nasal septum and the ethmoturbinal labyrinth (Greene, 1935).

The olfactory epithelium has a complex, multilayered structure (Fig. 2). At its base is the lamina propria, a vascular layer which supplies blood circulation to the sensory tissue above. Above the lamina propria are the basal cells, a mitotically active cell population from which new primary olfactory neurons are continuously generated. Olfactory neurons

deteriorate over time and are constantly replaced, a very rare process in the vertebrate nervous system. Above the basal cells are several layers of developing and mature olfactory neurons interspersed with occasional secretory cells. The number of layers of neurons varies among species. Olfactory neurons are bipolar with a single dendrite which extends from the cell body to the top of the epithelium, and ends in a terminal knob bearing 8-20 nonmotile cilia which are suspended in the olfactory mucus. The dendrites and cilia are supported by a layer of sustentacular cells overlying the neurons. The axons of the olfactory neurons project down through the lamina propria, where they form nerve bundles that thread through the perforations in the cribriform plate to the olfactory bulb, a paired organ located directly behind the plate (Gittleman, 1991). The outer layer of the olfactory bulb is the site of the first synapse in the olfactory system. There, the receptor neurons contact the dendrites of mitral cells, forming glomerular bundles. In mammals, in a single glomerulus about 25 mitral or tufted cells will synapse with tens of thousands of receptor axons. At deeper levels, granule cells and several categories of juxtaglomerular cells synapse with the mitral and tufted cells, allowing communication within and among glomeruli. These cells mediate lateral inhibition whereby more strongly activated mitral and tufted cells inhibit less strongly activated cells, and on a larger scale, strongly activated glomeruli inhibit weakly activated glomeruli. (Aungst *et al*, 2003, Mori *et al*, 1999). It is believed that this lateral inhibition plays an important role in enhancing and sharpening the spatial activation map in the bulb (Aungst *et al*, 2003, Yokoi *et al*, 1995). The axons of the mitral and granule cells form the lateral olfactory tract which projects to several regions of the brain, including the limbic system and the frontal cortex (Allison, 1953).

Olfactory Physics

The path traveled by inhaled air through the nasal cavity is very complicated and varies significantly with time over a single respiration cycle. Nasal flow varies among

species and individuals and also depending upon physiological state. Breathing rate, for example, increases during physical exercise and alters the flow patterns in the nose. Conscious behaviors of the animal also affect nasal air flow. It has been shown in several primates and Brown rat (*Rattus norvegicus*) that during normal breathing very little air passes through the olfactory region (Fig. 3a-c) (Kepler *et al*, 1998, Kimbell *et al*, 1993, Kimbell *et al*, 1997a, Morgan *et al*, 1991, Patra *et al*, 1986) but that during active sniffing (higher total flow rates) a greater proportion of inhaled air is diverted to the olfactory region (Chang, 1980, DeVries and Stuiver, 1961, Kimbell *et al*, 1997a).

A study of nasal flow rates in a dog (*Canis familiaris*) during an olfactory task (hunting) has revealed at least two strategies for olfactory detection: 1) While searching for a trail, running nose up, the subject maintained a constant slow, inward stream of air through the nose for 40 seconds, (concurrent with 30 cycles of mouth-breathing). While sniffing the ground, the subject sniffed (nasal inhale-exhale) at a frequency of 140-210 cycles/min (Steen *et al*, 1996).

Studies in humans have attempted to unravel how sniffing might be useful during olfaction. Schneider *et al* (1966) measured detection thresholds at different combinations of flow rate and sniff duration. They concluded that detection occurs when a critical number of molecules reach the olfactory epithelium within a given time window. In Schneider's study, the absolute number of odorant molecules inhaled in a 0.50 second period was the critical determinant of detection. A more prolonged pulse of lower

concentration resulting in the same total number of molecules inhaled failed to elicit a response. (Fig.4). However, within this window, sniffing faster for shorter periods eventually yielded poorer sensitivity. Moving from a 0.50 second sniff at 40ml/second to a 0.25 second sniff at 80 ml/second doubled the olfactory threshold. This suggests that for a given odorant, there is an optimal flow rate which will deliver the most molecules within the time window without washing them through so quickly that they fail to encounter the epithelial surface.

Sobel *et al* (2000) examined thresholds of the left and right nostrils separately and concurrently. It is well known that airflow resistance is usually greater in one nostril than the other (Widdicombe *et al*, 1986). The nostrils alternate accommodating high and low flow rates in a process probably governed by the nasal vasculature and referred to as the nasal cycle (eg.: Eccles, 1978, Haight and Cole, 1984). Sobel *et al* (2000) compared thresholds in the high flow vs. low flow nostrils and concluded that detection could occur through the low-flow nostril at the same threshold as the high flow nostril when the subject sniffed longer to compensate for the lower flow rate. The authors suggested that two simultaneous flow rates optimize detection of different kinds of odorant. Odorants that partition quickly into the mucus, (high diffusivity in air or mucus, or high solubility in mucus) will be better detected in fast flow, during which they are transported farther over the olfactory surface and activate a larger number of neurons. Odorants that partition

slowly will be better detected at lower flow rates because they have more time to contact the mucus before passing into the trachea.

Olfactory enzymes and transport proteins

There is enzymatic activity both in the olfactory mucus and in the nasal mucus that coats the respiratory epithelium. The respiratory mucus contains immunoglobins and lysozymes as well as other antiviral and antibacterial agents and is certainly an important line of defense against bacterial invasion in the respiratory system (Drettner, 1979, Jones, 2001). Both mucosae also produce a wide variety of enzymes that transform organic compounds either for detoxification or possibly, in the case of the olfactory mucosa, for rapid removal of excess odorant to prevent extended stimulation. (Bogdanffy *et al*, 1987, Bogdanffy, 1990, Dahl, 1988, Dear *et al*, 1991, Lazard *et al*, 1990, Lazard *et al*, 1991, Zupko *et al*, 1991) Activity of most enzymes is several times higher in the olfactory epithelium, and many biotransformation enzymes have been found only in the olfactory region. There are two plausible adaptive reasons for this. First, rapid transformation of stimuli in the olfactory region is necessary in order to terminate the stimulus; this of course is not necessary in the respiratory region. Second, toxin metabolism may be less important in the respiratory mucus simply because it is secreted rapidly and continually transported by the action of the cilia to the eosophagus for disposal by the digestive system. Olfactory mucus is secreted much more slowly and the sensory cilia do not affect mucus transport, so enzymatic biotransformation is the most important removal process

(DeSesso, 1993). This may explain the specific toxicity of many nasal carcinogens to the olfactory tissue where they are transformed into their active forms by localized enzymatic activity (Bogdanffy *et al*, 1987).

It is interesting to note that based on our current understanding of the olfactory mucosa enzyme system, it is not clear whether the compound that binds to the olfactory receptor is the same compound that was inhaled. However, for the purposes of this model, this question is not relevant. As long as a known compound contacts the olfactory epithelium, and a stimulus results, the precise chemical pathway does not matter.

Olfactory Genetics and Sensitivity

The relationship between olfactory genes and overall olfactory sensitivity remains uncertain, but there is wide variation in genomic investment in the olfactory system. Issel-Tarver and Rine (1997) defined olfactory receptor gene subfamilies by Southern blot hybridization of dog genomic DNA. Genes that cross-hybridized were assigned to a common lineage. They found in studies of humans, several artio- and perissodactyls (round- and split-hooved ungulates) and carnivores, that the number of lineages in the olfactory receptor protein superfamily was probably fixed in the mammalian ancestral line 60-100 million years ago and differs little among mammalian species. However, local duplication has since increased OR gene numbers in some lineages more than others. The human olfactory genome has been censused at 906 genes (Glusman *et al*, 2001), the mouse (*Mus musculus*) estimated (extrapolated from ~93% identified genes) at 1510 (Young *et al*, 2002) and the dog at 1322 (extrapolated from 50%) (Quignon *et al*, 2003). Furthermore, the complete olfactory genome is never functional. A large proportion of human olfactory receptor protein genes are pseudogenes (52-70%, Quignon *et al*, 2003, Rouquier *et al*, 2000,

Young *et al*, 2002, Gilad *et al*, 2003, Glusman *et al*, 2001, Niimura and Nei, 2003). Pseudogene counts up to 20% were found in the mouse (Zhang and Firestein, 2002, Young *et al*, 2002, Rouquier *et al*, 2000) and 18% in the dog (Quignon *et al* 2003). Rouquier *et al* (2000) and Gilad *et al* (2003) found elevated pseudogene counts also in a variety of primates although not to the extent reported in humans. However, there are published sensitivity data for only three species with measured pseudogene content, and the relationship between functional genome size and sensitivity must await further comparative psychophysical and genetic data.

Previous Anatomical Models of Olfactory Sensitivity

It is often suggested that olfactory sensitivity is related to the morphometry of the olfactory bulb, the first point of integration and potential amplification of transduced olfactory signals. A great deal of work has been done on comparative anatomy of the mammalian olfactory bulb. Published bulb dimensions are available for broad selections of the Carnivora, Primates, Insectivora, Chiroptera and also for mouse (Gittleman, 1991, Stephan *et al*, 1987, Williams *et al*, 2001, Hutcheon *et al*, 2002). However, a clear relationship between olfactory bulb dimensions and olfactory sensitivity has not yet been observed.

Bretting (1972) showed that olfactory bulb size need not correspond to olfactory acuity. Comparing the volume of the bulb relative to body mass in Insectivora, Bretting found it did not correlate with sensitivity as measured in behavioral studies. Sigmund and Sedlacek (1985), comparing neuroanatomy and sensitivity in shrews (*Sorex areneus*) and humans found very similar olfactory sensitivity despite the shrew's much larger olfactory bulb relative to brain volume. An independent contrasts analysis of olfactory bulb volume versus threshold using average threshold values and published neuroanatomical data implies but does not conclusively demonstrate a relationship (Fig. 5).

This is not surprising given the results of the visualization studies of Vassar *et al* (1994), Nagao *et al* (2000), and Ressler *et al* (1994) in mouse and rat. Attaching a molecular label to one olfactory receptor gene, they found that all of the labeled neurons expressing that gene project to a specific pair of glomeruli in the olfactory bulb. This specificity is further supported by the knock-in experiments of Mombaerts *et al* (1996) and Wang *et al* (1998). Substitution of the coding region of one olfactory receptor gene for the coding region of another will cause the axon of the altered neuron to project to the highly specific glomerular target of the substitute gene. It is by now generally accepted that all neurons expressing a given olfactory receptor type synapse in one lateral-medial pair of glomeruli in the olfactory bulb. If olfactory bulb structure is standardized in this way throughout the mammals, then bulb size must be at least partly constrained by the size of the functional olfactory receptor genome. Both bulb and genome size are no doubt related to olfactory distinction among the many thousands of odorants coded for by the genome. Olfactory epithelium dimensions are not similarly constrained, as the number of cells expressing the receptor type communicating with each glomerular pair is large and variable.

There is a great deal of published data on the histology and dimensions of the olfactory epithelium in mammals. It is reasonable to expect that density of olfactory neurons influences olfactory sensitivity. However, Sigmund and Sedlacek (1985) compared the shrew, hedgehog, and fox terrier and found that the dog had the highest sensitivity and the lowest neuronal density while the shrew had the lowest sensitivity and the highest neuronal density.

Leopold (1988) found among hyposmic humans that two morphometric variables, the volumes of two peri-olfactory regions of the nasal cavity, accounted for 58% of the variation in olfactory performance in clinical tests. The influential regions of the nasal cavity were the region just anterior to the olfactory cleft and the region just below the cleft. Based on their proximity to olfactory region, Leopold suggested that changes in airflow access to

the olfactory epithelia were the critical factors. The mathematical model below tests Leopold's hypothesis on an inter-species basis.

Nasal Airflow Modeling

The functional variable postulated to be important for absolute olfactory sensitivity is olfactory uptake efficiency (OUE), which is equal to the fraction of all inhaled molecules that contact the olfactory region's mucus layer. This quantity can be expected to depend on the geometry of the nasal cavity, the properties of the odorant, and several physiological variables.

It is well known that chemicals are filtered out of inhaled air as it passes through the nasal cavity (Bogdanffy *et al*, 1987, Gerde and Dahl, 1991, Kepler *et al*, 1998, Kimbell *et al*, 1993, 1997b, Morris, 1997a,b, Morris *et al*, 1993, Thornton-Manning and Dahl, 1997). The efficiency of this process varies among chemicals and among nasal cavity types. Uptake efficiency for different chemicals can range from 0 to 100% in any mammal species. Inhaled odorants diffuse through the nasal airstream, dissolve into the mucus layer, and diffuse through it to the olfactory receptors below. Diffusion in the air phase depends on the diffusivity of the odorant in air, its concentration gradient toward the wall of the nasal cavity, and the temperature and fluid dynamics of the airstream. Dissolution rate into the mucus will depend on the solubility of the odorant as well as temperature. Diffusion through the mucus layer depends on the diffusivity of the odorant in mucus and the steepness of the concentration gradient, and may be facilitated by

specialized transport enzymes (Lobel *et al*, 2002, Tegoni *et al*, 2000). Enzymatic transformation and removal of odorants render the process even more complex.

Olfactory uptake efficiency is thus a complicated function of geometry and physical and chemical properties. Achieving a simple and yet reasonable model of nasal and olfactory uptake is a difficult task. A number of mathematical models have nonetheless been proposed. (Hahn *et al*, 1994; Keyhani *et al*, 1997; Lamine and Bouazra, 1997). All of these have been based upon the assumption that inhaled volatiles dissolve into the olfactory mucus and reach a steady state in which an odorant partitions into the mucus layer at the same rate as it is removed by metabolic and circulatory processes.

The simplest version of this is the assumption that after molecules diffuse completely across the mucus layer, they are immediately removed at the bottom. Such models have successfully predicted several phenomena in olfaction, including the fact that while some odorants are more easily detected at relatively fast sniff rates, others are more easily detected at slower sniff rates (Hahn *et al*, 1994), which is in turn consistent with the proposal by Sobel *et al* (2000) described above. Therefore, this is the removal paradigm assumed in the model below. This is in some ways a crude approximation, but the variety of fates of the myriad of odorants entering the olfactory mucus are not sufficiently well described to warrant a more detailed approach for a model intended to describe the behaviour of any odorant. It must be pointed out however that several trends

in empirically obtained nasal uptake data contradict the predictions of a steady state, 'zero concentration at the bottom' model.

If dissolution of odorants into the mucus layer is governed by steady-state thermodynamics, then the equilibrium solubility of the odorant and its diffusivities in air and mucus should determine the differences in nasal uptake efficiency between different chemicals. Uptake was modeled quantitatively by Keyhani *et al* (1997) as a function of several physiochemical properties of odorant chemicals based on the steady-state assumption. However, for a small number of chemicals, uptake efficiency has been determined experimentally in the human nasal cavity (Landahl *et al*, 1950, as reported in Morgan and Monticello, 1990), and these data are not entirely consistent with the model's predictions (Fig. 6).

In addition, the steady state models predict that nasal uptake efficiency is independent of inhaled odorant concentration. It has been shown in several rodents that at high inhaled concentrations uptake efficiency decreases as inhaled concentration increases (Fig. 7) (Bogdanffy *et al*, 1998; Lang *et al*, 1996; Morris, 1997a, 1999).

The steady state assumption by definition implies no variation with time. Uptake efficiency measurements of nitrous oxide in human and vinyl acetate in rat nasal cavities reveal that uptake efficiency decreases significantly over the first 3-10 minutes of continuous exposure (Fig. 8) (Bogdanffy *et al*, 1998; Kelley and Dubois, 1998), a period many times longer than the time scale of olfactory stimulation.

These three points can all be explained by the influence of enzymatic biotransformation on uptake rate. First, solubility and diffusivity in mucus will not be good predictors of uptake if enzymatic processes in the mucus or the epithelium are more important than passive diffusion and removal. The latter two points were demonstrated in wide concentration ranges including relatively high inhaled concentrations. Decreasing efficiency at higher odorant concentrations almost certainly represents the saturation of the olfactory enzyme system. Morris *et al* (1991) found that three structurally similar esters and likely substrates of the same carboxylesterase enzymes, introduced to the nasal cavity simultaneously, were taken up with significantly lower efficiency than when individually introduced, probably as a result of competitive inhibition.

However, for Cytochrome P450 and several esterases in the olfactory mucus, inhaled concentrations of substrate required to saturate the enzyme systems are 1-5 orders of magnitude higher than typical olfactory threshold values (Dahl, 1988). Therefore, it is reasonable to assume that rates of odorant processing and uptake are not enzyme concentration limited at or near olfactory threshold. Decreasing efficiency with time may represent the introduction of new rate-limiting steps later in the removal process, either in the metabolic pathway or in the eventual removal by the circulatory system.

Proposed Model: Olfactory uptake efficiency at olfactory threshold

OUE, defined as the ratio of molecules contacting the olfactory epithelium to molecules inhaled, was estimated as the product of two factors: Q_{up}/Q_{tot}, the ratio of air passing through the olfactory region to air inhaled, and LUE_{olf}, the ratio of molecules contacting the olfactory mucus to molecules passing through the olfactory region.

Resistance and regional delivery

The nasal cavity in most mammals is divided into two geometrically distinct regions. A saggittal view of this division is shown in Fig. 1a, in the Pale spear-nosed bat. The lower nasal passage has a large hydraulic width, (being a single open compartment for up to half of its length). It is through this region that most nasal airflow passes. The upper passages, made up of the maxilloturbinal and ethmoturbinal labyrinths, contains the olfactory region, and is much more convoluted, with a smaller hydraulic width and correspondingly higher airflow resistance. The cross-sectional area of the upper passages varies significantly along the length of the nasal cavity (Fig. 1b,c). In the posterior 25-50% of the nasal cavity, the upper and lower passages are physically separated. In this study, where this separation became incomplete, a substitute landmark was assigned to represent the boundary. If present, the local minimium distance from the lateral wall to the septum, nearest the boundary as defined in the previous section was defined as the new boundary. If no such local minimum was present, the nearest local minimum distance between the two lateral walls of the cavity was used. If neither local minimum was available, the boundary was drawn between the nearest inflection point on either lateral

wall of the cavity (Fig. 1b,c). This division continued in the anterior direction until no suitable landmark was present; this occurred in the nasal vestibule in all specimens, approximately 10% of the nasal cavity length from the anterior tip of the rostrum. The length of the boundary if drawn in was excluded from the region perimeters in the calculation of perimeter.

At its broadest extent, the olfactory region occupies >95% of the perimeter of the upper cavity as defined above. In order to calculate the proportion of air passing through the upper region and hence the olfactory region, the nasal cavity was modeled as two parallel air flows, separating in the nasal vestibule and rejoining at the posterior end of the cavity. The cross-section of each flow was assumed to be an elliptical slit or bank of slits with maximum length ~2.5 x maximum width (Fig. 9). In order to calculate the resistance of each flow, the measured cross-sectional area and hydraulic slit width of the upper and lower nasal cavity regions were applied to this model geometry.

If two parallel nasal passages sharing a laminar flow have different hydraulic widths, and therefore different flow resistances, the air flow will be divided between them according to Poiseuille's Law. The force required to push fluid through a passage depends on the passage's cross-sectional area, the viscosity of the fluid, the mean flow velocity and the surface area of the passage wall which causes the drag. Assuming the length of and pressure drop along both passages are equal, and that the viscosity of the air is the same for both (as it would be for air at the same temperature), then the proportional flow velocities between the two sections would depend only on their cross-sectional areas and hydraulic widths, as outlined below. The following abbreviations will be employed:

w= width (m)

A= cross-sectional area (m^2)

p= perimeter (m)

η= kinematic viscosity of air (kg/(m·sec))

$\overline{V}$ = mean flow velocity (m/sec) $\approx$ 0.003 * (body mass$^{0.75}$(g)) (Kleiber and Rogers, 1961)

P= pressure difference (N/m^2, $kg/m \cdot sec^2$)

z= length (m)

Q = volumetric flow rate (m^3/sec)

Treating the passage cross-sections as straight rectangular slits with cross-section unvarying in the z direction, the effective or hydraulic width of the slit is defined as

$$w=2A/p \qquad \text{Eq. 1}$$

According to Poiseuille's law for laminar flow through a rectangular slit:

$$\overline{V} = \frac{Pw^2}{12\eta z} \qquad \text{Eq. 2}$$

Rearranging this expression allows the comparison of flow between two parallel slits of equal length, connected at either end and conducting the same fluid. If the slits are connected at either end, the pressure differences are equal and an expression for P in the

'up' slit will equal the same expression for P in the 'down' slit. Equal slit lengths and fluid viscosities also cancel out and a sumple flow distribution based only upon the slit hydraulic diameters results.

$$P = \frac{12\eta \overline{V} z}{w^2} \quad \text{Eq. 3}$$

$$P_{up} = P_{down} \quad \text{Eq. 4}$$

$$\eta_{up} = \eta_{down} \quad \text{Eq. 5}$$

$$z_{up} = z_{down} \quad \text{Eq. 6}$$

$$\frac{\overline{V}_{up}}{w_{up}^2} = \frac{\overline{V}_{down}}{w_{down}^2} \quad \text{Eq. 7}$$

$$\overline{V}_{down} = \frac{\overline{V}_{up} w_{down}^2}{w_{up}^2} \quad \text{Eq. 8}$$

Therefore, volumetric flow is distributed between the two regions thus:

$$Q = A\overline{V} \quad \text{Eq. 9}$$

$$Q_{up} + Q_{down} = Q_{tot} \quad \text{Eq. 10}$$

$$\frac{Q_{up}}{Q_{tot}} = \frac{\overline{V}_{up} A_{up}}{\overline{V}_{up} A_{up} + \overline{V}_{down} A_{down}} = \frac{\overline{V}_{up} A_{up}}{\overline{V}_{up} A_{up} + \frac{V_{up} w_{down}^2}{w_{up}^2} A_{down}} = \frac{A_{up}}{A_{up} + \frac{w_{down}^2 A_{down}}{w_{up}^2}} \quad \text{Eq. 11}$$

substituting in Equation 1:

$$\frac{Q_{up}}{Q_{tot}} = \frac{A_{up}}{A_{up} + \frac{A_{down} w_{down}^2}{w_{up}^2}} \quad \text{Eq. 12}$$

For this model, the values A and p were measured in the upper and lower nasal passages in each histological section (every 200μm in most specimens, beginning ~10% from the anterior end of the nasal cavity). The mean value for all measured sections was calculated for each variable and these values, A_{up}, A_{down}, p_{up} and p_{down}, inserted into Equation 12 to determine Q_{up}/Q_{tot} for the specimen.

Local Uptake Efficiency: uptake in laminar flow through a mucus-lined slit

In the upper nasal passage, odorant uptake in the respiratory region is neglected as discussed above. In each histological section containing olfactory tissue, modeled uptake is calculated as outlined below by approximating the olfactory mucus layer as a permeable wall through which odorant molecules are transported by passive diffusion, and immediately removed at the outside surface (the bottom of the mucus layer).

The shape of the nasal passage cross section is again approximated as a slit with width w defined as in Equation 1. The following abbreviations will be employed:

t=time

$\overline{C}$=cross-sectional mean concentration in air

C_{muc}=concentration in mucus

C_0=concentration in air at the air/mucus interface

C_w=concentration in mucus at the air/mucus interface

β=Henry's Law constant

x=variable depth measured from the air-mucus interface

H=total mucus layer height=0.0006cm

D_{air}=diffusivity in air

D_{muc}=diffusivity in mucus

G=transfer rate (mol/cm^{2}s)

h=mass transfer coefficient (cm/s)

Sh=Sherwood number (dimensionless constant reflecting duct cross-sectional shape)

The transfer rate G of molecules out through the permeable wall of a duct or nasal passage of arbitrary cross-section, when the concentration at the interior surface of the wall is constant, is

$$G = h(C_0 - \overline{C}) \qquad \text{Eq. 13}$$

where $(C_0 - \overline{C})$ is the difference between the concentration at the wall and the bulk mean concentration in the nasal passage, and h is the transfer coefficient which accounts for passage dimensions and fluid properties as follows:

$$h = Sh\ D_{air}/w \qquad \text{Eq. 14}$$

Here, Sh is the Sherwood number, a dimensionless constant which depends upon the shape of the passage cross-section, approximately 4.0 in the case of a slit 2-3 times longer than it is wide (Perry, 1963). D_a is the diffusivity of the odorant in air, and w is the width of the slit.

In the nasal cavity, the wall is the air-mucus interface, and the concentration at the wall, C_0 will be related to the solubility of the odorant in the mucus and the diffusion rate

through the mucus layer. Solubility is described by Henry's law, so assuming odorant solubility in mucus ≅ solubility in water, the mucus concentration of a given odorant in very close proximity to the interface will be a fixed proportion of the air concentration, with the proportion determined by Henry's Law:

$$C_0 = \beta C_w \qquad \text{Eq. 15}$$

Where _, the Henry's Law Constant, is empirically measured for a given compound at a given temperature.

Assuming passive diffusion of the odorant across the mucus layer and then immediate removal at the bottom, (a gross approximation of the actual removal processes which will be discussed below), the mucus concentration will decrease linearly from the air-mucus interface to the bottom of the layer, where the mucus meets the epithelial cells.

$$C_{muc} = C_w(1 - \tfrac{x}{H}) \qquad \text{Eq. 16}$$

Differentiating this expression with respect to x yields

$$\frac{dC_{muc}}{dx} = -\frac{C_w}{H} \qquad \text{Eq. 17}$$

Assuming steady state uptake at the air-mucus interface, the odorant flux in air must equal the flux in the mucus. The first variable is known from the transfer rate G. The

second is known from Fick's law of diffusion: diffusive flux = diffusivity x concentration gradient. Equating the two fluxes yields

$$D_{muc}\frac{dC_{muc}}{dx}|_{x=0} = \frac{ShD_{air}}{w}(C_0 - \overline{C}) \qquad \text{Eq. 18}$$

Substituting Equations 15 and 17 into Equation 18 yields an expression based on bulk mean concentration, the geometry of the nasal passage and the diffusive properties of the odorant:

$$\frac{D_{muc}C_0}{H\beta} = \frac{ShD_{air}(\overline{C} - C_0)}{w} \qquad \text{Eq. 19}$$

Solving Equation 19 for wall concentration C_0:

$$C_0 = \frac{\overline{C}}{1 + \frac{wD_{muc}}{\beta ShHD_{air}}} \qquad \text{Eq. 20}$$

Substituting Eq. 20 into Eq. 13 yields an expression for transfer rate based upon these same properties.

$$G = \frac{ShD_{air}\overline{C}}{w}(1 - \frac{1}{1 + \frac{wD_{muc}}{\beta ShHD_{air}}}) \qquad \text{Eq. 21}$$

This transfer rate is integrated over the wall surface area and residence time. This total odorant flux is divided by the calculation volume to yield the loss in concentration over a

given length of nasal passage. In this calculation, wall surface area is the product of the perimeter measured in the section and the intersection spacing, volume is the product of area measured in the section and the inter-section spacing, and residence time in the calculation volume is the intersection spacing divided by the mean flow speed.

$$\Delta C = (G \times \frac{z}{\overline{V}} \times pz)/(Az) = \frac{pzShD_{air}\overline{C}}{A\overline{V}w}(1 - \frac{1}{1 + \frac{wD_{muc}}{\beta ShHD_{air}}}) \qquad \text{Eq. 22}$$

Since the only permeable surface being considered is the olfactory mucus, the perimeter in this expression is the length of *olfactory* tissue in the cross-section, but in substituting for w using Equation 1, the total perimeter of the upper nasal passage is used:

$$\Delta C = \frac{p_{olf}p_{up}zShD_{air}\overline{C}}{2A^2\overline{V}}(1 - \frac{1}{1 + \frac{2A_{up}D_{muc}}{p_{up}\beta ShHD_{air}}}) \qquad \text{Eq. 23}$$

For any region of nasal passage, Local Uptake Efficiency (LUE) is equal to the number of molecules retained divided by the number that entered, the concentration lost divided by the original concentration, or:

$$LUE = 1 - \frac{\overline{C_{final}}}{C_{initial}} \qquad \text{Eq. 24}$$

In this study, this calculation was iterated at short intervals along the nasal passage length- one iteration per histologic section, every 200μm for most specimens. _C was

calculated for each section using perimeter and area values measured on the section and z=the length of the inter-section spacing. This concentration difference was subtracted from the initial C, and the calculation was repeated using the new value of C and the next histologic section. This process was repeated until the posterior end of the nasal cavity; thus, variation in morphometric values and changes in concentration were accounted for at a resolution of 200μm. LUE_{olf} was calculated relative to the unknown initial bulk mean concentration, in the most anterior section containing olfactory epithelium

Olfactory Uptake Efficiency

Assuming that initial concentration of odorant is the same in the upper and lower nasal cavity, then OUE is simply the product of the total Local Uptake Efficiency in the olfactory region and the proportion of inhaled air passing through the upper nasal cavity region.

$$OUE = LUE_{olf} \times \frac{Q_{up}}{Q_{tot}} \qquad \text{Eq. 25}$$

In this calculation there are several important simplifications and assumptions. First, it is assumed that temperature in the nasal cavity does not vary significantly among species. D_{air}, the diffusivity of a gas in air, varies with temperature. Temperature in the olfactory region in almost all cases approaches internal body temperature very closely (Schmidt-Nielson, 1999) and so will vary relatively little for mammals. Mammalian core

body temperatures lie usually between 36 and 40 °C, or 309 and 313 °K (Schmidt-Nielsen, 1997, Morrison and Ryser, 1952). Temperature in the respiratory region will grade from environmental temperature to body temperature.

The sensitivity data available for all species studied thus far were obtained under controlled laboratory conditions with ambient temperatures from 20-25 °C, or 293-298 °K. In humans, probably the least efficient mammalian nasal heat-exchanger, the temperature profile in the nasal cavity approaches core body temperature logarithmically from ambient temperature, with most of the temperature change occurring in the vestibule and valve area (Keck *et al*, 2000, Lindemann *et al*, 2004). In the most extreme case, ambient and core temperatures may vary among species by as much as 5°K. This difference will impact uptake rate in the nasal cavity. The effect of temperature change on diffusivity is described by:

$$D \alpha T^{3/2}$$

T=temperature (°K) (Wilke and Lee, 1955, as cited in Perry, 1963).

Therefore, a change in T of 5°K, in the range of 300 °K, or 1.66%, will have only a small effect on the diffusion rate, not exceeding 3%.

The second assumption is that the flow speed of inhaled air, (cm/s), is constant during sniffing, approximated as double the resting inhalation rate. In fact, linear flow rate

during active sniffing is under conscious control of the animal and can be highly variable, as noted above. Flow rate in each nostril is also subject to a nasal cycle (see above, Olfactory Physics). However, attempting to accurately represent such flow rate variation is beyond the scope of the present research effort. Resting inhalation rate was calculated from lung tidal volume which was estimated from body mass using Kleiber's law and the medians of body mass ranges reported in Walker's Mammals of the World (Kleiber and Rogers, 1961, Nowak, 1997).

Third, it is assumed that uptake is approximately zero in the respiratory mucosa, because odorant enzymatic biotransformation in this region is unimportant. Initial uptake will be significant as a new compound dissolves in the respiratory mucus. However, once mucus concentration rises and steady state is reached, removal will be limited primarily by enzymatic transformation. In fact, it has been demonstrated (Bogdanffy *et al*, 1987, Bogdanffy *et al*, 1990) that some toxic compounds such as formaldehyde and acetaldehyde are rapidly taken up and metabolized in the respiratory region. However, the enzymatic suites of the olfactory and respiratory regions are clearly distinct. There is abundant evidence for lower and less diverse enzymatic activity in the respiratory region. In addition, while toxin uptake is vital in both regions, odorant uptake would not be useful in the respiratory region. Therefore, respiratory region enzymatic biotransformation and, consequently, uptake by respiratory mucosa were neglected. This includes respiratory tissue in the upper nasal cavity region, where only olfactory tissue was treated as an absorbing surface.

Fourth, it is assumed that uptake in the olfactory region is instantaneous at the bottom of the mucus layer; i.e., that odorant molecules diffuse passively from the mucus-air interface to the mucus-epithelium interface and are instantly removed. This is an approximation for the great variety of removal processes taking place in the mucus and the epithelium in this region. The approximate 6 μm depth of olfactory mucus is threaded with nonmotile olfactory cilia (Menco, 1989, Menco *et al*, 1978, Reese and Brightman, 1970). Therefore, uptake by transmembrane cellular processes, as well as binding with olfactory receptors, could potentially take place at any depth and after any diffusion distance. In addition, secreted enzymes could effect biotransformation anywhere in the mucus. The modeled linear concentration profile decreasing to zero at the bottom of the mucus layer is a very rough approximation of these processes.

Fifth, it is assumed that inhaled air passes through the nasal cavity, and the olfactory region, directly from front to back. This is close to the real case, as demonstrated in the rat (Fig. 10) (Kimbell *et al*, 1993, 1997a). In the posterior olfactory region, airflow must double back and briefly flow in the anterior direction in order to reach the exit to the larynx. Therefore, in the real case the flow trajectory in this region is longer than in the calculated case, and because of the increased resistance of this route, some of the airflow is likely diverted into the lower cavity before it reaches the back of the upper cavity. Since the first mentioned airflow has increased residence time (and increased uptake) in the olfactory region, and the latter has decreased residence time and uptake, it

is difficult to say whether this simplification overestimates or underestimates uptake. However, since the region involved is fairly small, this will have only a slight effect on total olfactory uptake.

Sixth, the application of Poiseuille's law for calculating the division of flow between the upper and lower cavity assumes that the nasal passages are slits of uniform cross-section, which is not the case. Any linear error in this calculation that is systemic over the whole nasal cavity will have no effect on the ratio Q_{up}/Q_{tot}, so species in which the upper and lower cavity do not differ greatly in shape (the two primates, for example; see Fig. 20) are unlikely to be significantly affected. However, in cases where the shape or degree of longitudinal variability differs importantly between the upper and lower nasal cavity (this is true to varying degrees in the other specimens), differential error between the two regions will have an unknown effect on Q_{up}/Q_{tot}.

Seventh, fully developed parabolic laminar flow is assumed for the calculation of LUE. This assumption is reasonable in most but not all cases. Using the entrance length calculation of Bejan and Kraus (2003)

$$L=0.01w(Re)$$

where w=hydraulic width, Re=Reynolds number, and L is the length of the duct or nasal cavity after which the flow profile is fully developed, the flow profile in the human nasal cavity is expected to be fully developed after approximately 14 mm or 14% of its length. The human nasal cavity has by far the largest Reynolds number in the

dataset, approximately 500 at physiological flow rates. Therefore relative entrance lengths in other species will be even shorter and entrance region effects are not expected to be important.

Finally, a fully developed concentration profile is assumed in the olfactory region. Given the assumption of negligible uptake in the respiratory region, the concentration profile at the anterior end of the olfactory region must be flat. This is the point at which the concentration profile begins to develop. The point at which the concentration profile is fully developed varies among odorant compounds. The Schmidt number, or the ratio of kinematic viscosity of air to odorant diffusivity, determines how rapidly the concentration profile develops. A Schmidt number of 1 indicates that both profiles develop equally fast. Most volatiles have diffusivity values in air between 0.01 cm^2/sec and 1 cm^2/sec and corresponding Schmidt numbers between 0.17 and 17. For the odorants with diffusivities less than 0.1 cm^2/sec, the concentration profile will develop at least as fast as the velocity profile did, and only the very lowest diffusivity odorants will develop their concentration profiles significantly more slowly. Therefore, for nonhuman nasal cavities in which the velocity profile forms quickly, the concentration profile in the olfactory region will also form quickly for nearly all odorants and the fully developed concentration profile will be a reasonable assumption. For the human nasal cavity, the profile will take between 2mm and 20 cm to develop, depending on odorant diffusivity, so for many odorants the assumption will be reasonable, but for the lower diffusivity

odorants significant portions or the whole olfactory region will be a region of developing concentration boundary layers. In these cases the concentration in the middle of the air passages will be more uniform, the concentration gradient near the walls will therefore be steeper, and uptake will be higher than predicted. The diffusivity used to calculate OUE for comparison with olfactory sensitivity, 0.075 cm^2/sec, was selected to be representative of the odorants whose thresholds were used in the sensitivity comparison. For this diffusivity, the concentration profile in the olfactory region will develop in between 0.5 - 1 cm and the increased uptake in the developing region will not have an important effect on uptake.

All histological, morphometric and physiological characters implicated in potential model error: mucus chemistry, variability of inhalation rate, air temperature, and posterior division of the cavity, are similar amongst mammals. Therefore the error in the model results can be expected to be fairly uniform across species.

Methods

The following twelve species were measured: the House mouse (*Mus musculus*), the Brown rat (*Rattus norvegicus*), the Common European shrew (*Sorex araneus*), the Human (*Homo sapiens*), the Common squirrel monkey (*Saimiri sciureus*), the Vampire bat (*Desmodus rotundus*), Seba's short-tailed bat (*Carollia perspicillata*), the Mouse-eared bat (*Myotis myotis*), the Pale spear-nosed bat (*Phyllostomus discolor*), the Great fruit bat (*Artibeus literatus*), the Domestic dog (*Canis familiaris*), and the Sea otter (*Enhydra*

lutris). These species represent a wide range of habitat types as well as phylogenetic groups, allowing us to examine both variables.

Specimens were obtained from the American Museum of Natural History, the Whitehead Institute at the Massachusetts Institute of Technology, the Biology Department of MIT, the California Oiled Wildlife Network, the Harvard Museum of Comparative Zoology, the Institute for Hydrology and Ecology at Monk's Hood, Tufts Veterinary School, the New England Regional Primate Research Center, Lion Country Safari Zoo, and the Cameron Park Zoo.

Traditional studies of nasal anatomy have relied on light microscopic examination of serial sections. This technique, in conjunction with appropriate staining techniques, provides high-resolution histological data. However, it does not reflect the dimensions of undisturbed tissues. In order to obtain accurate morphometric measures as well as fine-level morphological detail, traditional light microscopy was combined with a nondisruptive imaging technique, computerized tomography (CT).

Radiologic techniques

CT imaging is based on measures of X-ray attenuation, which is closely related to tissue mineralization and density. Therefore it is most useful for distinguishing gradations of dense tissue and interfaces of bone with soft tissue or air. CT images have a pixel resolution of 100 microns, which is sufficient for comparison with conventional histologic sections. Consequently, CT data not only show undisturbed anatomical relationships but also provide measurements that can be directly compared with those from histologic examinations.

Nasal cavities were scanned using techniques established for both marine mammal and human cranial anatomy (Ketten, 1994, Ketten *et al*, 1998). Spiral and contiguous CT

scans were obtained in the transaxial plane, at 0.1 to 1 millimeter intervals. Scans of most specimens were obtained using a Siemens Volume Zoom CT unit in the WHOI CT facility. The house mouse, common European shrew, vampire bat and Seba's short-tailed bat specimens were scanned using an Siemens Emotion CT unit. Scan data and images were archived on magneto-optical disks. Transaxial and saggital section images were also archived as TIFF files as well as printed hard copies on radiologic film.

CT scans do not reveal fine detail or distinguish tissue types, but they accurately reflect the dimensions of undisturbed tissues. Measurements from the CT scans were compared with measurements of the identical feature (total nasal cavity length) from the histological sections in order to verify the latter and provide a correction factor if necessary.

Histology

Noses were sectioned for histology according to the method described for rats by Gross and colleagues (1982) with appropriate modifications for larger animals. Heads were skinned and the lower jaw removed. The nasal cavity was separated from the cranium immediately posterior to the cribriform plate. This operation was guided by landmarks obtained from the CT scans. The nasal cavity was decalcified in EDTA and embedded in celloidin. Sections were cut at 20 μm intervals in the transaxial plane. Every 10^{th} section was stained with haematoxylin and eosin, and mounted on a glass slide. In the two largest specimens, the dog and sea otter, section thicknesses varied from 20-36 μm

and every 100th and every 50th section, respectively, was stained and mounted. Epithelial lengths and lumen areas were obtained by light microscopy using an Olympus SZH10 stereomicroscope and an Olympus BX40 transmitted light microscope. Images of each section were acquired under magnification using an Hitachi CCD camera model KP- M1U and stored as TIFF files for measurement using Scion Image™.

Morphometry calculations

Olfactory and respiratory epithelia were distinguished by the following characteristics: differential staining in cell bodies, nuclei, and cilia, texture of cilia, packing of epithelial cells, and thickness of epithelial layer (Fig. 2b). The sea otter specimen had significant pathology and the epithelium was detached from the turbinates in many places. Therefore, approximately 30% of the tissue in the upper nasal cavity in the vicinity of the olfactory region could not be classified. However, where tissue was present it was still easily distinguishable as respiratory or olfactory (Fig. 2c,d). Conservative values of LUE and OUE were calculated using only the olfactory tissue that could be positively identified. Alternative values were calculated assuming that all the epithelium posterior to the first identifiable olfactory epithelium was also olfactory tissue. The means of the two values are the reported sea otter LUE and OUE.

Olfactory epithelial area was calculated as described in Gross *et al* (1982). Length of structures of interest was measured in the TIFF image of each histologic section using Scion Image™ 4.0. The length of epithelium in a single histologic section was multiplied by the section separation and the resulting section areas summed over the series to produce the total epithelial area. Air space cross-sectional areas were measured throughout the series and multiplied by section separation to produce lumen volumes.

Statistical analysis

The model was tested using chemical property values chosen to be representative of the compounds tested in the behavioral study: Henry's law constant=0.00001, diffusivity in air=0.075cm^2/s and diffusivity in mucus=0.00001cm^2/s, except where otherwise noted.

In order to compare general olfactory sensitivity among species, an Average Threshold was calculated. All threshold values were log transformed. The Average Threshold was defined as the mean of the log transformed threshold values of seven widely tested odorants, acetic acid (8 species), propionic acid (9 species), butyric acid (12 species), ethanol (7 species), butanol (6 species), ethyl acetate (5 species) and amyl acetate (7 species). These odorants were chosen in order to maximize the size of the dataset while equalizing the representation of the three available chemical groups, straight-chain aliphatic acids, alcohols and acetate esters. To compare fourteen mammal species using these seven compounds the total set of thresholds is 98. Of these, 55 were available in the literature and from this study. Due to inherent variation in detectability among these seven compounds, it was important to substitute approximations for the missing values. In all three chemical groups, an approximate logarithmic decrease in threshold with increasing carbon chain length is present in most species (Fig. 11). For species with missing values in a chemical group where two or more thresholds were available for related compounds, the missing value was estimated using the rate of increase with chain length among the known values. If only one threshold value for that species in that

chemical group was available, the missing value was extrapolated using the mean of the slope in question for all available species. Seventeen values were approximated in this way. The remaining 26 were approximated by the following value:

For species Q, odorant Y

Estimated Threshold = (mean [available thresholds(Q)] x mean[available thresholds (Y)])$^{1/2}$

AT was regressed on OUE. Plots of thresholds versus OUE with species values are included for inspection (Fig. 12). However, all species values were transformed using Felsenstein's method of independent contrasts (Felsenstein, 1985) to remove phylogenetic nonindependence before regression. The regressions were performed using Stata 8.0, with the constraint that the regression line pass through the origin. The topology of the phylogeny used to calculate the contrast values is shown in Fig. 13. Variances were estimated from the branch lengths in the Eutherian phylogenies of Goodman *et al* (1998) and Nikaido *et al* (2001) wherein branch lengths were calculated from molecular data. However, several branch lengths were unavailable. The basal and the Carnivora/Chiroptera/Soricidae nodes were left unresolved because there was no consensus in the literature. In those cases, two bifurcations were collapsed into one node of increased branch length and the extra bifurcation assigned a branch length of zero. In addition, branches within the chiroptera were arbitrarily assigned equal length between each bifurcation, because published branch lengths were not available. An identical

regression was performed for V_{olf}/V_{brain} vs. OUE. V_{olf}/V_{brain} values were log-transformed and OUE was arbitrarily assigned as the independent variable in order to permit the contrasts comparison.

Results

Model results

Variation in OUE was large and significant contributions were made by Q_{up}/Q_{tot} and LUE (R^2= 0.76 and 0.42, respectively). The two factors were not highly correlated (R^2=0.06).

Relative variation (standard deviation/mean) was slightly higher for Q_{up}/Q_{tot} than for LUE (Table 1). Most species were tightly grouped for both variables with several low outliers. The primates and sea otter had unusually low values of Q_{up}/Q_{tot}. This was attributable in all three cases to their small cross-sectional areas of the upper cavity relative to the lower cavity. For the squirrel monkey and the sea otter a small slit width in the upper region relative to the lower region was also an important contributing factor. The sea otter has unusually convoluted turbinal structure in the anterior nasal cavity, extending into the lower region, but persisting for a greater axial distance in the upper region. LUE values were also unusually low in both primates, reflecting their relatively small area of olfactory epithelium. This is consistent with the low neural investment made in olfaction in this highly visual lineage (Stephan *et al*, 1987, Gilad *et al*, 2004).

Respiration and chemistry effects on uptake

The model was tested against several empirically observed phenomena in respiration, nasal uptake and olfaction. In most cases the model was in qualitative agreement with empirical data. However, some limitations were revealed.

Empirical studies described above show that proportional flow through the upper nasal cavity increases with increased inhalation rate. The model fails to account for this as Q_{up}/Q_{tot} is independent of total inhalation rate.

The work of Schneider *et al* (1966) suggests a decrease in uptake efficiency with increasing flow rate in humans. This is the most parsimonious explanation for the decreasing sensitivity with sniff rate observed in the higher range of sniff rates tested. This is consistent with the model output in human as well as in mouse (Fig. 14), which shows continuously decreasing LUE in the olfactory region as inhalation flow rate increases. This effect is only likely to be important for low LUE species like humans since physiologically achievable flow rates for other species would produce only small decreases in LUE for most odorants.

Sobel *et al* (2000) suggested that fast-partitioning odorants would be optimally detected at faster flow rates than slow-partitioning odorants. At fast flow rates, uptake of slow-partitioning odorants would be small; at slow flow rates, uptake of fast-partitioning odorants would occur rapidly over a small area and activate fewer receptors. Three chemical properties are used as model input and affect LUE in the olfactory region: diffusivity in air, diffusivity in mucus, and Henry's law constant, ß. Diffusivity in air can

be predicted with reasonable accuracy from molecular formula (Fuller *et al*, 1966) and generally ranges from 0.01 to 1 cm/s^2. Diffusivity in mucus is problematic since in order to predict it theoretically it is important to know whether the odorant associates with the solvent, and the complex biochemistry of the olfactory mucus complicates this question. Nevertheless, the typical range of diffusivities of small molecules in any liquid is 10^{-4} to 10^{-6} cm/s^2 (Perry *et al*, 1997). Henry's Law constants have been empirically determined for a large number of small molecules, including all of the odorants used in this study (Yaws, 1999). This is the most variable property, ranging from 10^{-8} to 10^{1} (concentration in air/concentration in water at Standard Temperature and Pressure). The effects of all three variables are monotonic: increasing D_{air} or D_{muc}, increases uptake; increasing ß decreases uptake. The sensitivity of the model to these three variables varies with nasal morphometry (Fig. 15), and there are important interactions between them. In the high-uptake mouse morphometry, LUE_{olf} is almost invariant with D_{air} above approximately $2x10^{-2}cm^2/sec$, while in the human morphometry there is a strong dependence under all conditions of the other two variables that allow appreciable uptake. D_{muc} only has an important effect at values of ß greater than 0.01, in either species, which increases with increasing D_{air}. The most important effect of increasing ß is the aforementioned interaction with D_{muc} above ß values of 0.01, but at extremely high values (ß=0.1 in human, 1 in mouse), uptake is reduced to extremely low levels and dependence on both diffusivity terms becomes unimportant.

However, it must be borne in mind that *in vivo*, the solubility and diffusivity of odour molecules in mucus is subject to mucus biochemistry, and the behaviour of odorants in mucus will be difficult to predict until the mucus enzyme system is more completely understood. For this reason the interaction of physiochemical properties with sniff rate was examined along a gradient of D_{air} values in the human nasal cavity. D_{air} appears twice in the LUE calculation: once in the calculation of odorant concentration just above the mucus layer, C_0, a negligible effect, and again in the calculation of the transfer rate G of molecules into the mucus surface (molecules/area/time). This is later integrated over the mucus surface area (molecules/time), and then divided by the upper nasal cavity air flow rate Q_{up} (volume/time) to determine total concentration change (molecules/volume); therefore, the ratio of D_{air} /Q_{up} is the only important uptake consequence of D_{air} (Fig. 16).

The model results in the human nasal cavity support Sobel's theory: at extremely high values of D_{air} /Q_{tot} (fast-sorbing odorants at low flow rates) uptake is nearly complete but over 75% of it occurs in the anterior 25% of the olfactory region, 90% in the anterior half, potentially limiting the number of receptors activated. At very low values (slow-sorbing odorants at fast flow rates) uptake is evenly distributed but reduced to less than 5% (Fig. 17). For the odorants used in this study at double the resting inhalation rate, uptake is distributed moderately evenly over approximately half the length of the

olfactory region, with LUE_{olf} ranging from 0-20%. According to the model output, these odorants could be taken up with greater efficiency at slower inhalation rates.

Model output for LUE was compared with the empirical values for whole nasal cavity uptake measured by Morgan and Monticello (1990) for four compounds (Fig. 18). A direct comparison is not strictly valid: Morgan and Monticello tested significantly higher inhaled concentrations and longer exposures than those for which the model is intended. This introduces the possibility of saturation of the nasal enzyme systems which could have differing effects on the substances tested. The model, by contrast, relies on physiochemical parameters for all four compounds (Henry's law constants and diffusivities in air and water from Perry and Green, 1997 and Dean, 1999). The model output was consistent with the empirical data for carbon monoxide (extremely low solubility, no uptake), and ethanol and acetone (small, mobile, highly soluble molecules, mid-range diffusivities in air and water, moderate uptake) but not for ammonia. Ammonia diffusivities and solubility were not dramatically different from ethanol or acetone and moderate uptake was predicted (24%). Actual uptake was 80%. Ammonia is a weak base that can be found in significant concentrations in nature and is an important respiratory system irritant and toxin (Pyatt, 1970, Kirkhorn and Garry, 2000). A robust pathway for removal of this compound from the nasal mucosa would be adaptive for the protection of the lower respiratory tract. Such a system, if it exists, would explain the unexpectedly high nasal uptake of ammonia after prolonged exposure.

The fact that the model is consistent with observed physical and chemical trends and the quantitative comparison with empirical uptake measurements suggest that the model varies at least qualitatively with olfactory uptake efficiency. Model output can therefore be used as a proxy in order to determine the effect of OUE on sensitivity.

Morphometry effects on threshold

Model output for the study species is summarized in Table 1. Tabulated values are for a single specimen of the Domestic dog, Sea otter, Mouse-eared bat, Spear-nosed bat, Vampire bat, Common squirrel monkey, Brown rat and European shrew, and the mean of two specimens for the remaining four species. The Mouse-eared bat AT value was deemed an outlier and this species was excluded from the analyses involving AT.

There are obvious and significant phylogenetic effects on AT and particularly on OUE (Fig. 12a). The Primates form a distinct group at low AT values and extremely low OUE, separated from the nearest nonprimate OUE value by nearly a factor of three. The shrew, the Rodents and the Chiroptera form a large cluster with similar, high OUE values and widely varying AT. The carnivora have widely separated OUE values intermediate between the primates and the rest of the mammals.

Contrast values are in Fig. 12b. Linear regression of AT vs. OUE among the eleven mammals shows a strong although not statistically significant trend ($R^2 = 0.27$; $P = 0.10$).

A regression of AT on the two factors of OUE showed that LUE was the more important factor due to its higher variation. However, most of this variation was

contributed by the extremely low LUE values of the human and squirrel monkey. Excluding them from the analysis, the important factor in the remaining variation in OUE was Q_{up}/Q_{down}.

Morphometry effects on neuroanatomy

Linear regression shows that OUE is significantly related to the ratio of olfactory bulb volume to total brain volume, V_{olf}/V_{brain} ($P=0.02$, $R^2=0.43$, Fig. 19).

Discussion

Significance of OUE

In light of the quality of the data, and especially considering the small size of the dataset (11 species), the results for AT and OUE are difficult to interpret. The regression of AT on OUE appears correlated but is not significant at the 5% level. The R^2 value indicates that this relationship explains 27% of the variation in the threshold dataset. This is remarkable, particularly considering the many sources of error described below, that contribute to the large variance of AT. This suggests that nasal cavity morphometry does play a role in determining general olfactory sensitivity, in a fashion consistent with its role as a physical collector of the stimulus. A larger dataset will be necessary to determine whether this relationship is indeed significant. Estimating the power of this experiment is problematic since there is no independent reference for the magnitude or variability of the effect examined. A first-order power analysis of the regression based on the signal to

noise ratio, as described in Cohen (1977) shows that under these conditions a sample size of thirty species would be 89% likely to show a relationship significant at the 5% level (Fig. 20).

It is interesting to note that even this small sample showed a highly significant relationship between OUE and neural investment in brain volume, as represented by V_{olf}/V_{brain}. While the relationship between nasal and brain morphometry is striking, neither variable appears to be strongly related to directly measured olfactory sensitivity. The high variability in intra-species values of AT, as well as the many obvious sources of error in the measurement of behavioral olfactory thresholds and the calculation of a representative average suggest that these are the limiting factors in predicting olfactory sensitivity from anatomy. Modern neurophysiologic theory and computer-aided flow modeling techniques currently available could increase the sophistication of the anatomical model, in fact, to a point unwarranted by the quality of the threshold data available currently for testing it. Future research should, ideally, both broaden and standardize the psychophysical dataset. Such work is difficult, expensive and practical only for a few species. However, a comprehensive comparison of anatomy with sensitivity may eventually permit informed sensitivity estimates of mammals for which direct measurements are not available.

Nonolfactory morphological features

Three important nonolfactory biological features may have impacted measurement of OUE. The first is body mass. Total inhalation flow rate, to which we have seen that OUE is extremely sensitive, was predicted from body mass. It is worth noting that the four largest species have the four lowest values of OUE. However, beyond this grouping the pattern breaks down. The smallest of the four, the squirrel monkey, has a nearly identical OUE to the largest (human). The two most similar sized species, the dog and sea otter, have very dissimilar OUE values. The dog, the larger of the two as well as the second largest in the whole dataset, has the highest OUE of the four, a value similar to those of the small mammals in the dataset.

The other two features are both non-olfactory functions of the nasal cavity. There is extremely wide variation in gross nasal cavity morphology among the species examined (Fig. 21). In the case of the sea otter, highly derived turbinal structure was observed which greatly increased surface area through most of the nasal cavity. This feature is likely to have evolved for the respiratory functions of heat and water retention. Among its adaptations to a marine existence, the sea otter has unusually thick fur, a variety of behavioral and metabolic adaptations for heat conservation (Costa and Kooyman, 1984) and a highly derived respiratory system, including a lung volume 2.5 times that of similarly sized terrestrial mammals, which is believed to be adaptive both for long dives and for buoyancy regulation (Kooyman, 1973, Leith, 1976, Lenfant *et al*, 1970).

All but one of the bat species studied utilize nasal echolocation. In the posterior nasal cavity of each of these species is a large sinus or pair of sinuses, varying in shape and unique to the Chiroptera. This sinus communicates with the surrounding olfactory region but does not contain olfactory epithelium. A function in the modification or directing of the echolocation signal is likely, analogous to the melon in echolocating odontocete whales. This postulated function is supported by the absence of this sinus in the Mouse-eared bat, which is a buccal emitter in which the echolocation signal passes through the open mouth rather than the nasal cavity.

Variation and error

Several simplifying assumptions in the model may produce systematic errors. However, the purpose of the model is not to predict actual uptake quantitatively but only relatively across varying morphometries. It is likely that other sources of variation are collectively more important than the deviations of the model from explicit flow and transport conditions.

There was significant inter-individual variation in OUE in species for which more than one specimen was measured. Variation between conspecifics ranged from 1.4 to 30% (Table 2). The most similar animals were two female *Mus musculus* of the same strain. In that case, turbinate morphometry was nearly identical, and considerable differences in the extent of the olfactory region resulted in only slightly different LUE values.

Olfactory receptor cell numbers decrease with age (Hinds and McNelly, 1981, Ohta and Ichimura, 2000). While this process begins relatively young, the model output suggests that significant loss of uptake efficiency will not be proportional orimmediate, particularly in high-uptake species like the mouse, but losses will have a much larger uptake effect in low-uptake species including primates. Therefore, the effect of age both within and across species is likely to be substantial and complex.

The most extreme difference was between the two humans. Human turbinate morphometry data was taken from Kelly *et al* (2000) and Keyhani *et al* (1995) and was measured by similar radiographic methods (CT scan). The difference in humans arises entirely from turbinate structure, in particular a difference in nasal passage width (w). Olfactory tissue distribution data from the same source, an *in vivo* biopsy sampling study (Feron *et al*, 1998) was superimposed on the two morphometries obtained from Kelly *et al* (2000) and Keyhani *et al* (1995). The difference in LUE was larger than in Qup/Qdown and also opposite in sign. This is expected to be typical of this kind of morphometric difference. Increased relative passage width in the upper nasal cavity will increase flow through the olfactory region, but as Q_{up}/Q_{tot} increases, residence time decreases and so, correspondingly, does LUE_{olf}. Since the two effects are in opposition, the net effect of increased width can be positive or negative. The uptake effect is more important in the human case. Therefore, the net result of wider upper nasal passages is increased OUE. However, in nasal cavities where uptake is near completion (LUE_{olf} close to 1) the relative

importance of the flow distribution effect will increase. While the human turbinate morphometry differences may have been an artifact of differences in technique between the two sources, intra-species variation in nasal passage width due either to turbinate morphology or to occlusion is likely to be an important source of intra-species variation in OUE in humans and possibly other species.

Surface area and volume measurement error due to tissue shrinkage during histological processing is a possibility that must always be considered in work of this kind. In this case the importance of these effects should be unimportant. While the resolution of the CT scans do not permit measurement of very small features, comparisons of overall nasal cavity length showed that the calculated length from the histological series does not differ systemically from that measured in the undisturbed tissue from the scans (Fig. 22). The three largest differences observed are largely attributable to lack of resolution in the scans. These specimens, the house mouse, Seba's short-tailed bat and common European shrew, are all very small and were scanned on the less high-resolution model scanner. Counting only the specimens scanned on the Volume Zoom, the largest difference was 5% and the mean difference was 1% (shorter in CT scan).

Intra-species variation in olfactory sensitivity is well documented. It has been shown in humans, (Lehrner *et al*, 1999, Stevens and Cain, 1987), lemurs, (Aujard and Nemoz-Bertholet, 2004) and rats (Kramer and Apfelbach, 2004) that many aspects of

olfactory function, including sensitivity and ability to distinguish between odorants, decline throughout adulthood. Among females, seasonal or hormonal variation in sensitivity must also be considered. Navarrette-Palacios *et al* (2003) found in humans that significant changes in olfactory sensitivity occur over the course of the menstrual cycle, with lowest thresholds during ovulation and highest thresholds during menstruation. Schmidt, (1978) found similar variation in female mice based on hormonal state.

Sexual dimorphism in olfactory sensitivity is also common but not uniform across species and compounds. Among humans, better performance by females in olfactory tasks has been reported often (Doty, 1986, Yousem *et al*, 1999, Oberg *et al*, 2002, Dalton *et al*, 2002). However, this finding is not robust among other mammals. Because of the cyclic variations in female sensitivity, most nonhuman studies have simplified their analyses by testing only males. Among the five quantitative studies cited here that tested both sexes, three reported individual results for each sex. Myers and Pugh (1985) tested 12 dogs, 5 female, 7 male, and found no significant difference in performance, noting that there was no estrous among the females nor any sign of sexual interest on the part of the males that would indicate an estrous female. Moulton *et al* (1960) tested two dogs, and the male was uniformly more sensitive than the female. In neither canine study is age specified beyond the description 'mature'. Hubener and Laska (2001) tested two adult and one subadult male and one adult female pig-tailed macaque. The female acquired the task in approximately 200 practice trials before the first of the males, or approximately

50% faster but her threshold values were not significantly different from any of the corresponding mean male thresholds. It is unproven but reasonable to consider that sexual dimorphism will eventually be found to vary widely among species and among compounds.

Aside from differences in age and sex of the subject animals, largely unquantifiable differences in experimental conditions and technique contributed to 'noise' in the dataset. Olfactory masking effects, training & reinforcement schedules, dilution medium, temperature, trial timing and resulting olfactory acclimation, concentration measurement and potentially many other experimental conditions varied among the four decades of studies used for developing and testing the model. To illustrate the importance of this variation, see Fig. 23 for a comparison of the range of threshold values for the 12 tested mammals for butyric acid (6 orders of magnitude) and the range of published values in the 17 studies measuring human threshold for butyric acid, (4.4 orders of magnitude) which were combined by Devos and colleagues (1990) to yield the value utilized herein. The published mammalian olfactory threshold dataset is particularly susceptible to this source of variation compared with the OUE and olfactory bulb volume datasets because of the large variety of sources from which it is derived. The thresholds used in this study were obtained from 17 studies conducted over 43 years. In contrast, OUE data came from four sources (this study and the three human anatomy references used to calculate human

OUE) dating from the past nine years and olfactory bulb volumes were drawn from four sources dating from the past 13 years.

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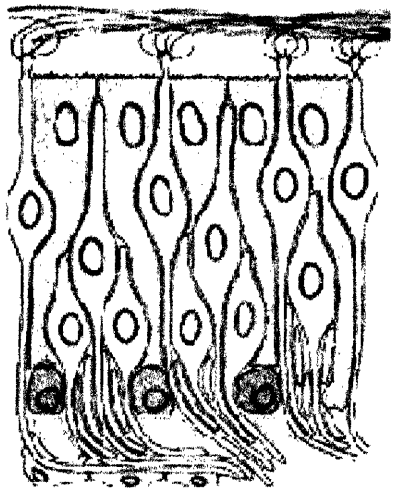
Fig. 1a: Sagittal CT scan section through the skull of the Pale spear-nosed bat (*Phyllostomus discolor*). The upper nasal cavity containing the olfactory epithelium is highlighted in yellow. Directly underneath is the lower-resistance region of the lower nasal cavity, which conducts the bulk of the nasal airflow.

b c

Fig. 1: Transverse histological sections through the nasal cavity of the House mouse (*Mus musculus*). The olfactory epithelium is highlighted in yellow. b: an anterior section, where the olfactory epithelium is not extensive. The separation of upper and lower nasal cavity is indicated by the blue line. c: a posterior section, where the olfactory epithelium nearly fills the upper nasal cavity. Here, the lower nasal cavity is physically separated. Identical scale, bar=1 mm



a)

b)

c)

d)

Fig. 2:a) Olfactory epithelium, schematic; b) House mouse (*Mus musculus*) nasal epithelium, respiratory on left, olfactory on right; c) Sea otter (*Enhydra lutris*) nasal respiratory epithelium; d) Sea otter (*Enhydra lutris*) olfactory epithelium

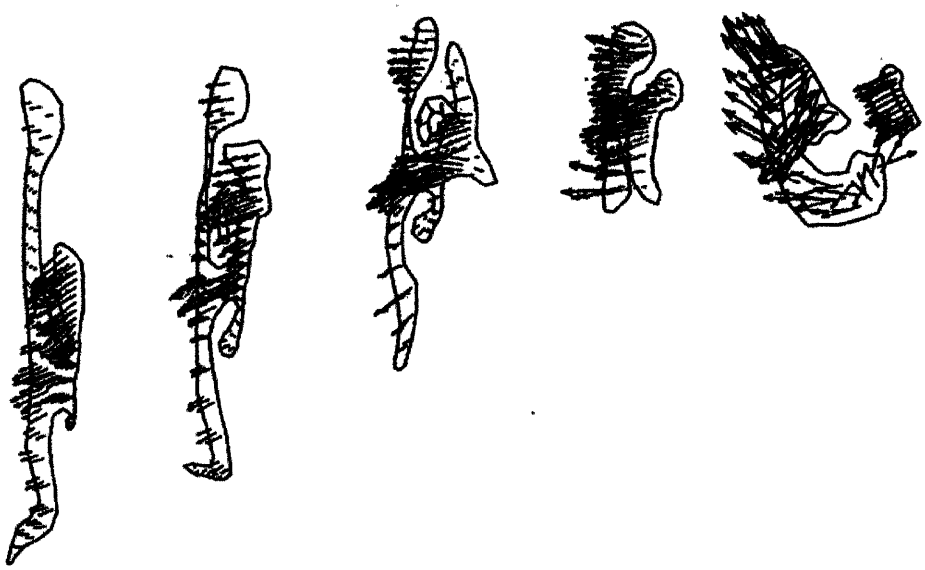


Fig. 3: Nasal airflow patterns. a)baboon (*Papio sp.*), video analysis of dye flow in transparent nasal cast, from Patra *et al*, 1986

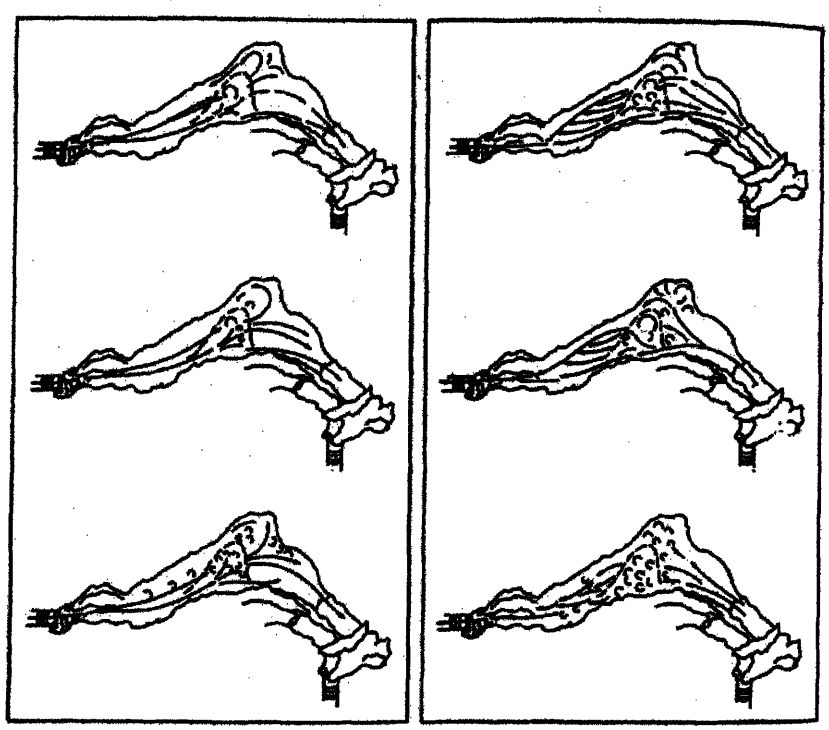


Fig. 3: Nasal airflow patterns. b) rhesus monkey (*Macaca mulatta*), video analysis of dye flow in transparent nasal cast, from Morgan *et al*, 1991.

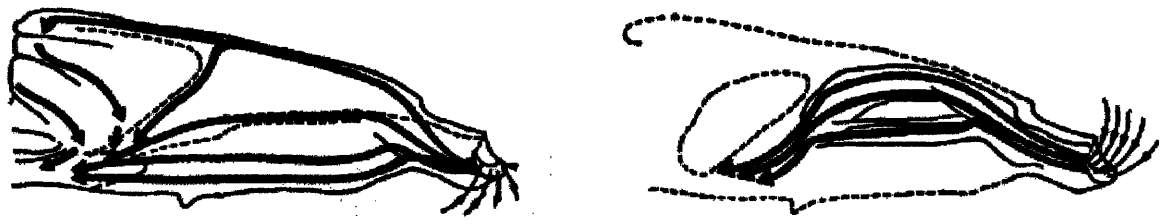


Fig. 3: Nasal airflow patterns. c) F344 rat (*Rattus norvegicus*), video analysis of dye flow in transparent nasal cast, from Morgan *et al*, 1991.

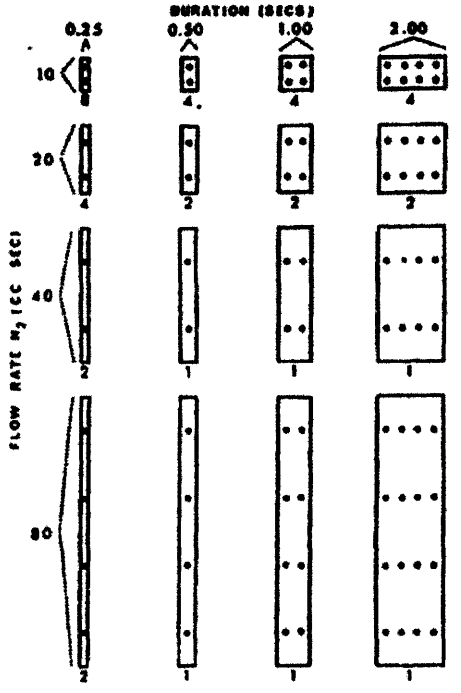


Fig. 4: The interaction of flow rate and time in olfactory detection, from Schneider *et al*, 1966

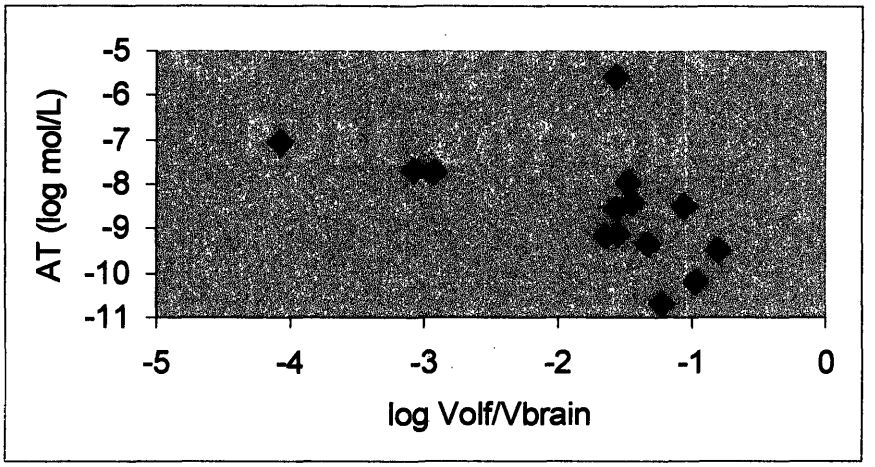


Fig. 5a: Average Threshold (AT) versus olfactory bulb volume, all available species values. From Hutcheon *et al*, 2002, Gittleman, 1991, Stephan *et al*, 1987, Williams *et al*, 2001

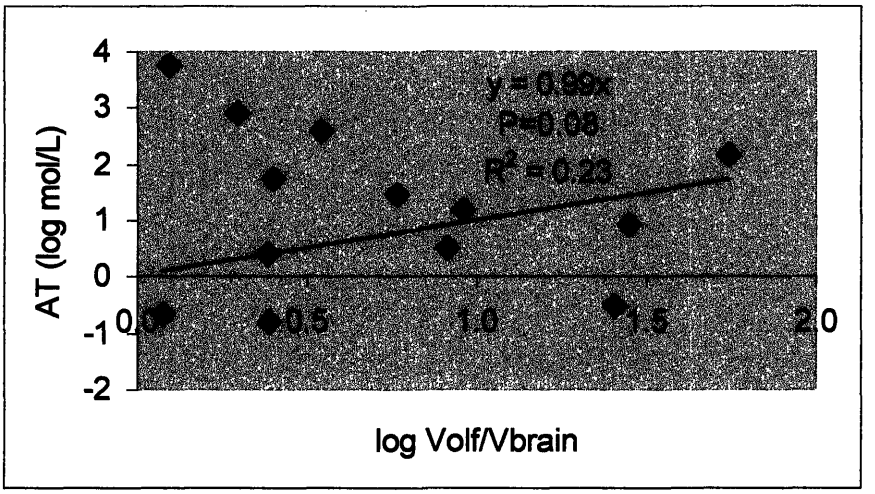


Fig. 5b: Independent contrasts analysis, AT versus olfactory bulb volume, all available species

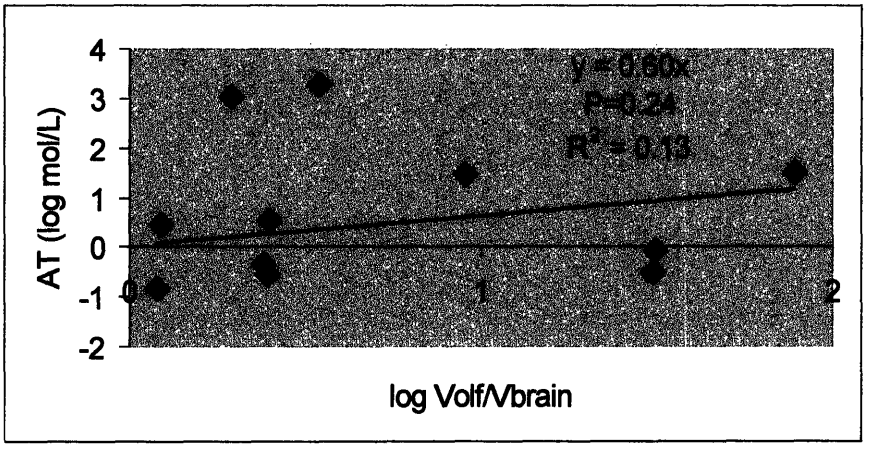


Fig. 5c: Independent contrasts analysis, AT versus olfactory bulb volume, OUE study species

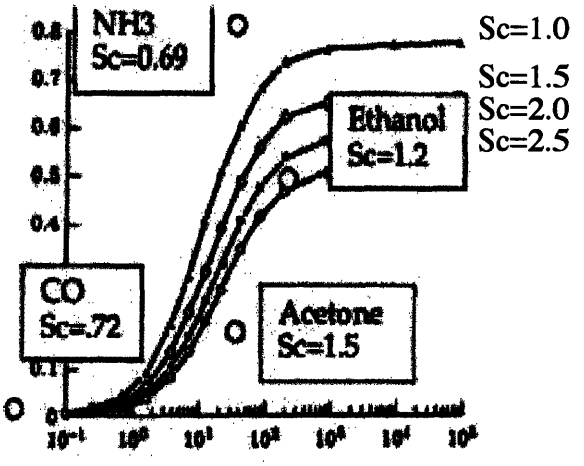


Fig. 6: Modelled human (*Homo sapiens*) nasal uptake compared with empirical values, model from Keyhani *et al*, 1997, empirical values from Morgan and Monticello, 1990.
x=physicochemical parameter
y=nasal uptake efficiency
Sc=Schmidt number (inversely proportional to diffusivity in air)

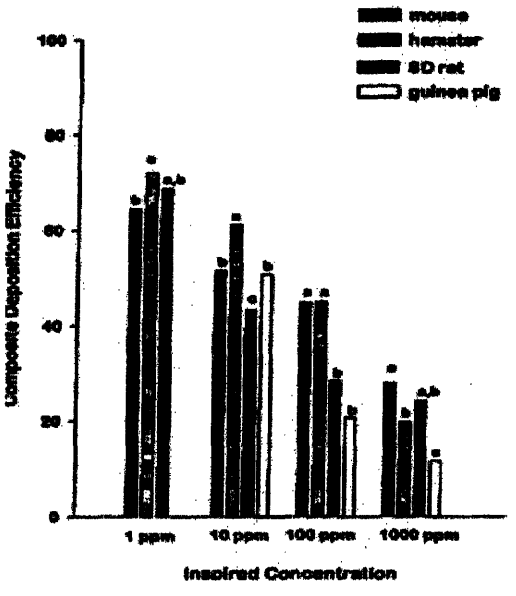


Fig. 7: Nasal uptake efficiency for acetaldehyde in four rodents at four odorant concentrations. House mouse (*Mus musculus*), Hamster (*Mesicricetus sp.*), Brown rat (*Rattus norvegicus*) and Guinea pig (*Cavia porcellus*). From Morris, 1997a

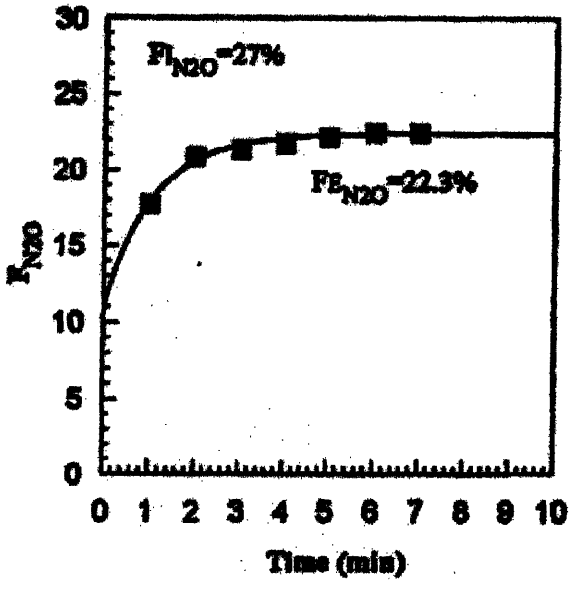


Fig. 8: Human (*Homo sapiens*) nasal tissue uptake efficiency kinetics. From Kelley and Dubois, 1998

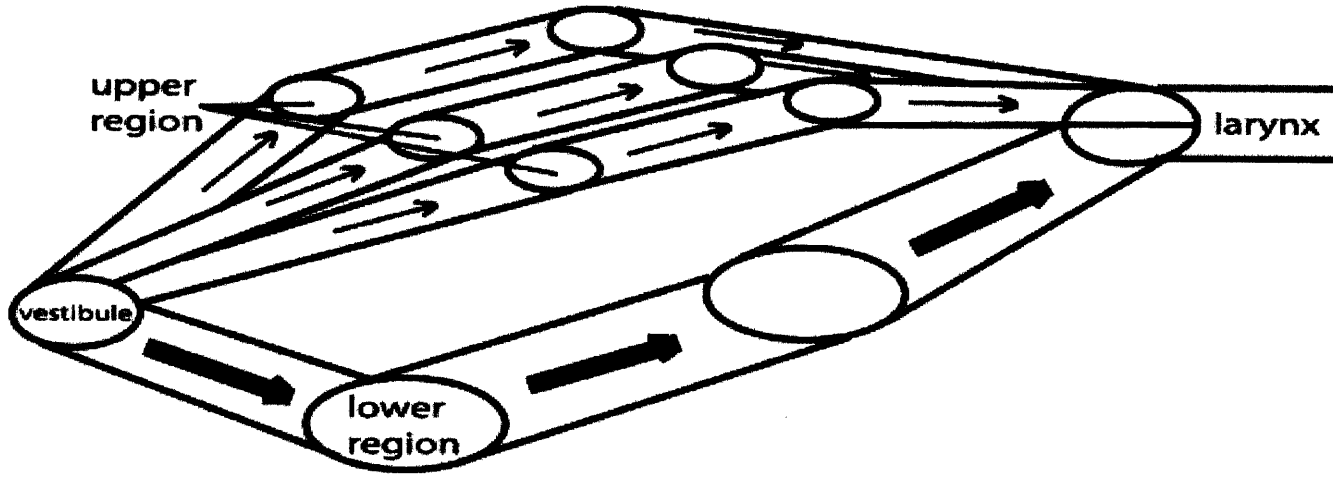


Fig. 9: Schematic representation of the mammalian nasal cavity as modeled herein.

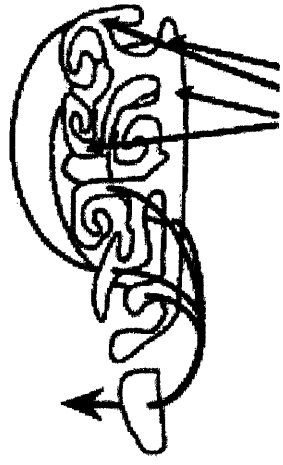


Fig. 10: Posterior nasal airflow in the brown rat, (*Rattus norvegicus)*
From Kimbell *et al*, 1997a

Table 1. Olfactory uptake values for 14 mammal species for a compound of Henry's law constant=0.00001, diffusivity(air)=0.075cm^2/s and diffusivity(mucus)=0.00001cm^2/s, at a total nasal flow rate of 2 x resting inhalation flow rate
Q_{up}/Q_{tot} =flow through upper cavity/total nasal flow
LUE = molecules encountering olfactory tissue/molecules in upper cavity flow
OUE (Olfactory Uptake Efficiency)= molecules encountering olfactory tissue/total molecules inhaled

Species	Q_{up}/Q_{tot}	LUEolf	OUE
House mouse (*Mus musculus*)	0.729	0.909	0.663
Brown rat (*Rattus norvegicus*)	0.616	0.839	0.517
Human (*Homo sapiens*)	0.262	0.188	0.049
Common squirrel monkey (*Saimiri sciureus*)	0.135	0.408	0.055
Mouse-eared bat (*Myotis myotis*)	0.647	0.897	0.580
Pale spear-nose bat (*Phyllostomus discolor*)	0.652	0.855	0.558
Great fruit bat (*Artibeus literatus*)	0.719	0.764	0.542
Seba's short-tailed bat (*Carollia perspicillata*)	0.710	0.937	0.664
Vampire bat (*Desmodus rotundus*)	0.747	0.901	0.673
Domestic dog (*Canis familiaris*)	0.616	0.658	0.405
Sea otter (*Enhydra lutris*)	0.176	0.850	0.150
European shrew (*Sorex araneus*)	0.617	0.987	0.608
mean	0.552	0.766	0.455
standard deviation	0.215	0.229	0.227
std. dev./mean	0.389	0.299	0.498

a)

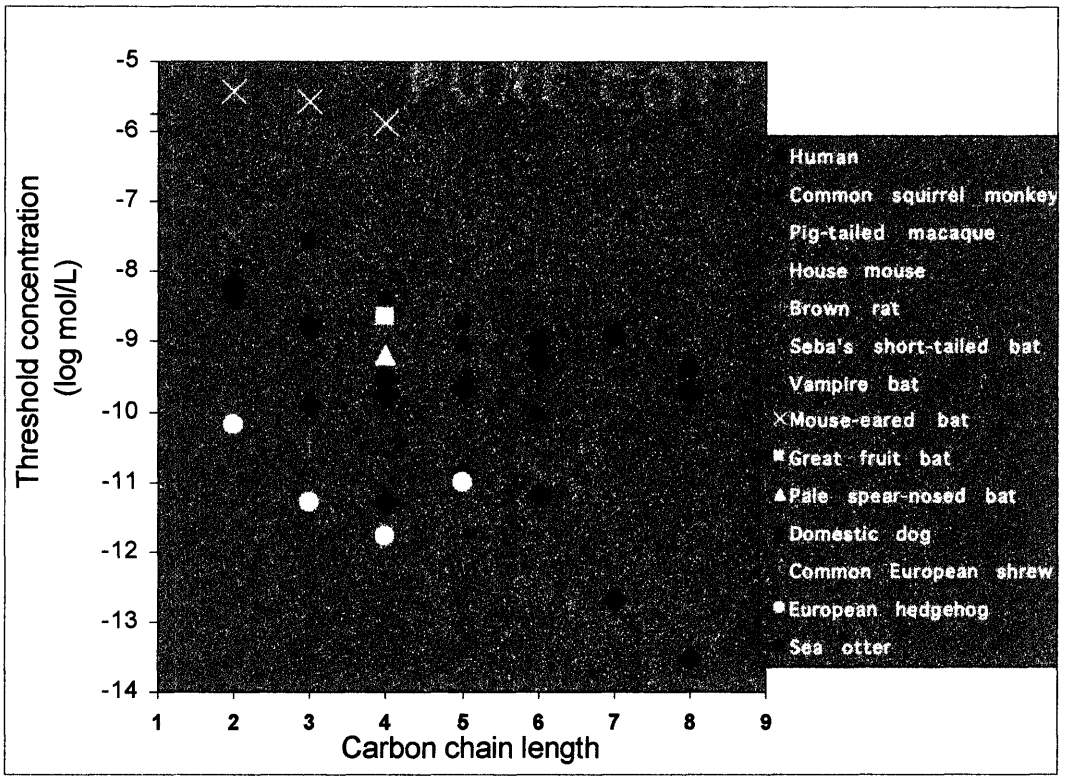
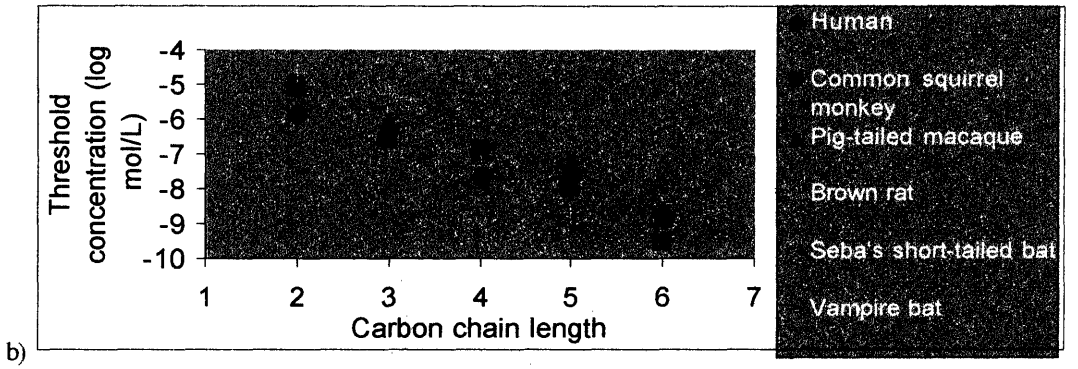


Fig. 11: Previously published olfactory thresholds organized by chemical group a) carboxylic acids. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), Pig-tailed macaque (*Macaca nemestrina*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), European hedgehog (*Erinaceous Europaeus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). Data from: Bretting, 1972; Devos *et al*, 1990; Hubener & Laska, 2001; Laing *et al*, 1989; Laska, 1990; Laska *et al*, 2000; Moulton *et al*, 1960; Obst *et al*, 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985, this study



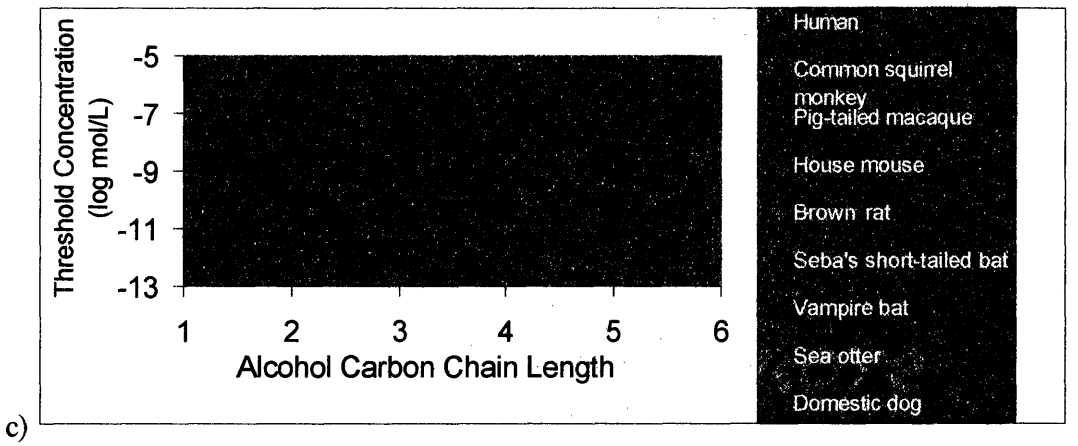


Fig. 11: Previously published olfactory thresholds organized by chemical group b) alcohols; c)acetate esters. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), Pig-tailed macaque (*Macaca nemestrina*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), European hedgehog (*Erinaceous Europaeus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). Data from: Devos *et al*, 1990; Krestel *et al*, 1984; Laska, 1990; Laska and Seibt, 2002a,b; Moulton, 1960; Moulton and Eayrs, 1960; Obst *et al*, 1976; Schmidt, 1975; Yee and Wysocki, 2001; this study

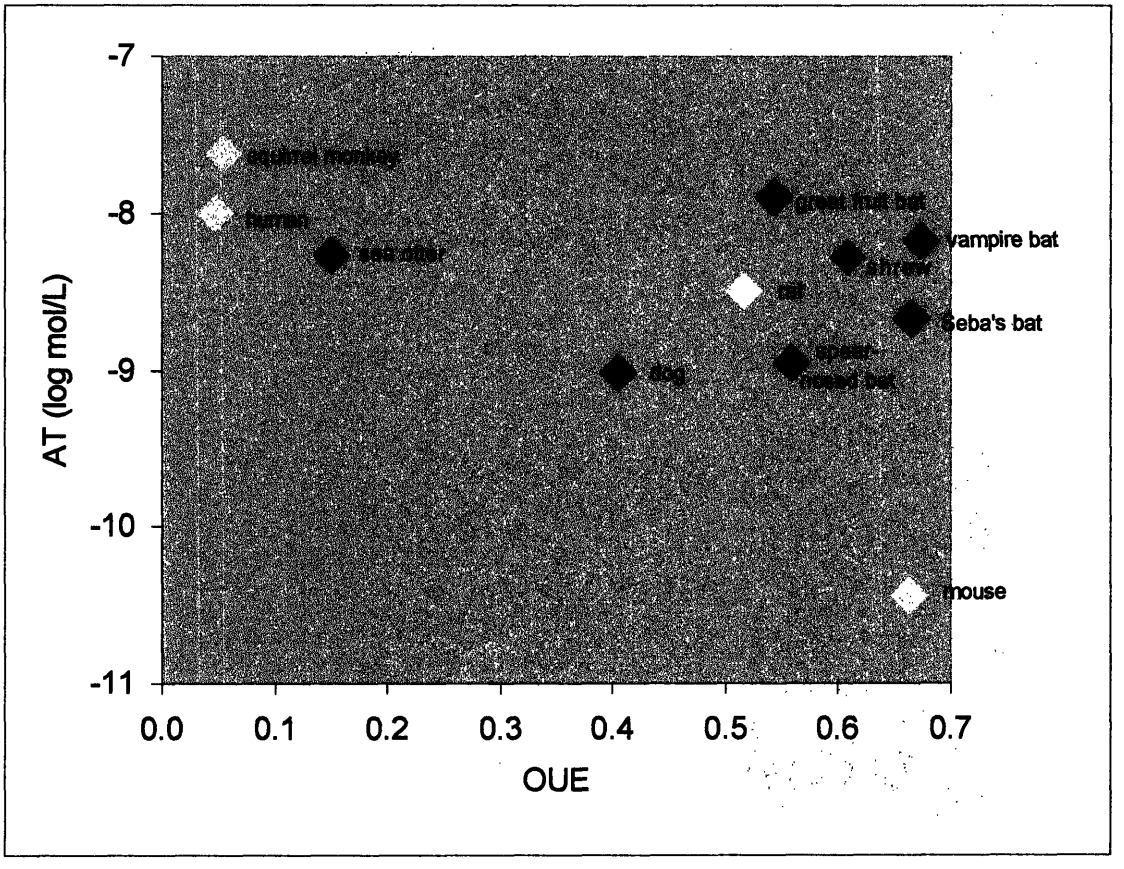


Fig. 12a: AT vs. OUE, species values. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), European shrew (*Sorex araneus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*)

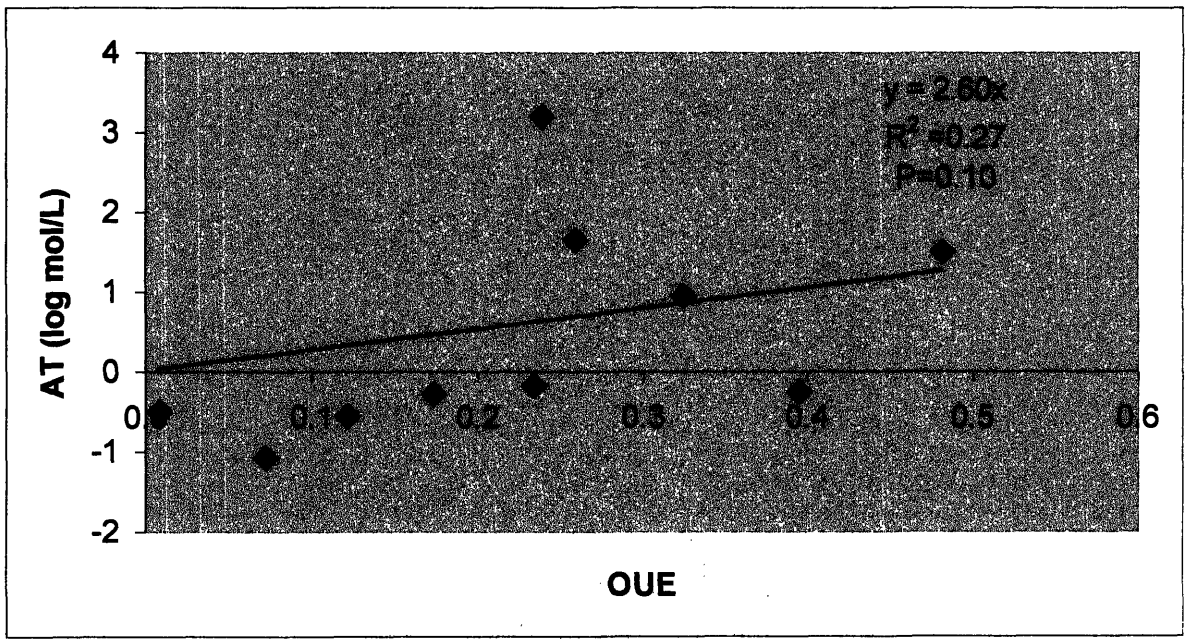


Fig. 12b: AT vs. OUE, contrast values

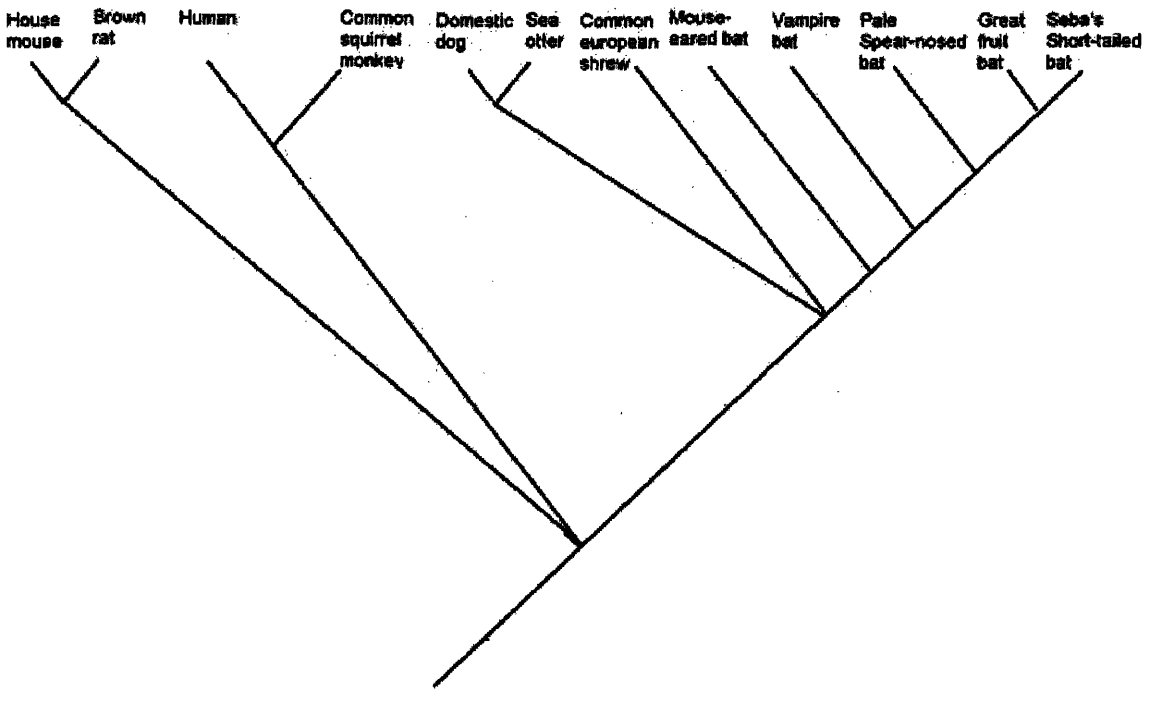


Fig. 13. Complete phylogeny of morphometric study species. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). Data from: Goodman *et al*, 1998, Nikaido *et al*, 2001.

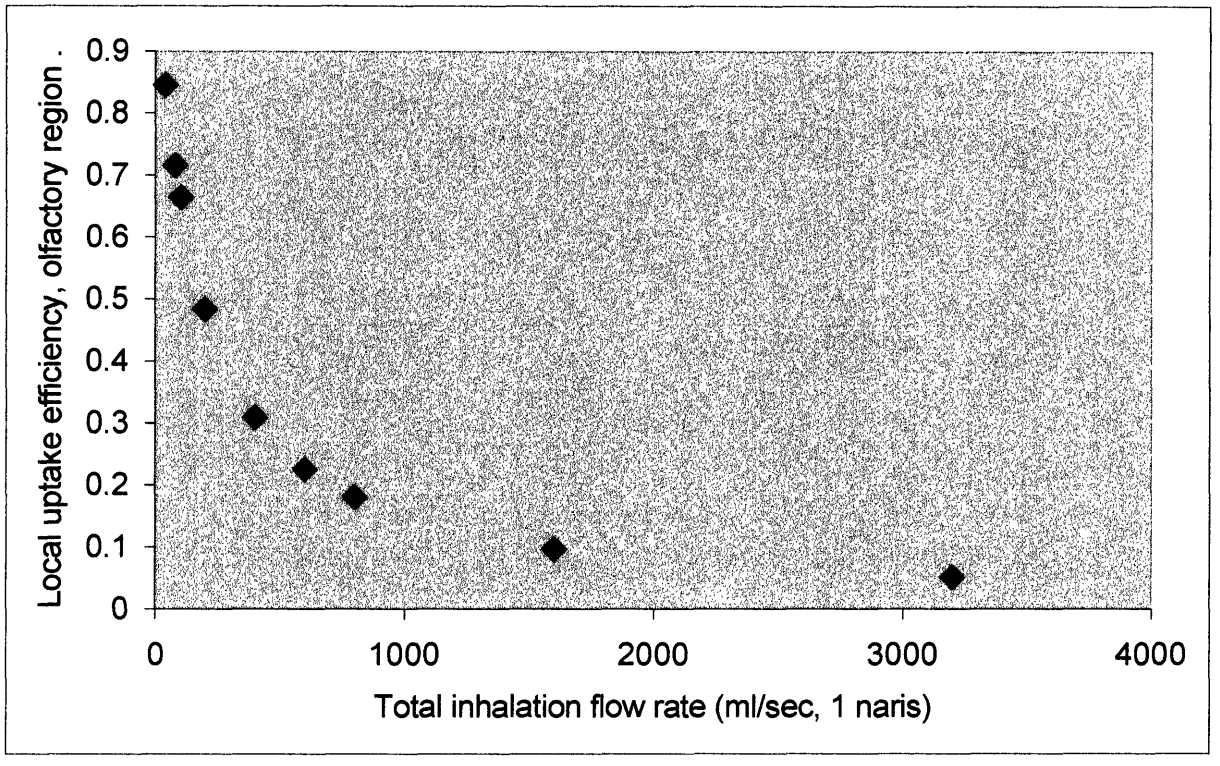


Fig. 14a: LUE versus sniff rate, human (*Homo sapiens*) nasal morphometry. Red point: rate double resting inhalation rate

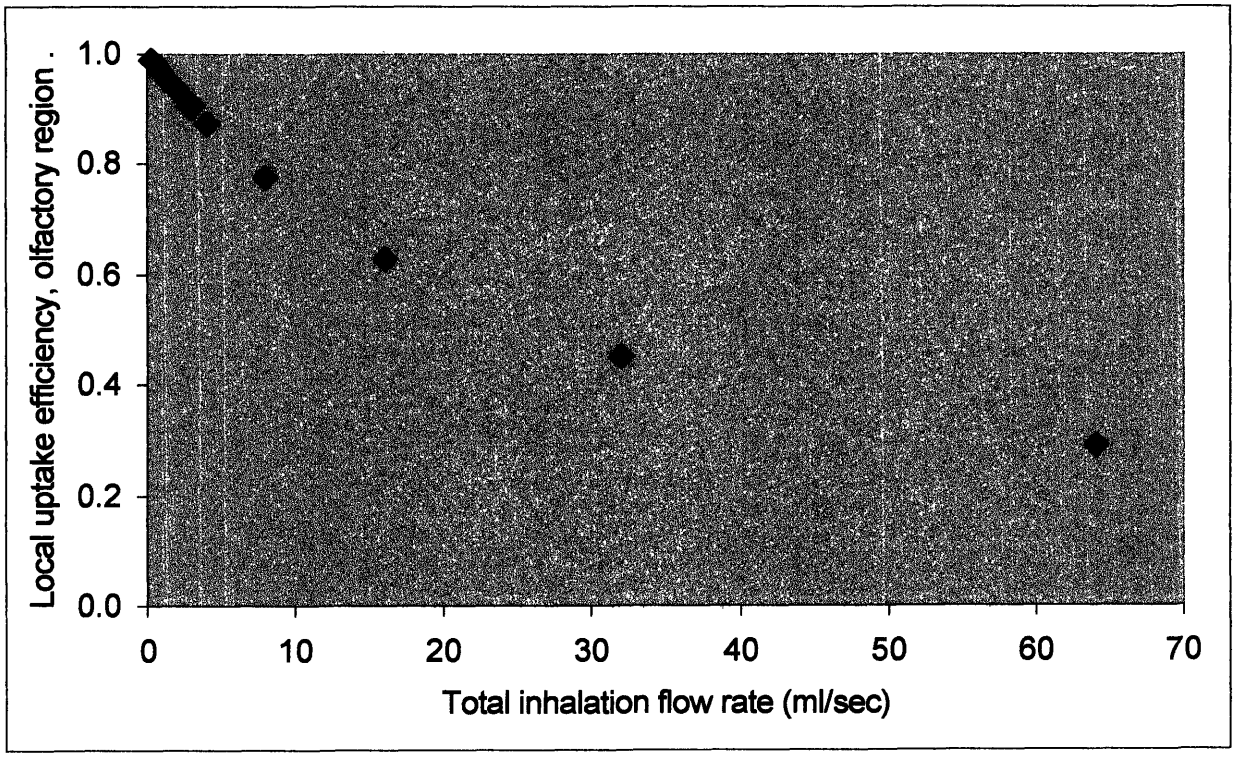
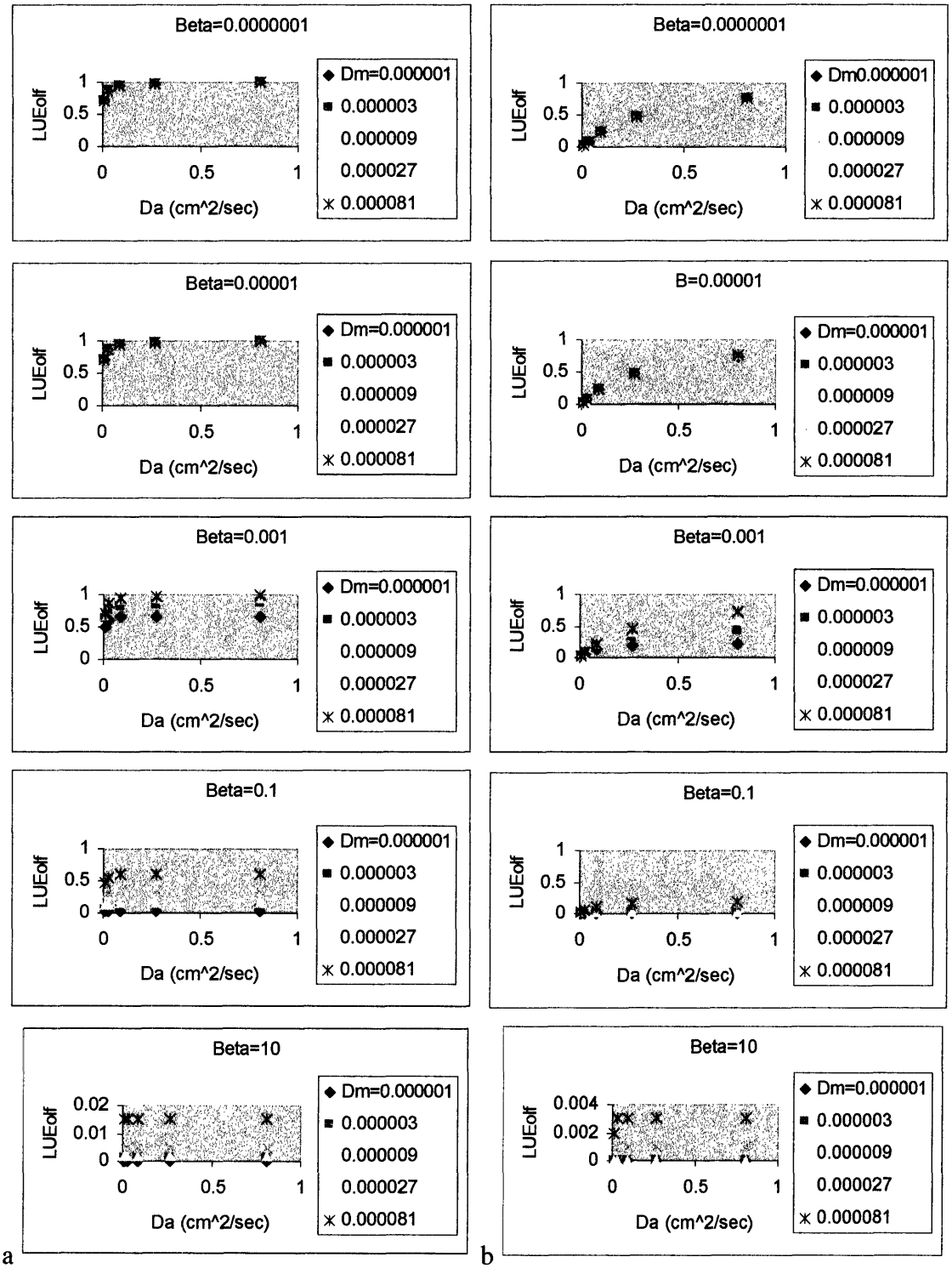
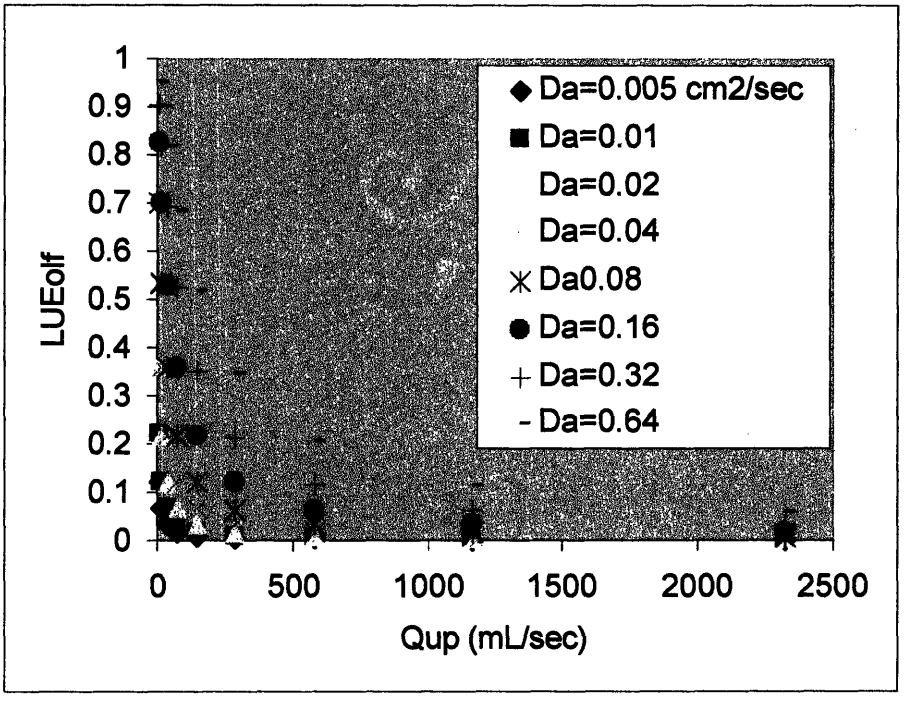


Fig. 14b: LUE versus sniff rate, mouse (*Mus musculus*) nasal morphometry. Red point: double resting inhalation rate



a b

Fig. 15: Interactions of Henry's law constant (Beta), Diffusivity in air (D_a) and mucus (D_m) on Local Uptake Efficiency (LUE_{olf}) a) Mouse (*Mus musculus*) b) Human (*Homo sapiens*)



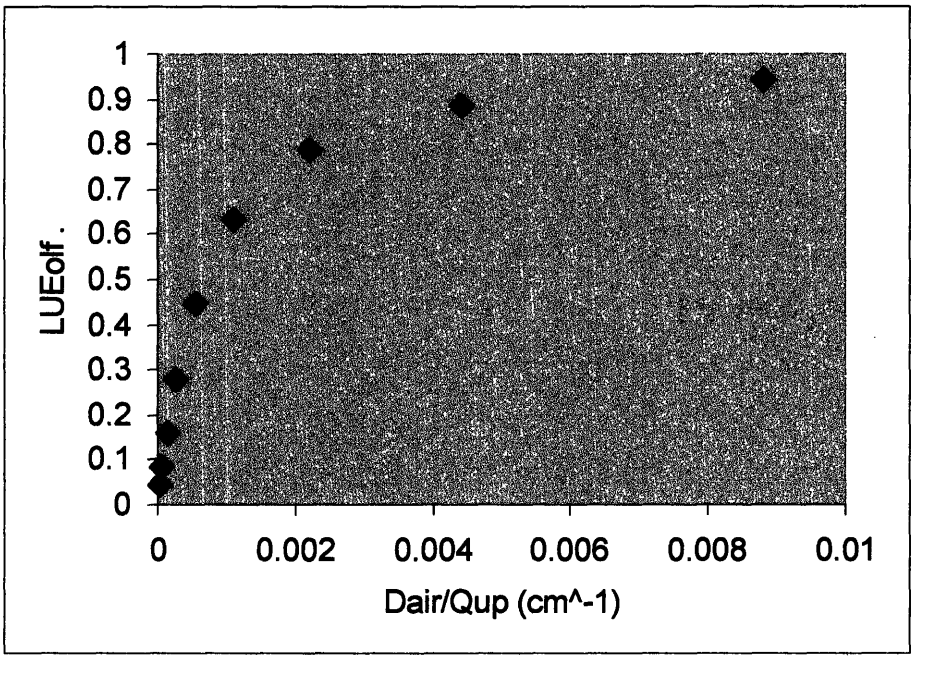
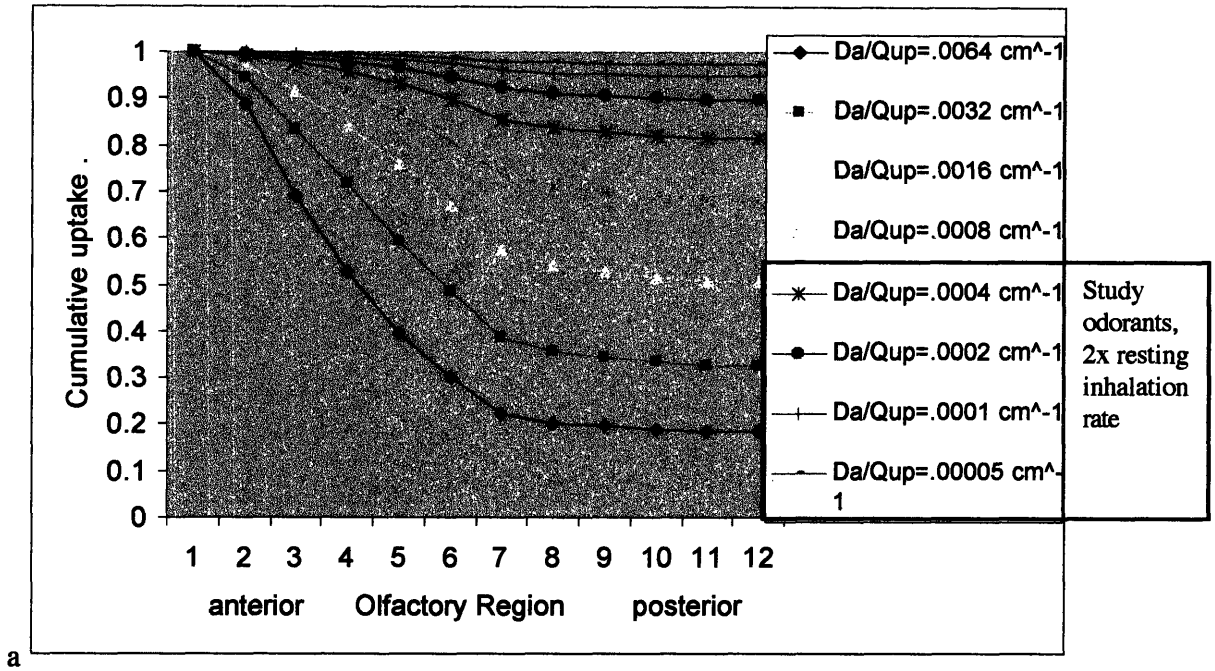
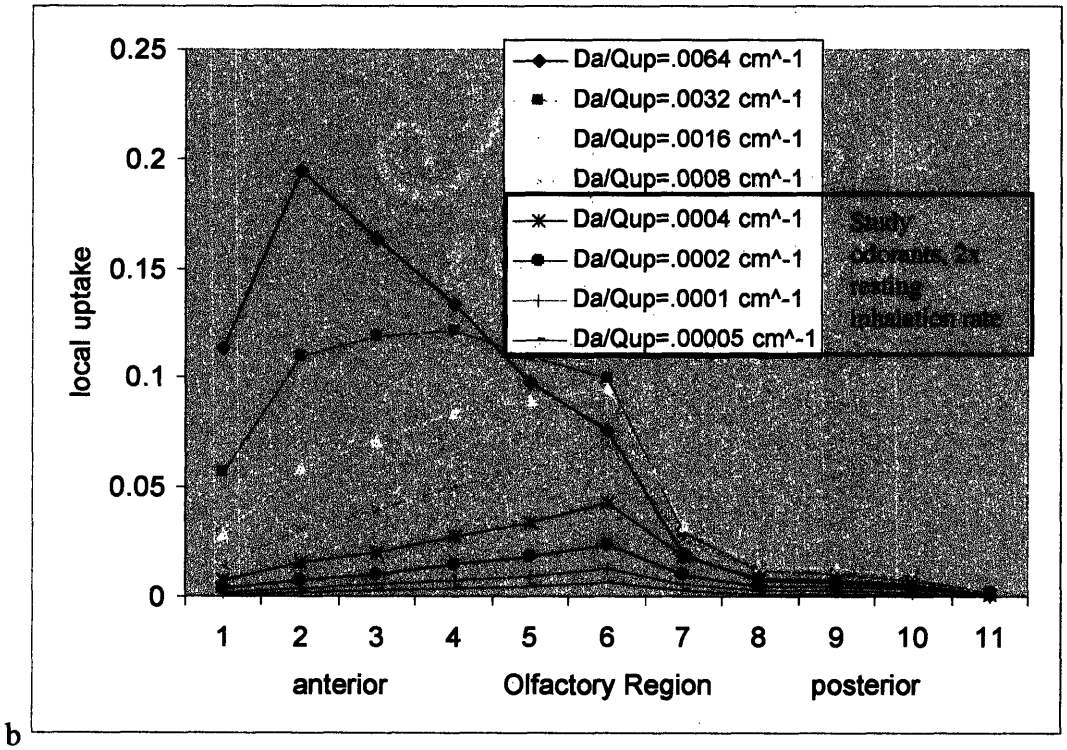


Fig. 16: Effects of Diffusivity in air (Da, cm^2/sec) and inhalation flow rate (Qup, mL/sec) on Local Uptake Efficiency of the olfactory region (LUEolf). Human (*Homo sapiens*) nasal cavity



a



b

Fig. 17: Effect of diffusivity in air (Da, cm^2/sec) and upper nasal cavity flow rate (Qup, mL/sec) on distribution of uptake in the human (*Homo sapiens*) nasal cavity. a) cumulative uptake, b) fractional uptake

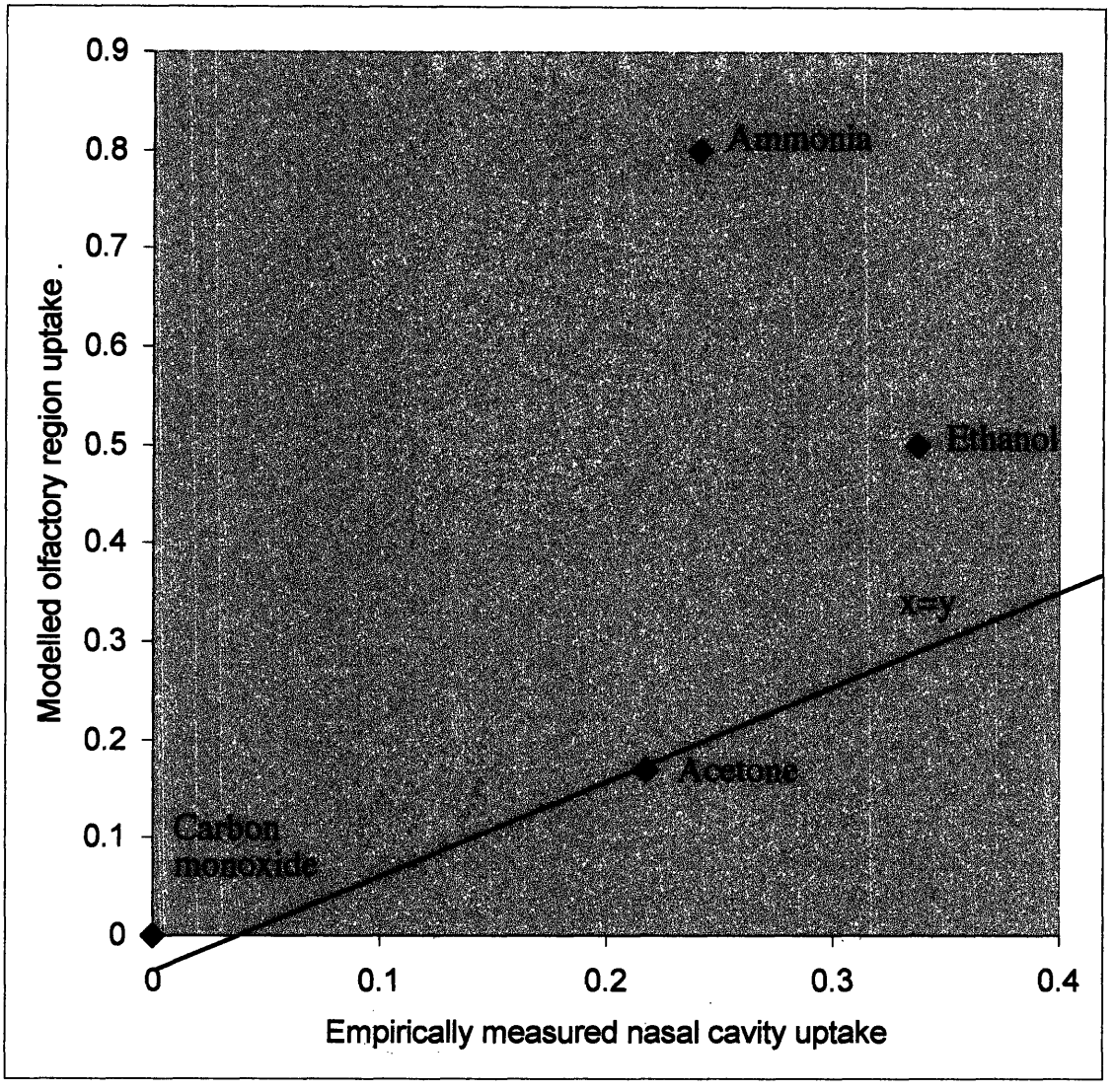
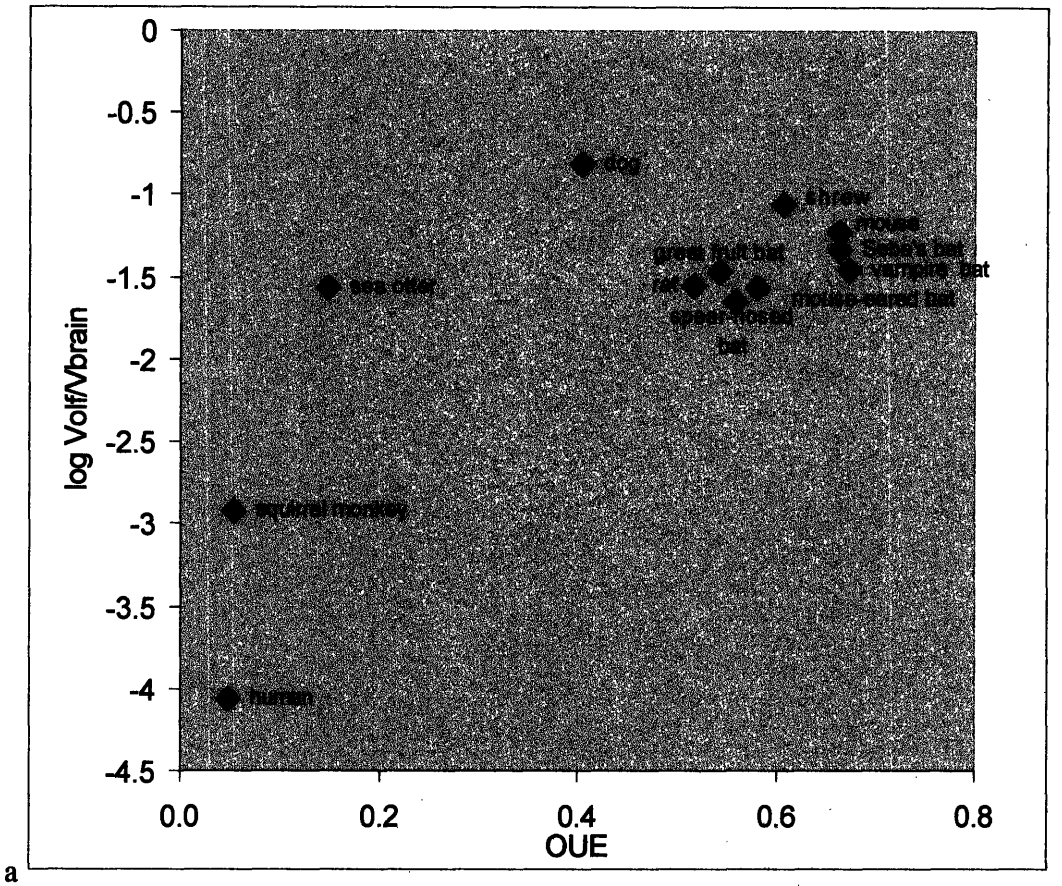


Fig. 18: Model output versus empirical results for proportional uptake of four compounds in the human (*Homo sapiens*) nasal cavity.

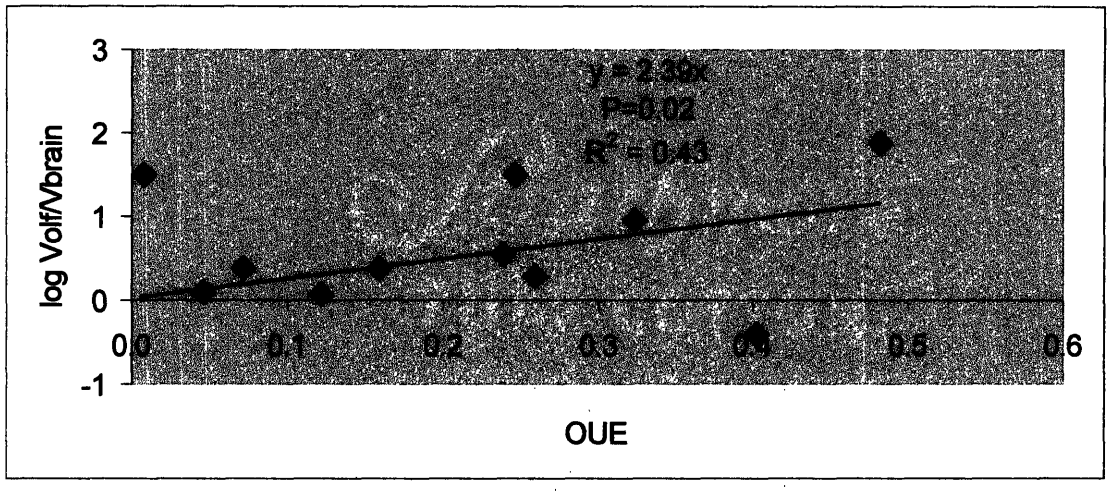
Uptake=1-(concentration inhaled/concentration exhaled)

Data from Morgan and Monticello, 1990.



a

Fig. 19a: Log-transformed ratio of olfactory bulb volume to brain volume vs OUE. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). a) species values



b

Fig. 19b: Log-transformed ratio of olfactory bulb volume to brain volume vs OUE. Human (*Homo sapiens*), Common squirrel monkey (*Saimiri sciureus*), House mouse (*Mus musculus*), Brown rat (*Rattus norvegicus*), Seba's short-tailed bat (*Carollia perspicillata*), Vampire bat (*Desmodus rotundus*), Pale spear-nosed bat (*Phyllostomus discolor*), Great fruit bat (*Artibeus literatus*), Mouse-eared bat (*Myotis myotis*), European shrew (*Sorex araneus*), Domestic dog (*Canis familiaris*), Sea otter (*Enhydra lutris*). b) contrast values

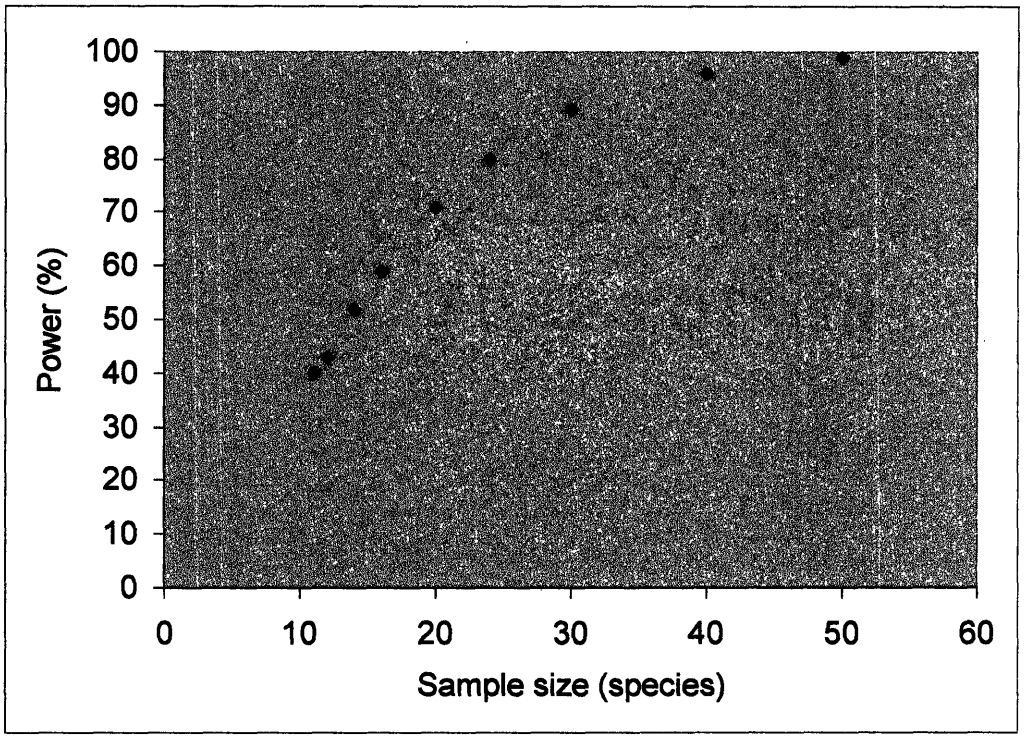
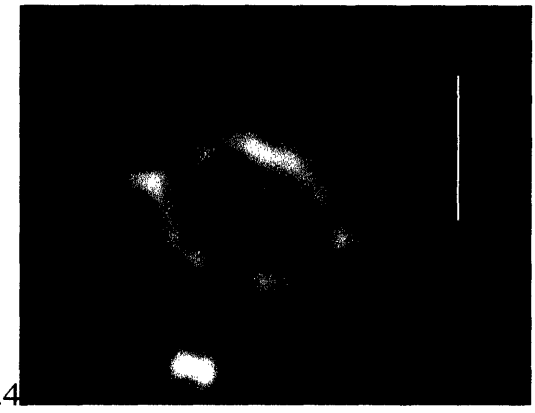
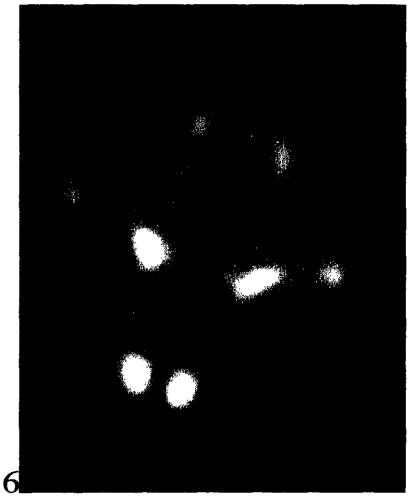


Fig. 20: Power analysis of OUE vs. AT regression based on signal to noise ratio, $R^2/(1-R^2)$. Power=probability that an experiment of a given sample size will yield a P value of <0.05



0.4



0.6

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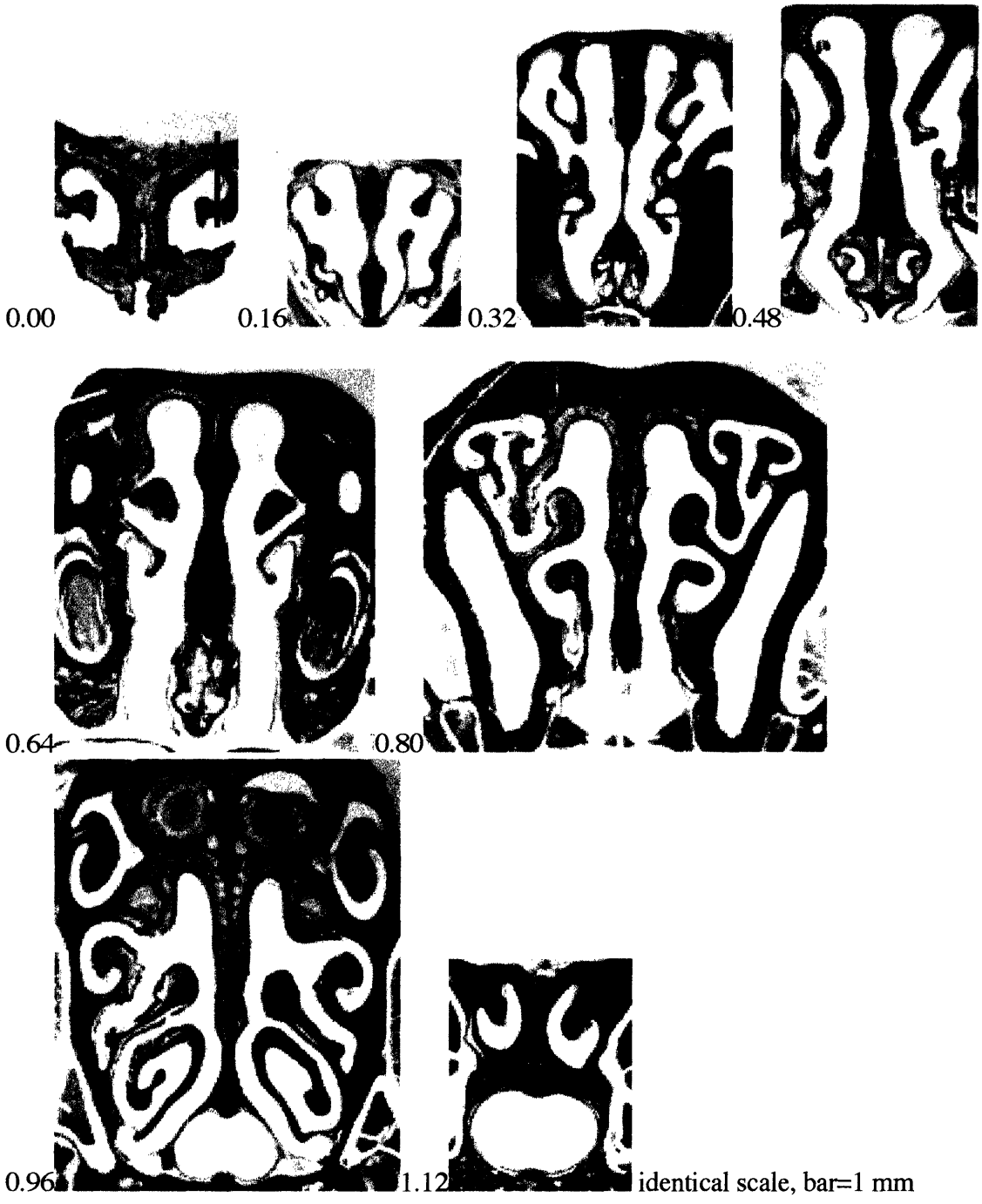
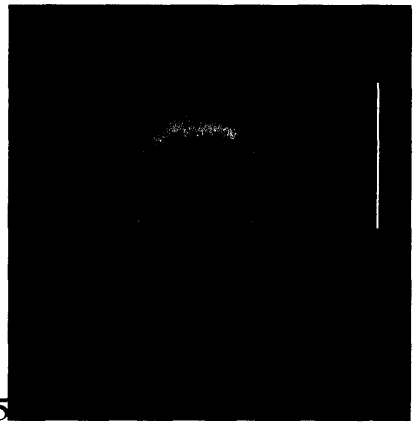


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. a)House mouse, *Mus musculus* (distances measured in cm from the rostral end)

0.25



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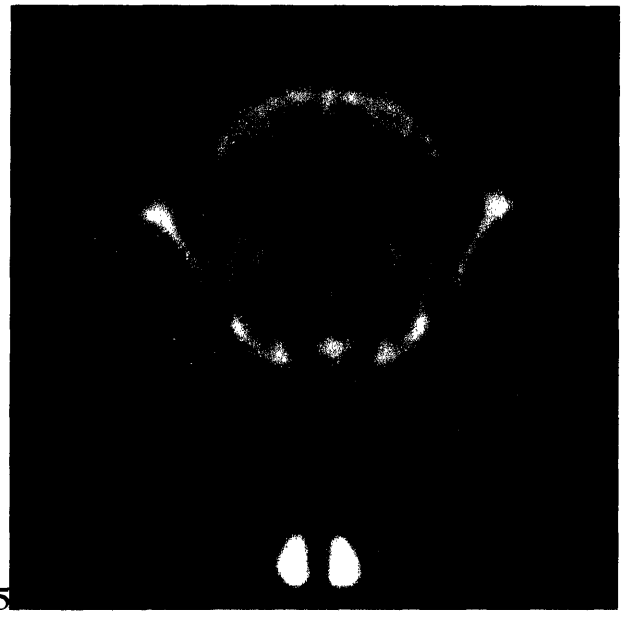

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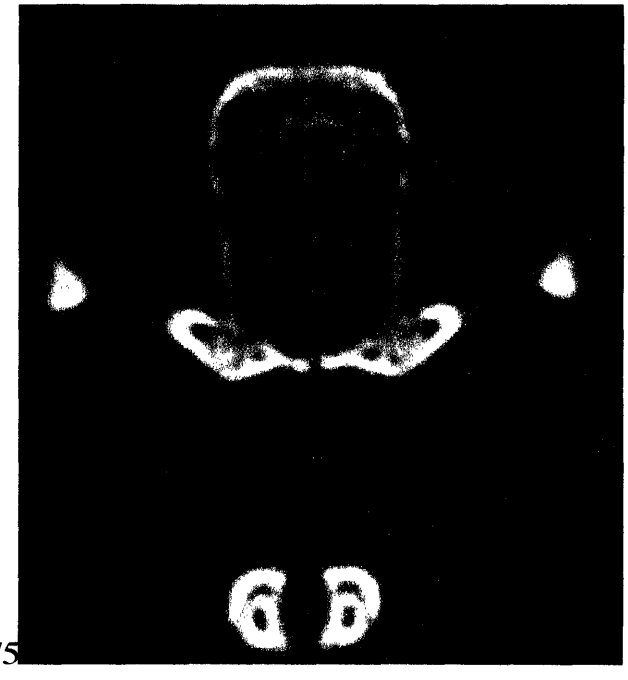

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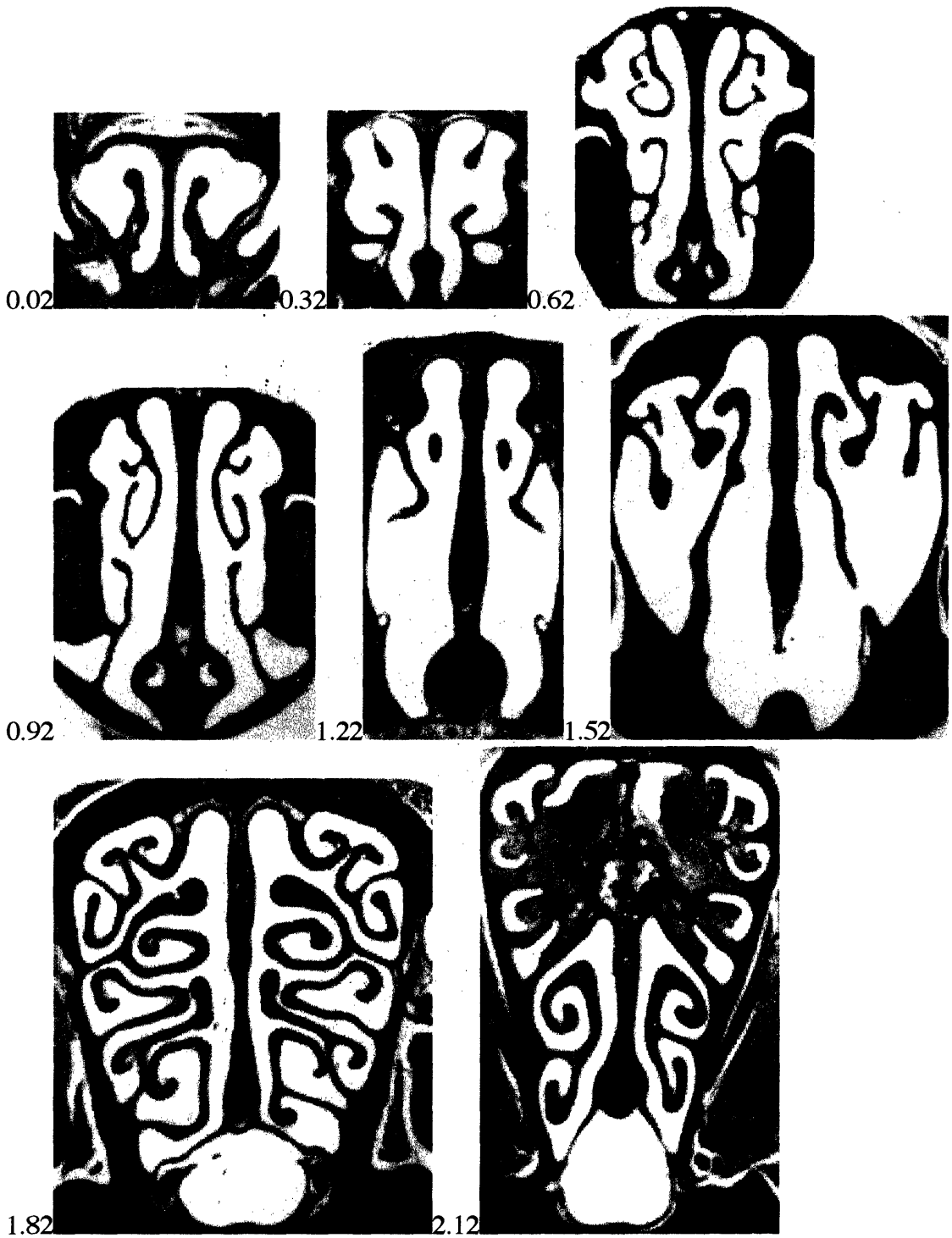

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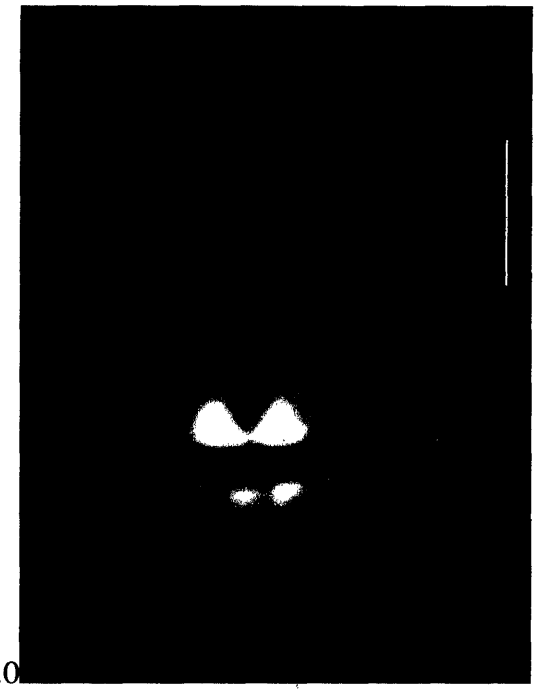
Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. b) Brown rat, *Rattus norvegicus* (distances measured in cm from the rostral end)



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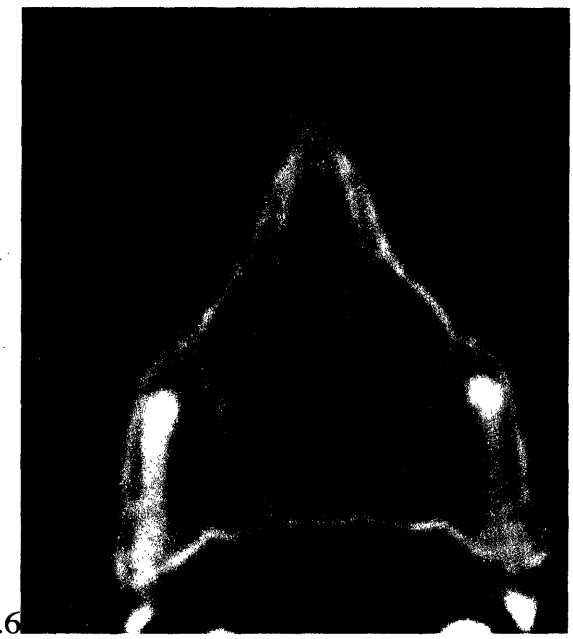

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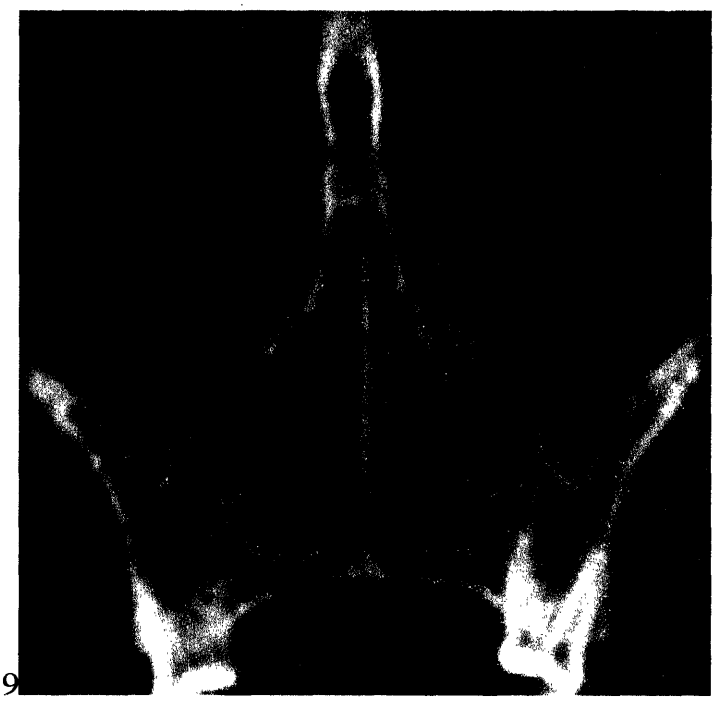

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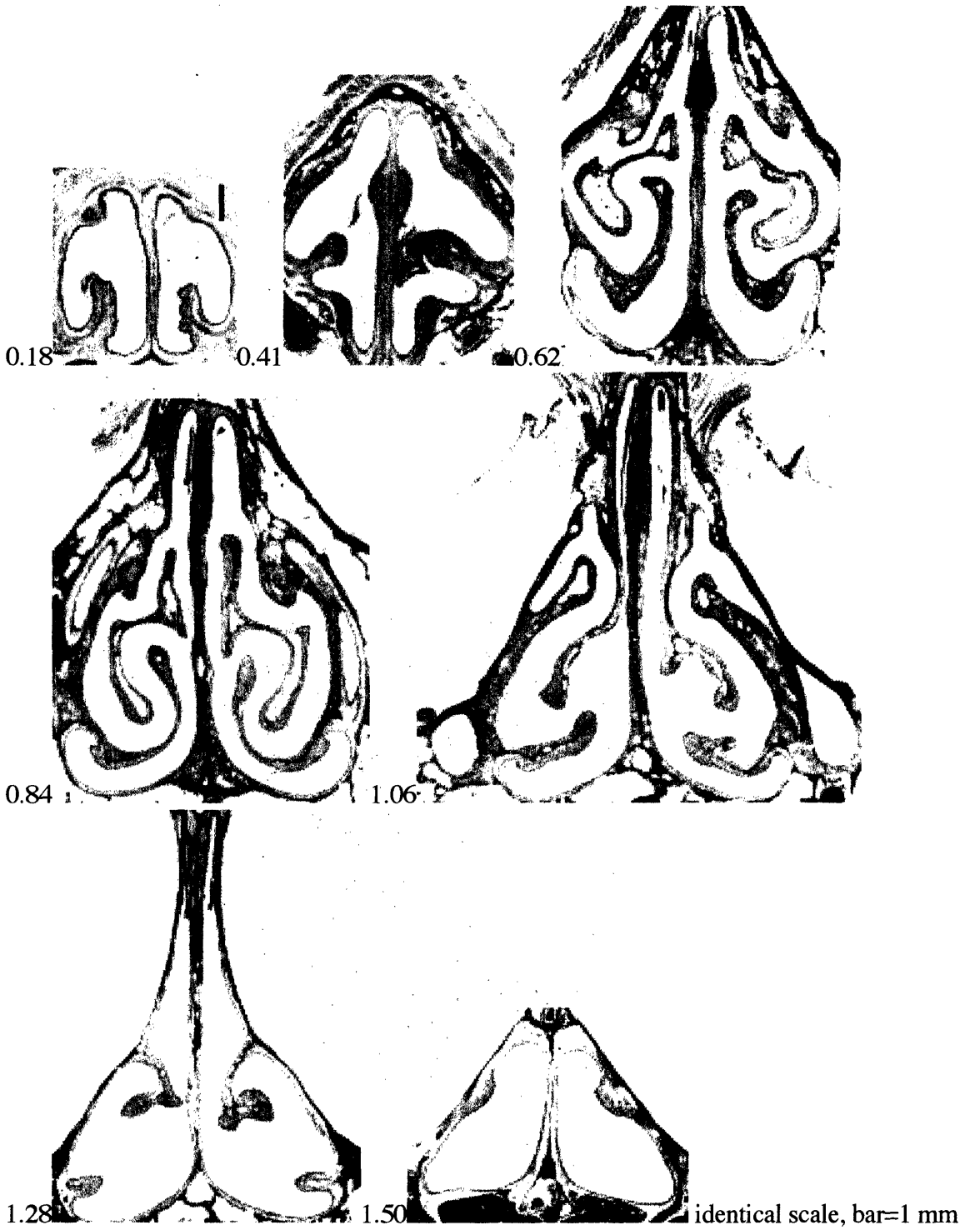
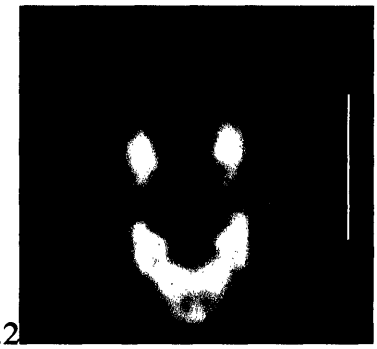


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. c) Common squirrel monkey, *Saimiri sciureus* (distances measured in cm from the rostral end)



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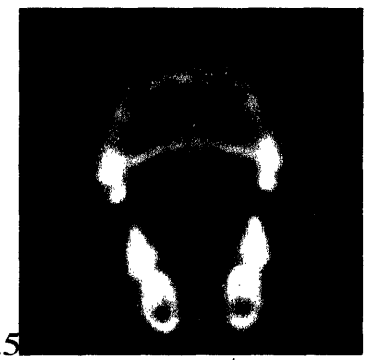

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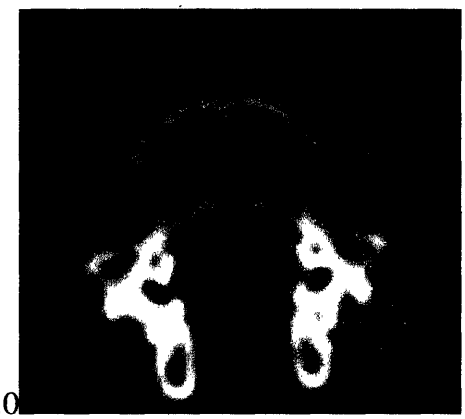

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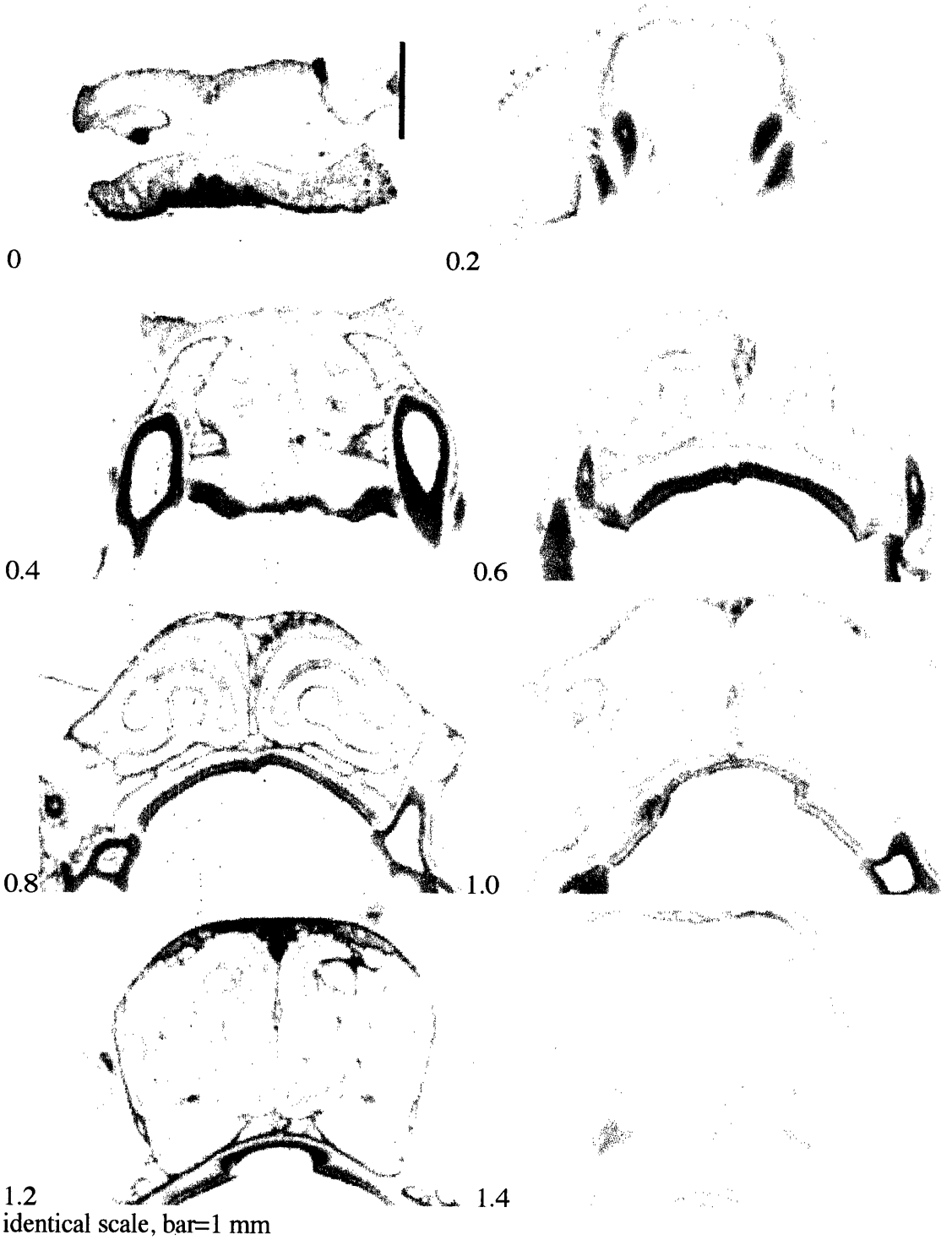

Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page.
d) Mouse-eared bat, *Myotis myotis* (distances measured in cm from the rostral end)

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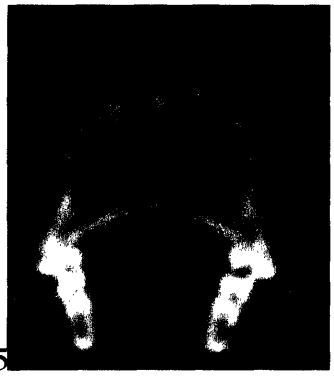

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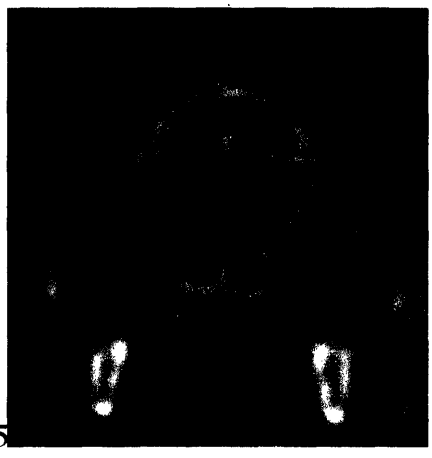

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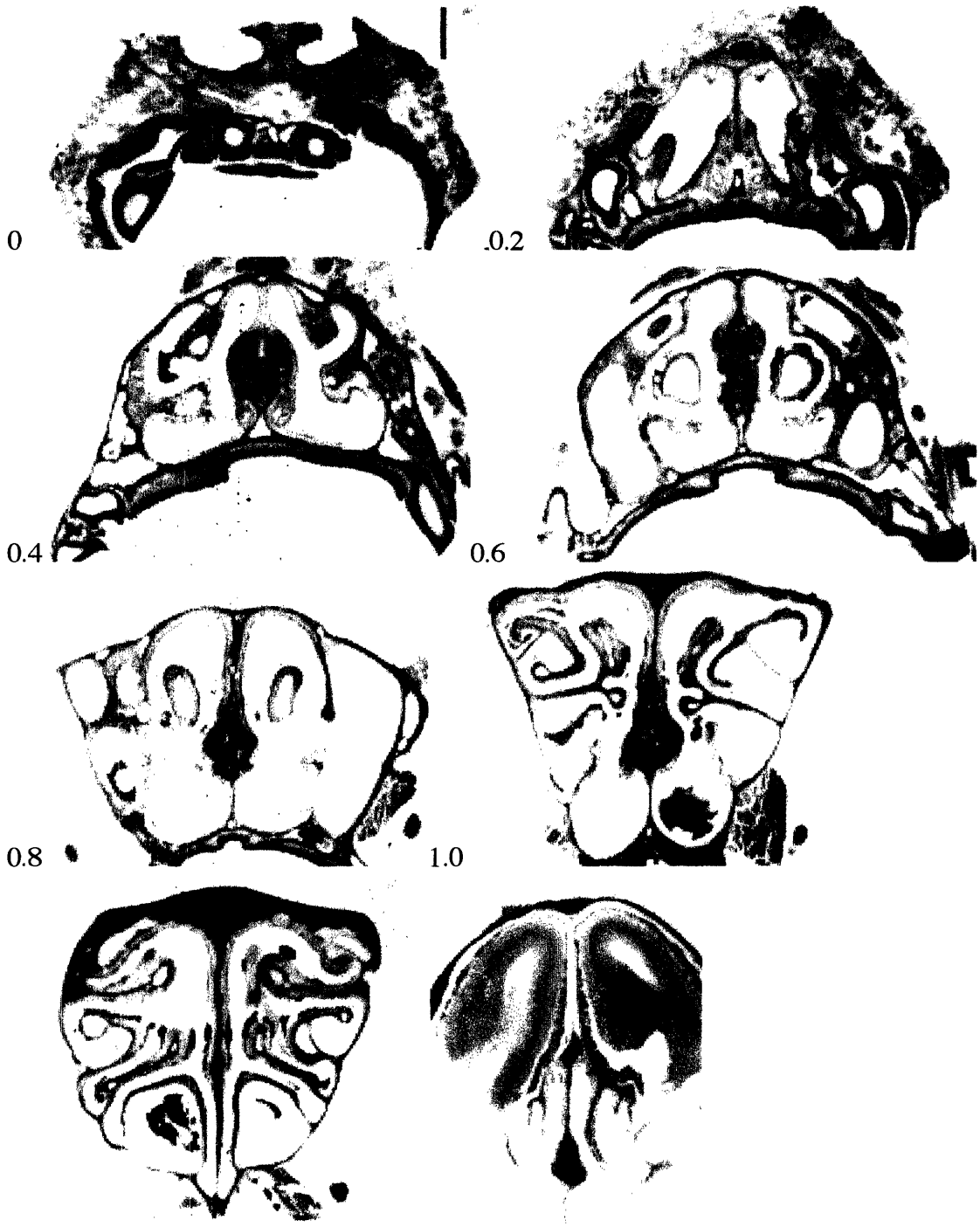
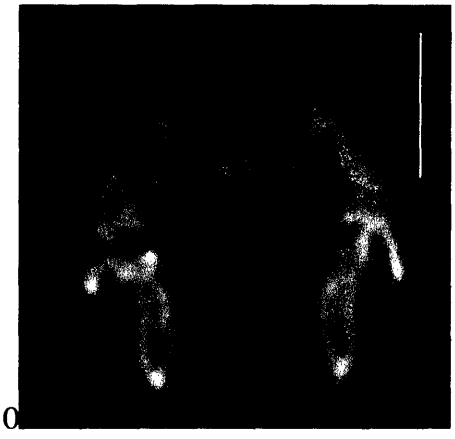


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. e) Pale spear-nosed bat, *Phyllostomus discolor* (distances measured in cm from the rostral end)



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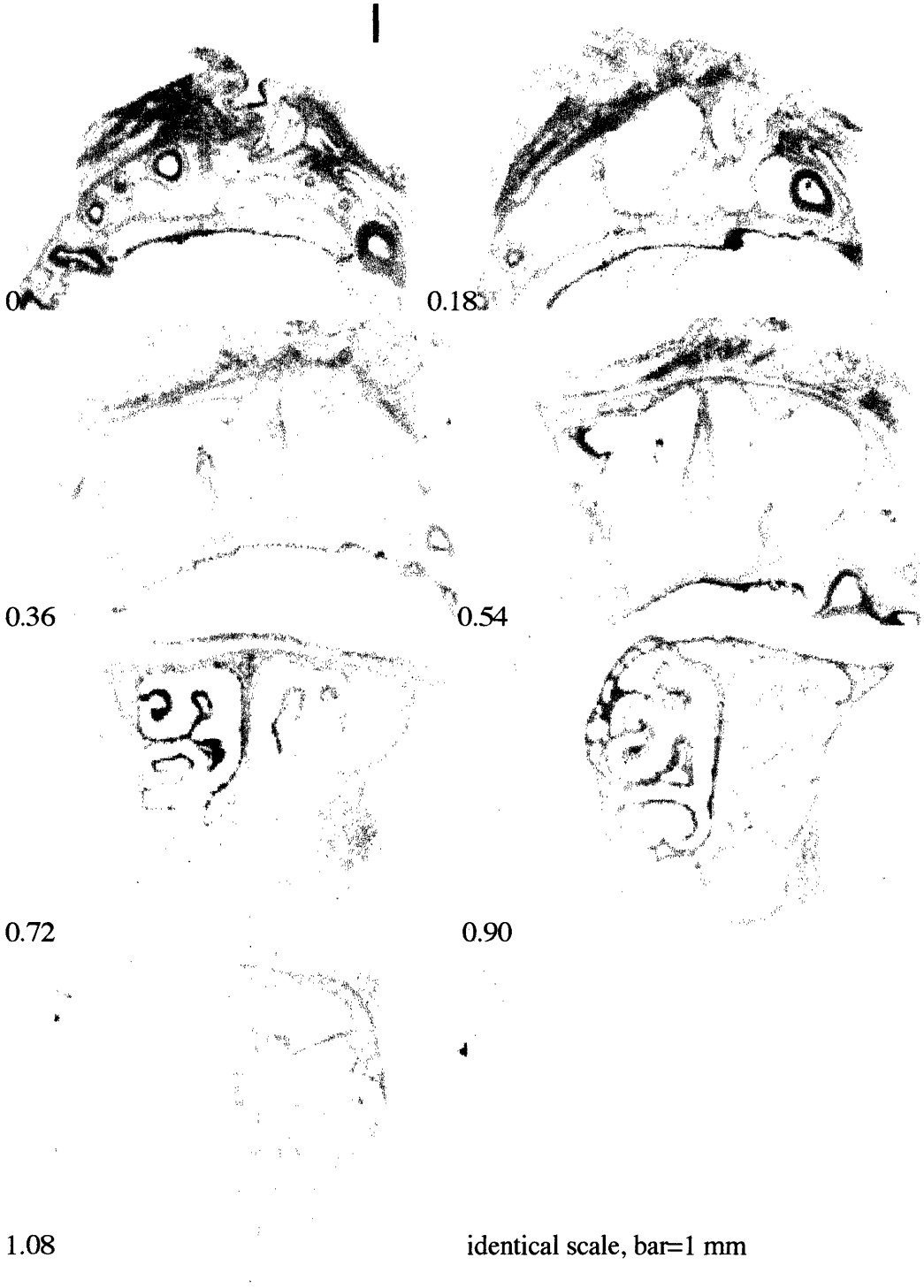
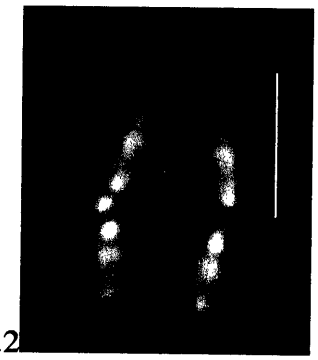


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. f) Great fruit bat, *Artibeus literatus* (distances measured in cm from the rostral end)



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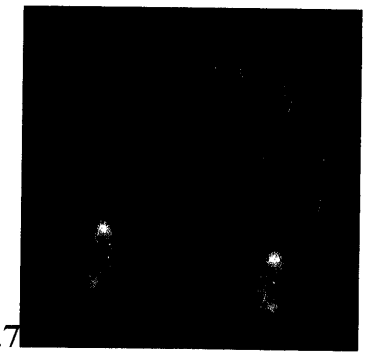

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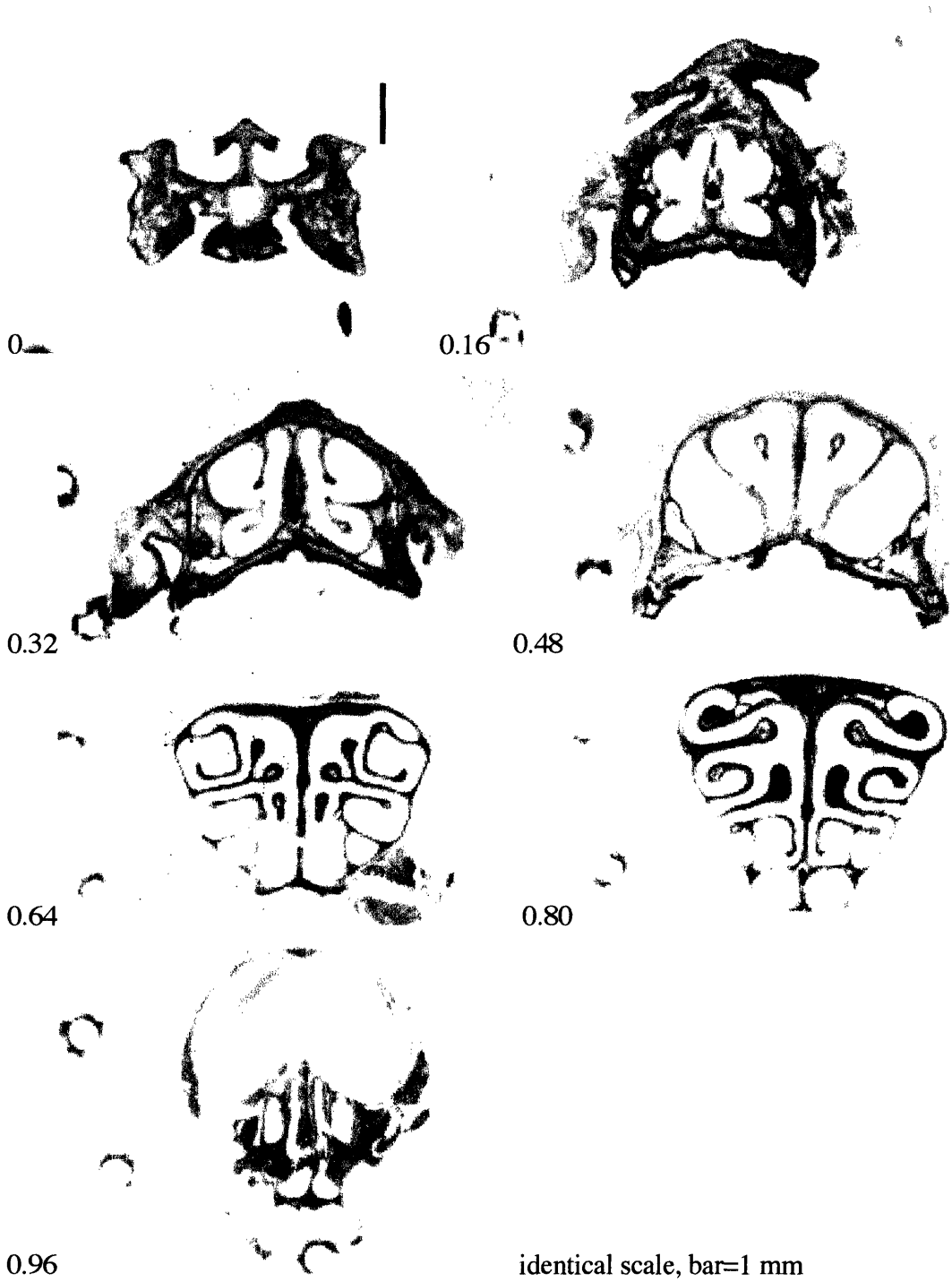
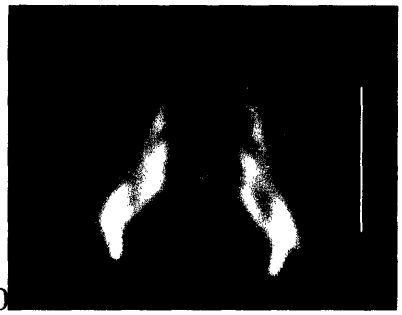


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page. g) Seba's short-tailed bat, *Carollia perspicillata* (distance measured in cm from rostral end)



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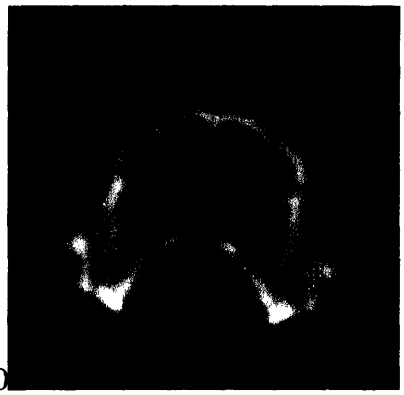

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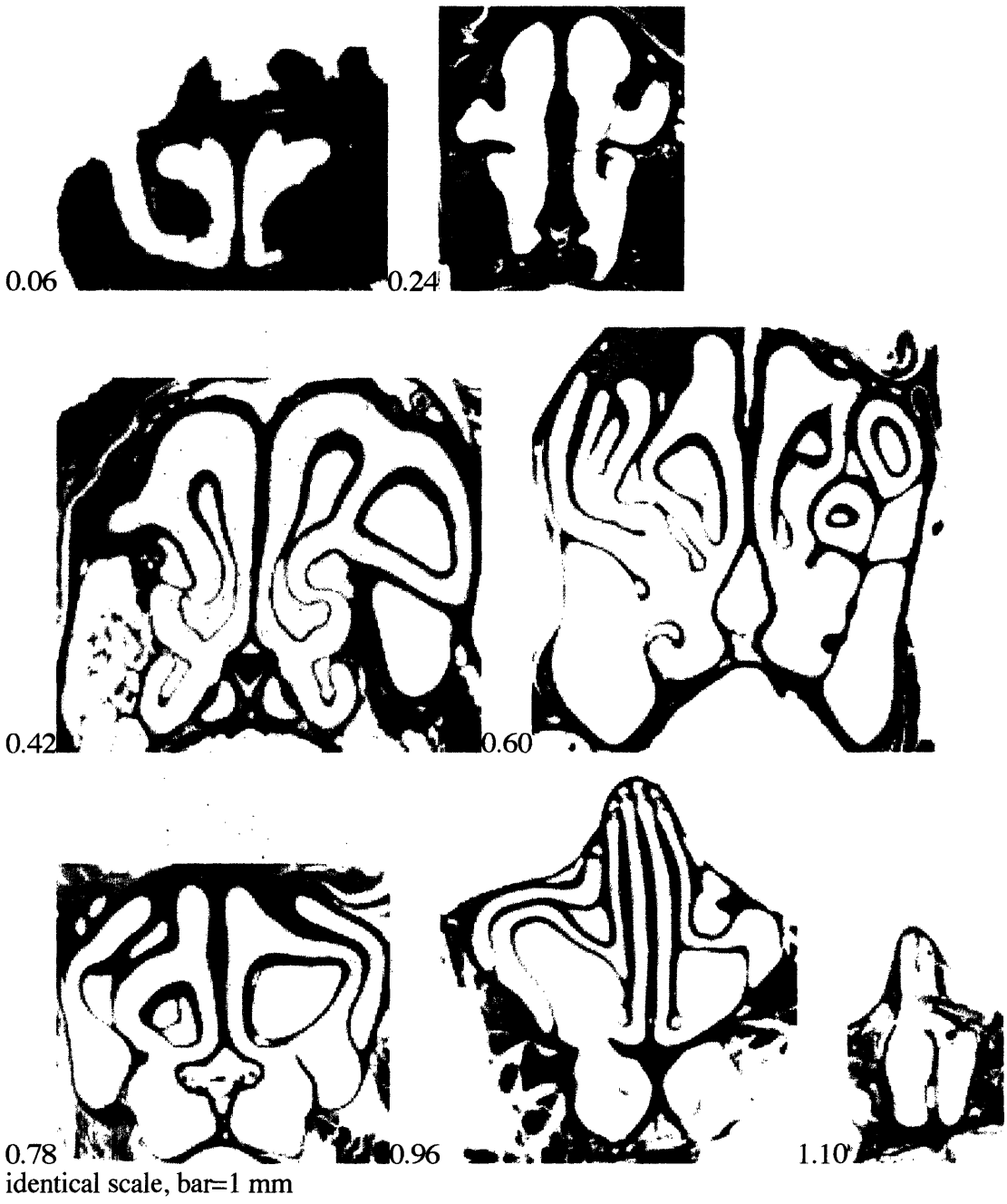
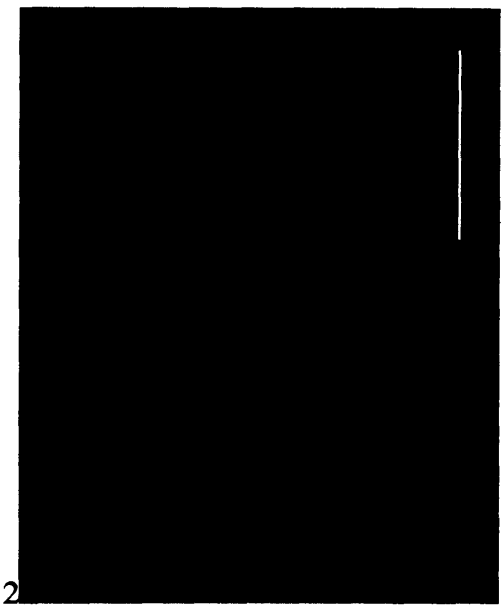


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page.

h) Vampire bat, *Desmodus rotundus* (distances measured in cm from the rostral end)



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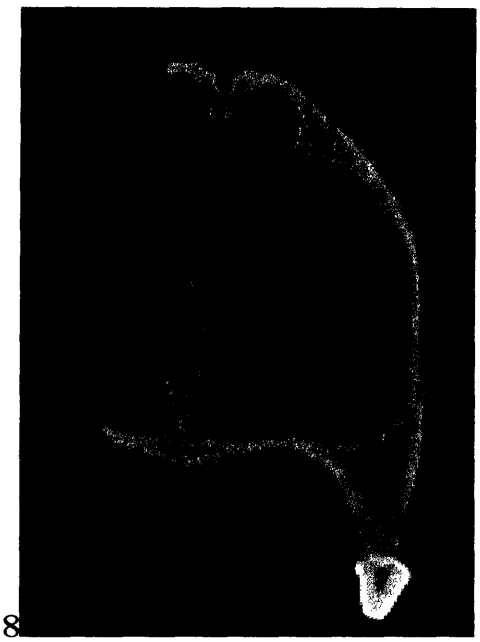

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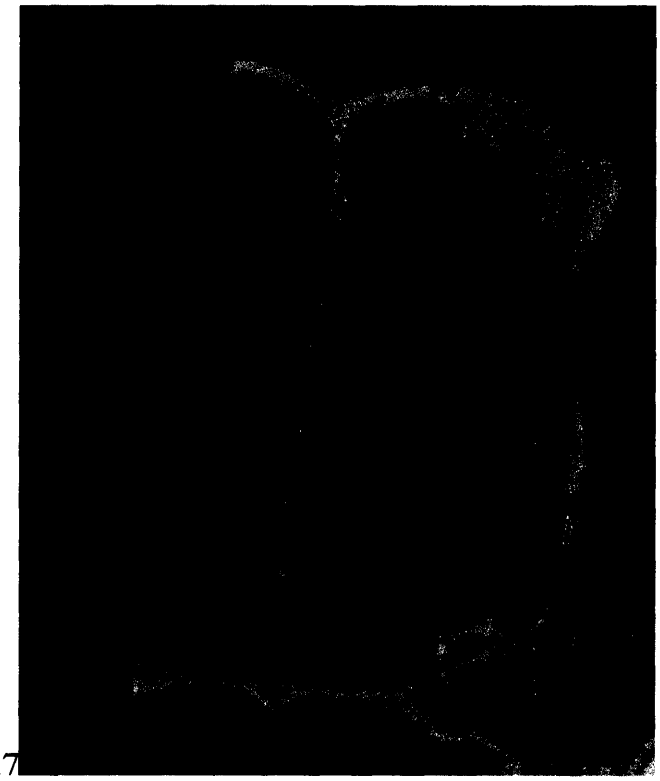

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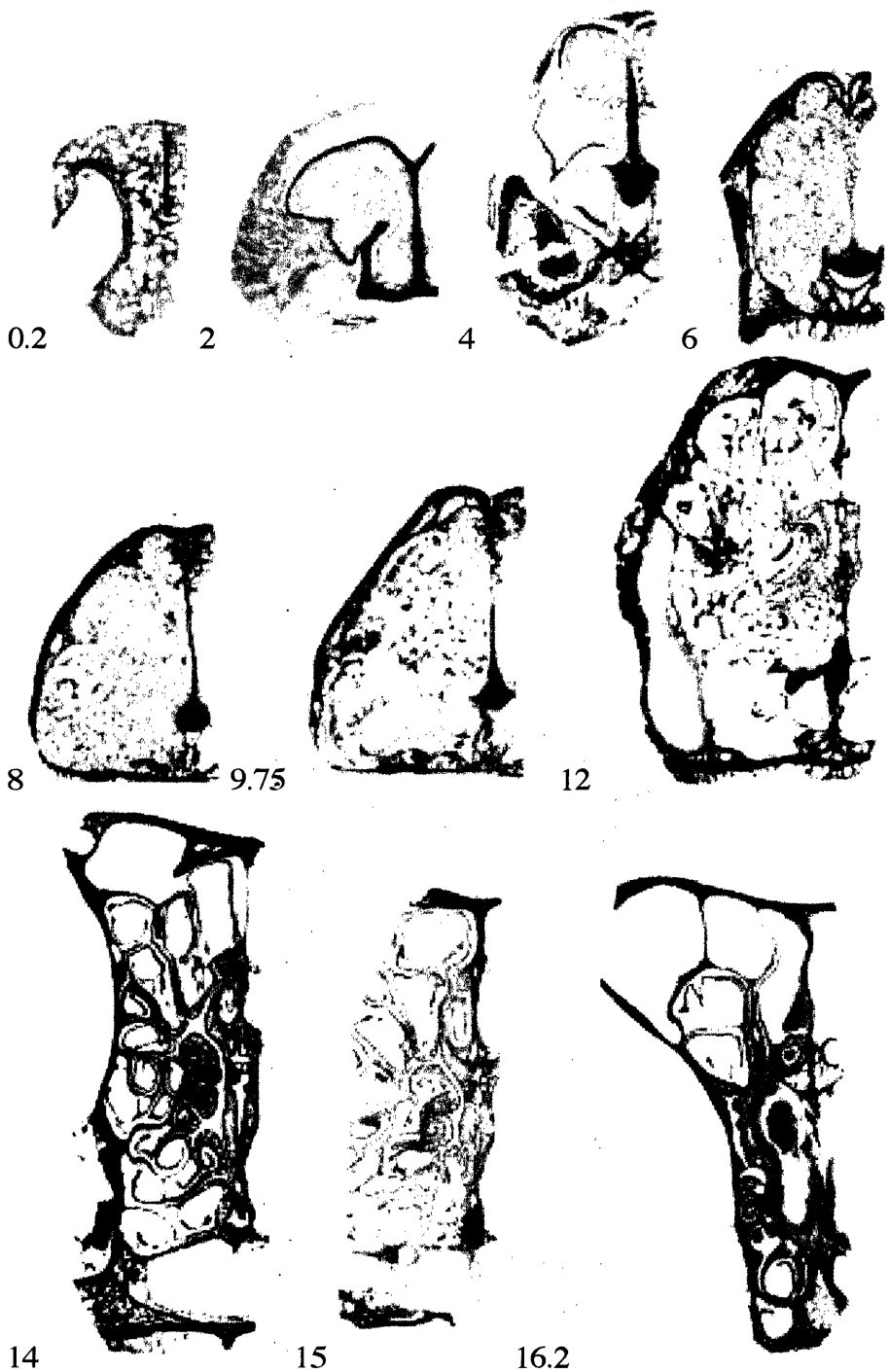

Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page.

i) Domestic dog, *Canis familiaris* (distances measured in cm from the rostral end)

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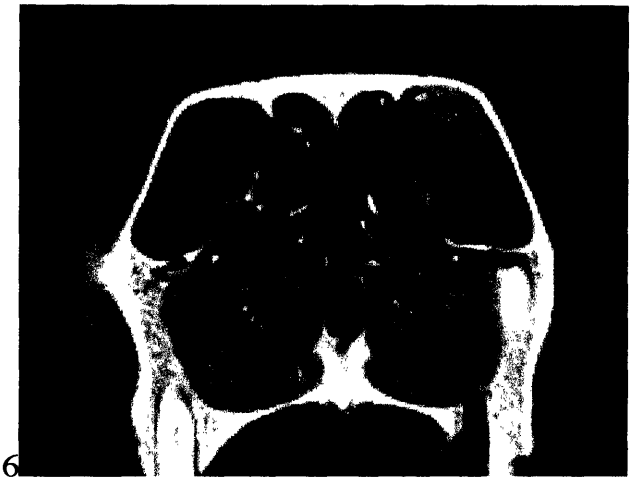

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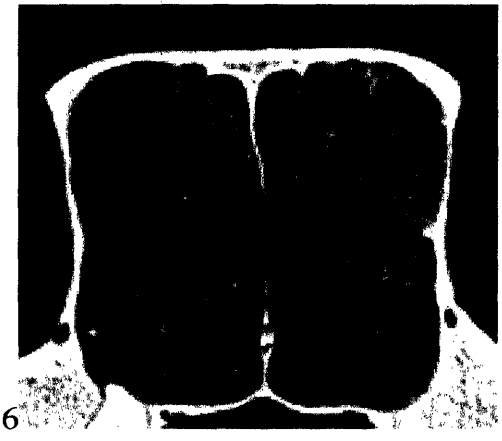

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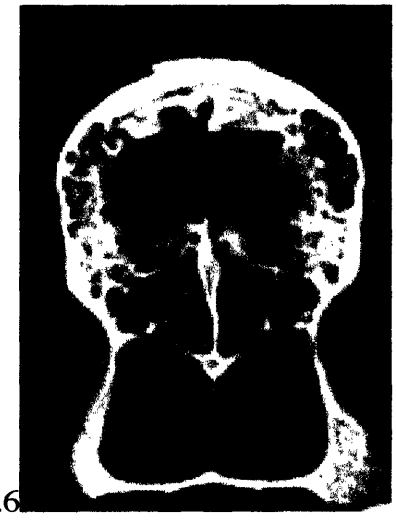

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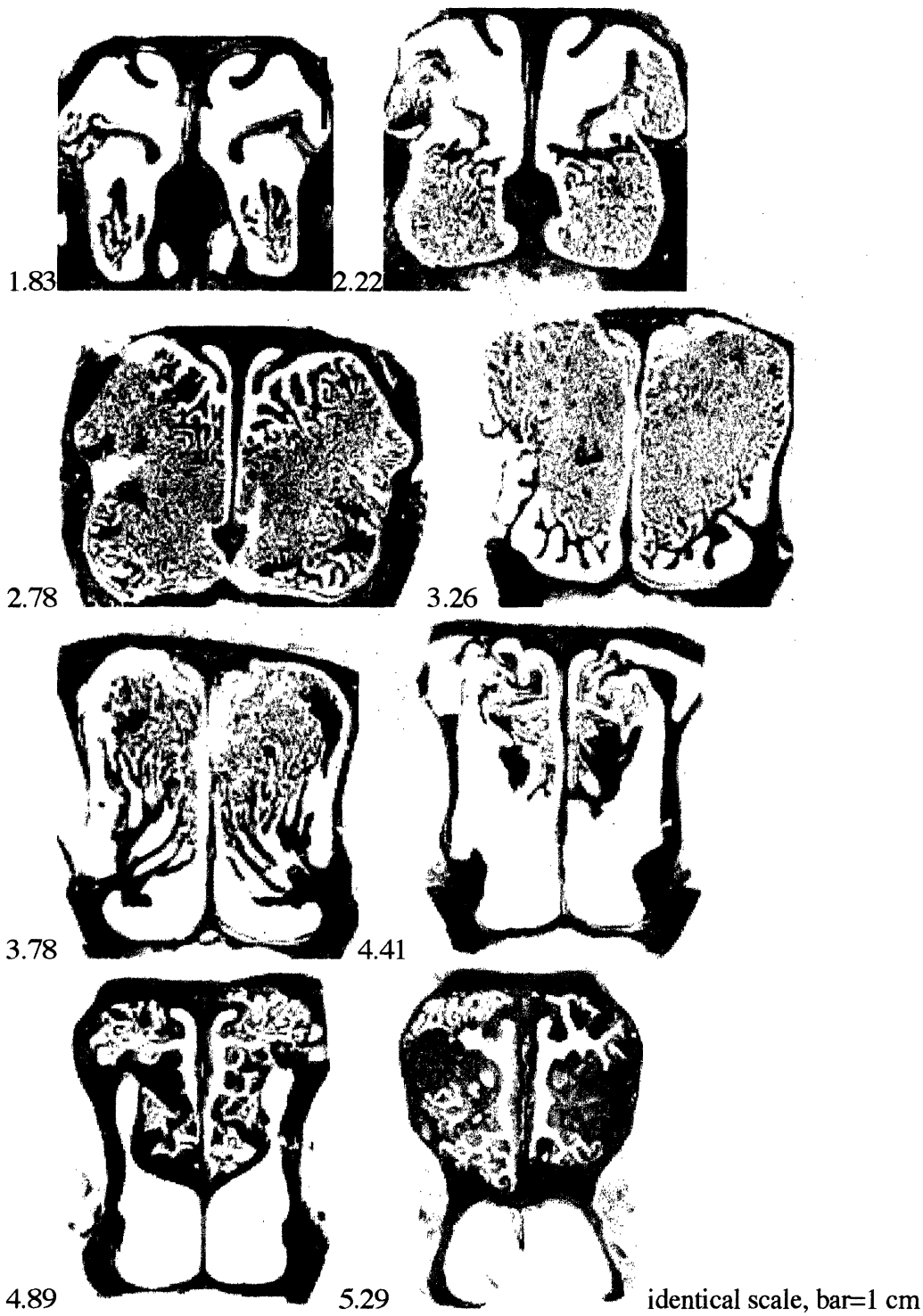
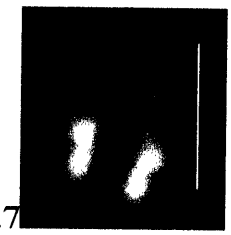


Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page.

j) Sea otter, *Enhydra lutris* (distances measured in cm from the rostral end)



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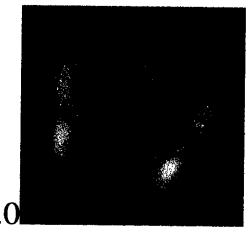

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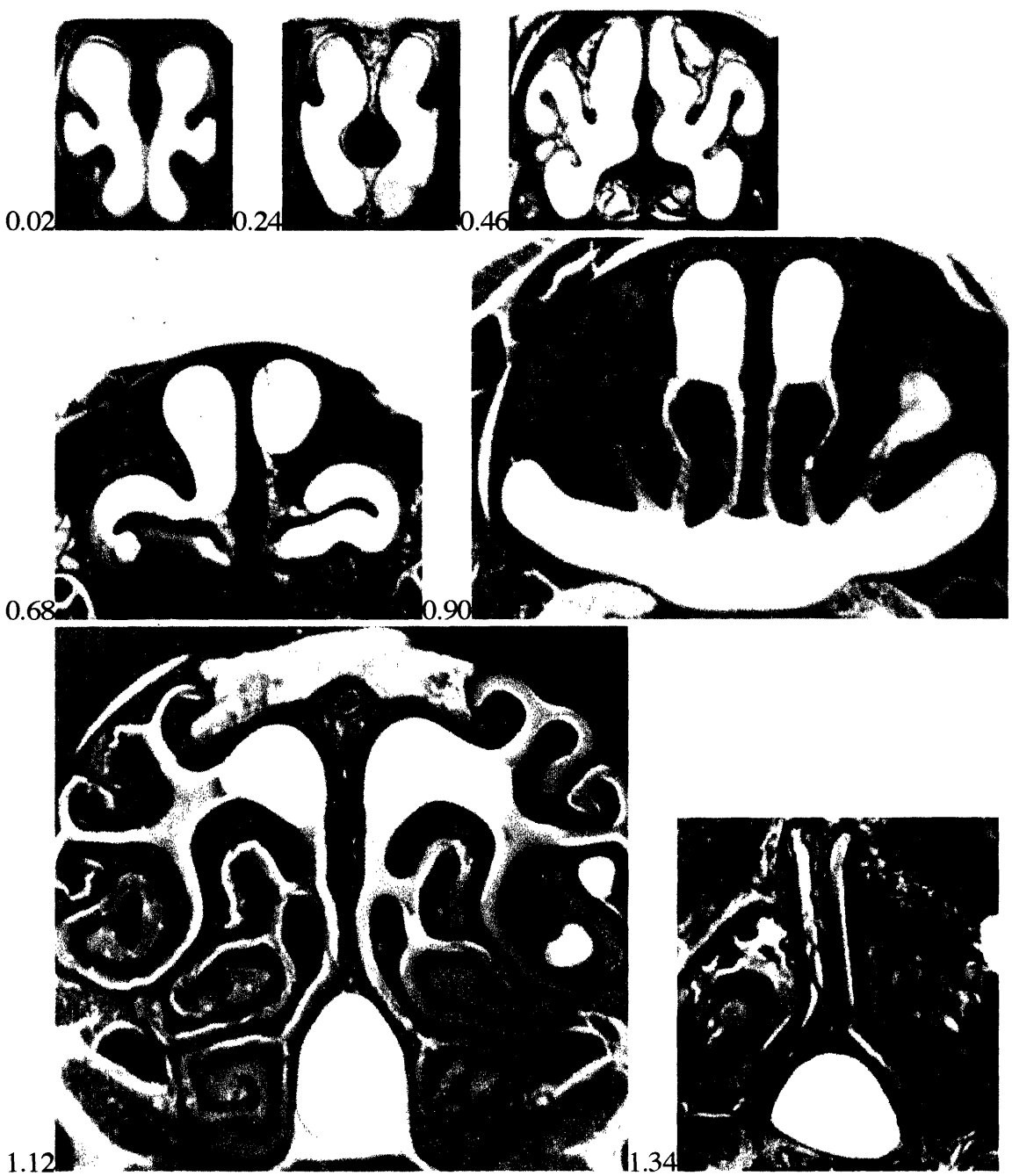

Fig. 21: sample nasal cavity sections; histological sections above, CT scans on facing page.

k) European shrew, *Sorex araneus* (distances measured in cm from the rostral end)

	Great fruit bat (*Artibeus literatus*)	Human (*Homo sapiens*)	House mouse (*Mus musculus*)	Seba's bat (*Carollia perspicillata*)
Qup/Qtot	0.821	0.246	0.732	0.642
	0.617	0.279	0.726	0.778
LUE	0.691	0.227	0.912	0.950
	0.838	0.149	0.906	0.923
OUE	0.567	0.558	0.667	0.610
	0.517	0.414	0.658	0.718

Table 2: Individual values of model output for species of which two specimens were measured.

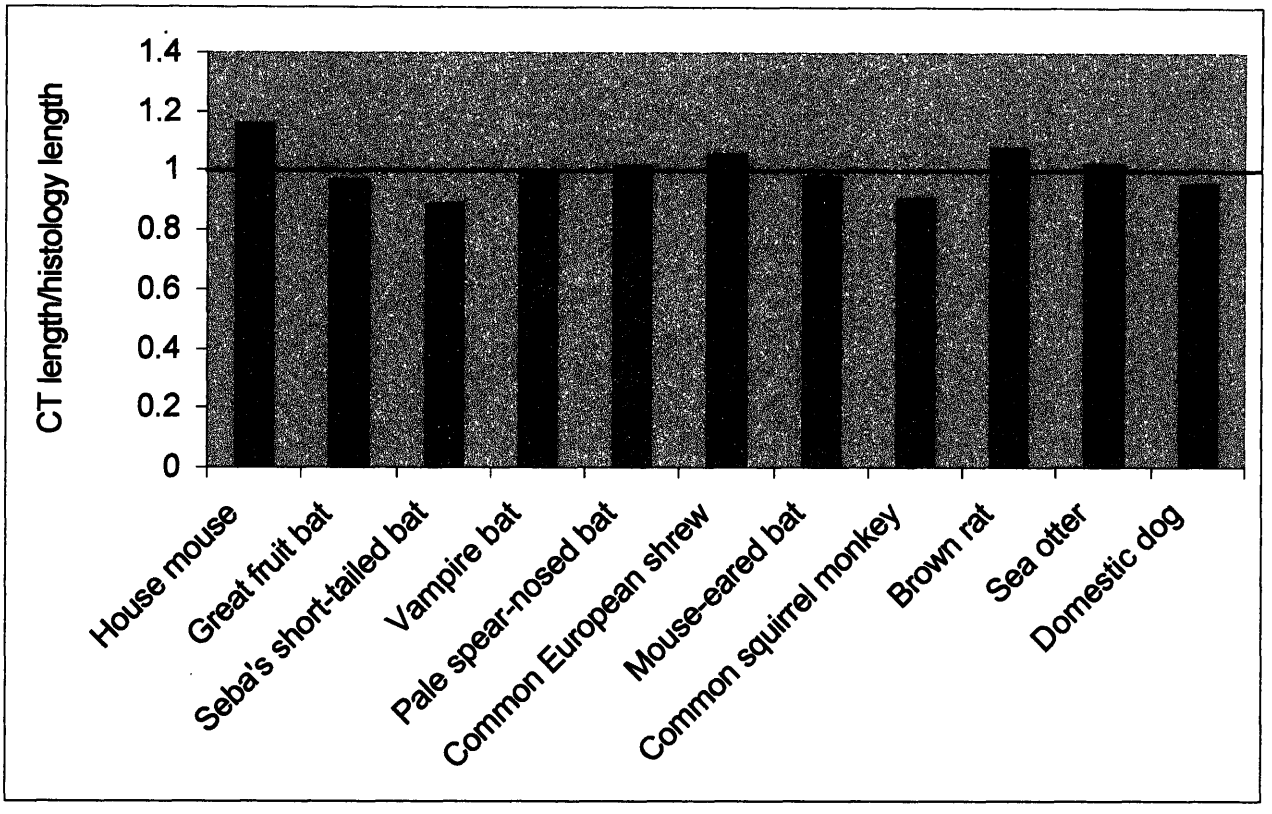

Fig. 22: Ratio of nasal cavity length measured from CT scans to nasal cavity length measured from histological sections. Species arranged in ascending order of nasal cavity length.

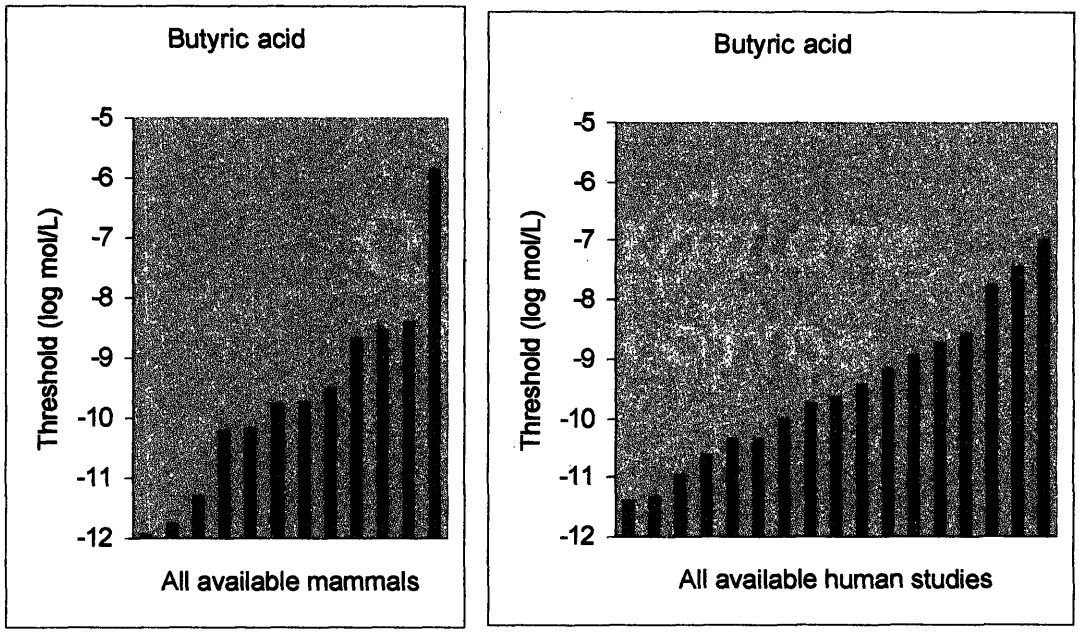

Fig. 23. Variation in published olfactory thresholds for butyric acid. Data from Bretting, 1972; Devos *et al*, 1990; Hubener & Laska, 2001; Laing *et al*, 1989; Laska, 1990; Laska *et al*, 2000; Moulton *et al*, 1960; Obst *et al*, 1976; Schmidt, 1981; Schmidt, 1975; Sigmund & Sedlacek, 1985, this study

Chapter 5: Summary and Conclusions

This project utilized such data as is available for mammals to test a candidate source of selection pressure for specific sensitivity, (dietary chemical ecology), a candidate source of selection pressure for general sensitivity (terrestrial versus marine habitat) and a candidate mechanism of increasing general sensitivity (nasal cavity morphometry).

The question of presumed olfaction-eroding habitat, specifically marine habitat, was addressed through the sea otter. Many marine mammal species appear to have vestigial or dysfunctional olfactory systems. If it is the marine habitat that reduces the importance of nasal chemoreception, then the sea otter should also have shown impaired olfactory function. However, if it is the particular dive and respiration habits of the Cetacea that are responsible, the sea otter should have unimpaired olfactory function. The typical mammalian olfactory thresholds measured in the sea otter and reported in Chapter 2 show that reduced olfactory function need not occur in a marine species that breathes freely at the surface most of its life.

Considering the widely varying natural distributions of volatile chemicals, the adaptive importance of detecting each must also vary widely for any animal, depending on the value of the information that the chemical can provide, for example about the location and nature of its source. If specific sensitivities to different odorants evolve independently, as is suggested by our current knowledge of the molecular biology of olfaction, then it is reasonable to expect sensitivity to different compounds to be related to their usefulness in detecting and identifying objects of importance such as food items.

The results of the specific sensitivity comparisons reported in Chapter 3 show that in some cases, notably between a marine carnivore, the sea otter, and a terrestrial carnivore, the domestic dog, and between two primates with divergent dietary habits, the omnivorous human and the frugivorous squirrel monkey, differences in dietary importance are reflected in specific sensitivity. In other cases, however, exemplified by the chiroptera, diet leaves no signal in the olfactory sensitivity repertoire. These cases may reflect competing odorant sources of greater ecological importance than diet, especially if food searches are conducted primarily in other sensory modalities. In no case did the dietary significance signal swamp out sensitivity trends related to odorant chemical structure which may plausibly result from overlap between sister odorants in the combinatorial olfactory receptor code.

No measure has yet been described that is strongly related to olfactory sensitivity differences among species. The results of Chapter 4 clearly show that the morphometry of the nasal cavity is strongly related to olfactory neuroanatomy in the brain. This striking result implies a balance of anatomical investment in olfactory structures presumably adaptive for maximizing functional return on that investment. However, neither of these important anatomical features is as strongly related to measured sensitivity as they are to each other. Considering the relative difficulty of accurate behavioral sensitivity measurement compared with morphometric measurement, variation in the behavioral dataset is likely to be largely responsible for this difference.

Chapter 6: Glossary

Olfactory threshold: lowest airborne concentration of odorant that can be distinguished from odourless air (specific to individual olfactory and odorant)

Average Threshold (AT): a representation of average olfactory sensitivity for a species, the mean of log-transformed values of seven widely available olfactory thresholds: acetic acid, propionic acid, butyric acid, ethanol, butanol, ethyl acetate and amyl acetate

Incidence (I): A property of a particular odorant for a particular animal: the proportion of food items in the diet of the animal that contain the odorant

Olfactory Uptake Efficiency (OUE): the ratio of odorant molecules taken up by the olfactory mucus to total molecules inhaled

Local Uptake Efficiency (LUE): the ratio of odorant molecules taken up in an area to total molecules entering the area; e.g.: the olfactory region

Relative olfactory bulb volume (Q_{up}/Q_{tot}): ratio of the volume of the olfactory bulb to total brain volume

Chapter 7: Bibliography

Cited references are also listed immediately following each chapter.

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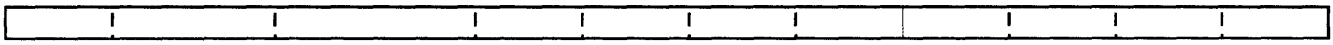
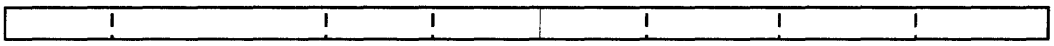
	MARINE ANIMAL						
COMPOUND\SOURCE	FISH		CRAB		SHRIMP		
SPECIES	whitefish	ayu (sweet smelt)	?	blue	?	Sergia lucens	Euphausia pacifica Hansen
methanol			9		9		
ethanol			9		9	3	14
propanol							
butanol		28				3	
1-pentanol (amyl alcohol)	5			4			
hexanol	5						
1-heptanol				4			
1-octanol	5			4			
1-nonanol	5			4			
decanol							
undecanol							
dodecanol							
tridecanol							
benzaldehyde	5			4		3	14
acetic acid	5						
propanoic acid							
butanoic acid							
pentanoic acid (valeric acid)							
hexanoic acid	5						
heptanoic acid							
octanoic acid							
nonanoic acid							
decanoic acid							
undecanoic acid							
dodecanoic acid							
tetradecanoic acid (myristic acid)					15		
pentadecanoic acid					15		
hexadecanoic acid (palmitic acid)					15		
heptadecanoic acid (margaric acid)					15		
octadecanoic acid (stearic acid)					15		
ethyl formate							
methyl acetate							
ethyl acetate		28				3	
propyl acetate							
butyl acetate							
amyl acetate							
hexyl acetate							
DMS/DMSP			9		9		
a-pinene							
B-pinene							

Eugenol							

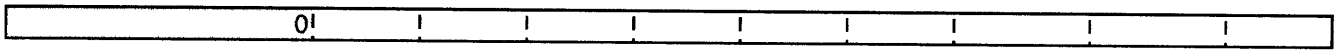
	OCTOPUS	CUTTLEFISH	OYSTER				CLAM			
Parapenaeus fissurus	?	?	?	west coast	east coast blue point	Crassostria gigas	C. virginica	west coast butter	east coast littleneck	Mya arenaria (soft-shell)
				6	6					
				6	6					
						83				
						83				
			10							
			10							
			10							
30				6	6					
	29	29	10	6	6		31			
	29	29	10	6	6		31			
			10							
			10				31			
							31			
30					1	16				1
							31			
							31			



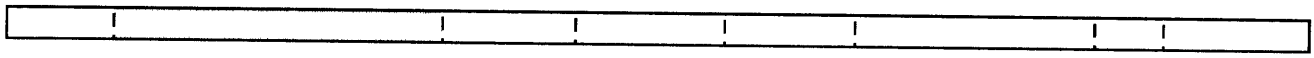
	SCALLOP		MUSSEL			DONAX	URCHIN	PRAWN
Short-necked (Tapes philippinarium)	Placopectin megallanicus	Placopecten yessoensis	Mytilus edulis	Volsella demissa	Volsella modiolus	?	?	?
			11					
		26						
						10	10	
						10	10	
						10	10	
						10	10	
							10	
27						10	10	
			11			10	10	
			11			10	10	
			11			10	10	
						10	10	
			11					
27		26						
27			11					
27		26						
27								
27								
			11					
27			11					
	1	26	1	1	1			



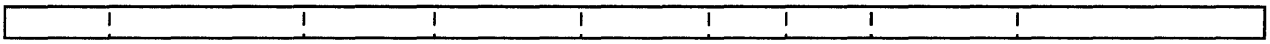
TOTAL MARINE ANIMAL	MARINE PLANT								
	KELP						SYNURA	GREEN	RED
24							S. petersenii	Scenedesmus incrassatulus	Corallina elongata
0.083333333									
0.291666667							230		
0									
0.083333333									
0.208333333									
0.166666667	7	7		7		7			
0.083333333								227	
0.208333333								227	
0.208333333								227	
0	7								226
0.125									
0.041666667									226
0	7			7		7			
0.416666667									
0.416666667							230		
0.375							230		
0.166666667									
0.166666667							230		
0.083333333									
0									
0.083333333									
0.083333333									
0.083333333									
0									
0									
0.083333333	7	7	7	7	7	7			
0.041666667									
0.083333333	7	7	7	7	7	7			
0.041666667									
0.041666667								227	
0.041666667									
0									
0.208333333							230		
0									
0									
0									
0									
0.375									
0.041666667									
0.041666667									


0

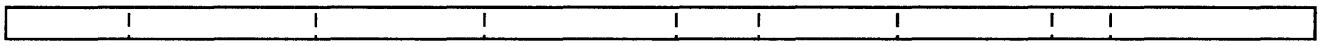
	TOTAL	LAND ANIMAL						
	MARINE PLANT	TURKEY	CHICKEN	RABBIT	CATTLE		PIG	MUTTON
Palmaria palmata	10			European (Oryctolagus cuniculus)		Milk, Italian Simmental	Lean or Iberian	
	0.000							
	0.100				24	232		
	0.000							
205	0.100			206	23,24	232		
	0.000		21		23,24	232		
	0.400				23,24	232	50	
	0.100			206	23,24		50	
	0.100				23,24		50	
	0.100							
	0.200							
	0.000							
	0.100							
	0.300		22					
				206	23,24			
	0.100						50	
	0.100			206				
	0.000			206			50	25
	0.100							25
	0.000							25
	0.000							25
	0.000						50	25
	0.000						50	25
	0.000							25
	0.000							25
	0.000							
	0.600							
	0.000							
	0.600							
	0.000							
	0.100							
	0.000							
	0.000					232		
	0.100					232		
	0.000							
	0.000							
	0.000							
	0.000							
	0.000							
	0.000			107				
	0.000							



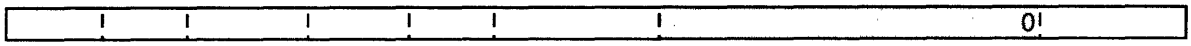
LAMB	MARMOSET	HUMAN	BADGER	PANDA	FOX	LION	BOBCAT	GARTER SNAKE
	Callithrix jacchus	H. sapiens	Meles meles	Giant, Ailuropoda melanoleuca	Vulpes vulpes	Panthera leo	Lynx rufus	Thamnophis sp.
						180		
				122				
		136		122				
		136		122				
				122				
		136						
	192							
	192							
		95						
	192	95	111	122		94		
	192	95, 136, 234		122	94	94		99
	192	95, 234		122	94	94		99
25	192	136, 234		122	94	94		99
25	192	234		122	94			
25		95, 234		122				
25		95, 234		122				
25		95, 136, 234		122				
25		95, 136, 234						
25		95, 225, 234						
25		95						
	192	95, 225, 23	111					
	192	234	111					
		225						
	192	225, 234	111				102	
		225	111					
		225, 234	111					
		136						
				122				
		136		122				



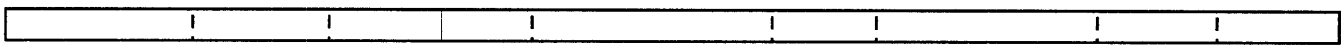
LIZARD	CROCODILE	HAMSTER	BINTURONG	BAT	BEAVER	BUFFALO	OX	WATERBUCK
Liolaemus fabiani	Crocodylus acutus	Dwarf (Phodopus sungorus sungorus)	Arctictis binturong	Big brown (Eptesicus fuscus)	Eurasian (Castor fiber)	Syncerus caffer	Bos indicus	Kobus defassa
				104				
	105							
					116			
		138						
		138						
		138						
		138						103
123		138						103
								103
123								103
123								103
123								103
123					116			
123	105	138			116			
123	105				116			
123	105	138, 139			116			
123								
123	105	138, 139			116			



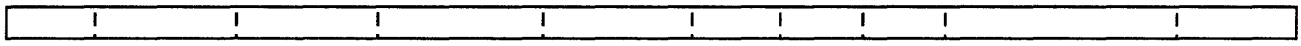
						TOTAL	PLANT
ORIBI	SUNI	HARTE	DEER	SIKA	PECCARY	LAND VERTEBRATE	CARROT
Ourebia ourebi	Neotragus moschatus, male	Alcelaphus buselaphus caama	White-tailed (Odocoileus virginianus), estrous female	Cervus nippon	White-lipped (Tayassu pecari	29	
						0	
						0.068965517	
						0.034482759	
						0.137931034	
						0.068965517	
			100			0.206896552	
						0.206896552	
						0.137931034	
97						0.034482759	
97						0.068965517	
						0.034482759	
				118		0.034482759	
				118		0.137931034	
			100			0.310	
			101			0.344827586	
						0.275862069	
						0.379310345	
	110	121		118		0.344827586	
	110	121		118	117	0.344827586	
	110	121		118		0.24137931	
	110	121		118	117	0.344827586	
		121		118	117	0.310344828	
				118	117	0.206896552	
				118		0.103448276	
				118	117	0.24137931	
		121		118	117	0.344827586	
		121		118	117	0.24137931	
97	110	121		118	117	0.448275862	
97						0.137931034	
97	110	121		118		0.344827586	
						0	
						0.034482759	
						0.068965517	
						0	
						0	
						0	
						0.034482759	
						0	
						0.034482759	55
						0.068965517	55


0

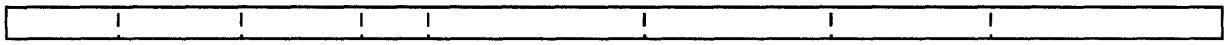
ROSE ROOT	LOVAGE	ONION	LEEK	BEAUTYBERRY	CHIVE	ASTERACEAE	APPLE	LEMON
		Allium cepa	Allium porrum	Callicarpa americana	Chinese (Allium tuberosum Rottle. Ex Sprengel)	Anthemis melampodina, Pluchea dioscoridis	Malus domestica cv. Earligold	Citrus limon
	56							
64	56							
64				208				
64								
64						224		
64	56							
64								
	56							
	56							
	56							
64	56							
64	56							
	56							
64								
64								
64								
64								
64								
64				208		224	183	186
64				208		224	183	186



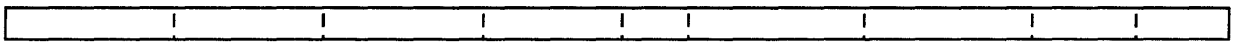
LIME	CHERRY	POPLAR	HEMLOCK	CONIFER	PINE	OAK	ELM	COTTONWOOD	ASPEN
Citrus aurantifolia	Prunus avium	Populus nigra	Eastern (Tsuga canadensis)	Picea abies	Pinus pinea	Quercus robur	Field (Ulmus minor)	Populus trichocarpa	P. tremuloides
						140	137	218	218
						140			
		181							
		181							
						140			
						140			
186	183		182	222	164	140	137	218	
186	183		182	222	164		137	218	



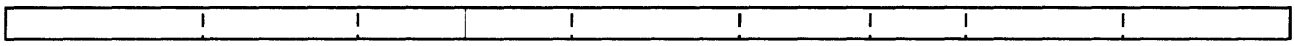
BIRCH	MAPLE	ALDER	FIR	WILS CHERVIL	EUPATORIA	TRIMENIA	OILSEED RAPE
Betula spp.	Bigleaf, Acer macropyllum	red, Alnus rubra, Sitka, A. viridis	Douglas (Pseudotsuga menziesii	Anthriscus sylvestris	Ageratina adenophora Spreng	T. moorei	Brassica napus
93, 218			177				96
93							
218	218	218	98, 17	129	128		150
218	218	218	98, 1	129	128		



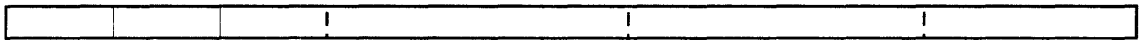
CABBAGE	TOMATO	TARBUSH	PEANUT	FIG	LAVENDER	OREGANO	BASIL	MINT
Brassica oleracea	*Lycopersicon esculentum* Mill.	*Flourensia cernua*	*Arachis hypogaea*	*Ficus hispidia*	*Laveandula* spp.	*Origanum vulgare*		
160	156							
				114				
160								
	156, 171	115	109		133	130, 135	58	135
	171	115	109		133	130	58, 135	135



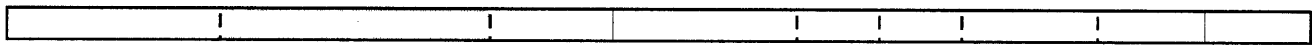
MELALEUCA	PARSLEY	SAGE		CHARRUA	CATNIP	ALOE	ARUGULA	RHUBARB
M. alterniflora	Petroselinum crispum	Greek (Salvia fruticosa)	Salvia officionalis					
								67
						61		67
						61		67
						61	77	67
						61		
							77	
							77	
							77	
							77	67
						61	77	67
						61	77	67
							77	67
						61		67
						61		67
								67
								67
				84				67
								67
								67
						61		67
			187					
								67
190	188	141	187	84	60			
190	188	141	187		60			



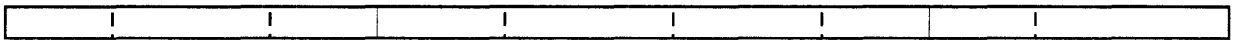
ECHINACEA			CHRYSANTHEMUM	BEEFSTEAKPLANT	EUCALYPTUS
74	74	74			
74	74	74		82	
74	74	74			
74	74	74			
74	74	74	81	82	87
74	74	74	81	82	87



	TOTAL	FLOWER						
TALL FESCUE	LAND PLANTS	UNSHIU	MANDARIN	OAK	BAY	LOVAGE	ECHINACEA	
	47		Citrus deliciosa	Blossom, Quercus robur				
	0							
	0.021276596							74
	0.021276596							
	0.063829787							
	0.085106383							
89	0.340425532			140		56	74	74
	0		127					
89	0.085106383			140				
89	0.085106383							
	0.063829787							
	0							
	0.042553191							
	0							
	0.042553191							
	0.021276596							
	0.021276596					56		
	0.021276596							
	0.106382979					56		
	0							
	0.127659574					56		
	0.063829787					56		
	0.063829787							
	0							
	0.085106383							
	0.042553191							
	0.042553191							
	0.085106383							
	0							
	0.021276596				73			
	0.021276596							
	0							
	0.042553191	65						
	0							
	0.021276596							
	0.021276596							
	0.106382979			140				74
	0.021276596						74	74
	0.765957447	65	127	140	73		74	74
	0.70212766	65	127		73		74	74



	MICHELIA	ANAXAGOREA		DAGUETIA	ROLLINIA	XYLOPIA		CARNATION
	M. alba	A. brevipes	A. dolichocarpa	D. asterotricha	R. insignis	X. aromatica	X. benthamii	
74								200
74								
74	145	175	175	175	175	175	175	
74	145	175	175			175		



CHRYSANTHEMUM	MAHONIA	CLUSIA	TOBACCO	ROSE	LANTANA	BAT-POLLINATED		
	M. japonica		Nicotiana	Rosa hybrida	L. camara	Bauhinia ungulata	Calyptrogyne ghiesbrechtiana	Cobaea scandens
				144				
	165	185						
		185						
		185						
		185						
		185						
		185						
		185						
		185						
		185						
		185						
				144				
				144				
81			191	144	118	112	112	112
81			191	144	118	112		112

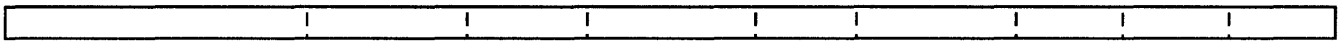
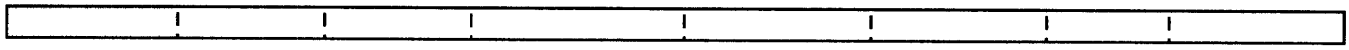
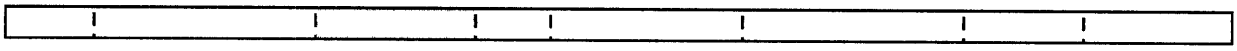


							FIG	POLEMONIACEAE		BABY KIWI
Hippeastrum calyptratum	Musa sp.	Parmentiera aculeata	Parmentiera alata	Vriesea gladioliflora	Weberocereus biolleyi	Weberocereus tunilla	Ficus spp.	Ipomopsis aggregata	Polemonium foliosissimum	
										86
										86
										86
										86
										86
							166, 114		106	86
							166			86
										86
										86
										86
										86
							166			86
										86
										86
							114			
							114			
										86
										86
	112			112						
					112	112				
112	112	112	112	112			108, 166	106	106	86
	112	112	112	112			166, 114	106	106	86

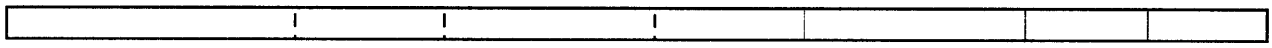
TOTAL	HONEY						
FLOWER	CASHEW	ORANGE	EUCALYPTUS	ROSEMARY	LAVENDER	THYME	CHESTNUT
29		Citrus sp.	Eucalyptus spp.	Rosmarinus officinalis L.	Lavedula latifolia	Thymus spp.	Castanea sativa
0.034482759							
0.068965517		113	113	113	113	113	
0.034482759							
0.034482759							
0.034482759							178
0.275862069					113		178
0.034482759							
0.172413793							
0.068965517							
0							
0							
0.034482759							
0							
0.034482759		113	113	113	113	113	
0	88						
0.034482759							178
0							
0.103448276							
0.034482759							
0.137931034							
0.103448276	88						
0.034482759							
0							
0.068965517							
0.034482759							
0							
0.068965517							
0							
0.068965517							
0							
0.034482759							
0.068965517							
0							
0.103448276							
0							
0.137931034							
0.034482759		113	113	113	113	113	
0.827586207							178
0.655172414							178



		TOTAL	FRUIT				
LIME	MARMELEIRO	HONEY	KIWI	RAMBUTAN	HYUGANATSU	GUAVA	BANANA
Tilia spp.		9	Actinidia deliciosa				Musa spp.
		0					
		0.55555556				57, 71	223
		0				71	223
		0				57, 71	223
		0.11111111	54				
178		0.33333333		78		57, 71, 80	223
		0				57	
		0			79	57, 71, 80	
		0					
		0				71	
		0					
		0					
		0					
		0.55555556				57, 71, 80	223
	88	0.22222222					223
178		0.22222222		78			
		0					
178		0.11111111	54	78		57, 71, 80	142
		0		78			
178		0.11111111	54	78		71	
	88	0.22222222		78			
		0		78		57, 71	223
		0				57	
		0				57, 71	223
		0		78		57, 71	223
		0				57, 71	
		0		78		57, 71	223
		0					
		0					
		0				71	
		0				57	
		0				57, 71, 80	223
		0				57	72
		0		78		57, 71, 80	72, 223
		0		78		57	
		0		78		57	223
		0.55555556					
178		0.22222222	54		79	57, 71, 80	
178		0.22222222	54, 142		79	71	

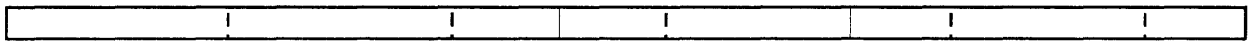


BULLOCK'S HEART	TOMATO	MUSKMELON	KI-MIKAN	HYUGANATSU	LEMON	DAIDAI
	Juice, fresh,		Citrus flaviculpus	Citrus tamurana	Citrus limon	Citrus aurantium
76		59				
76						
		59				
76	231	59				
76		59				189
		59				
76		59	179	179		
						189
76		59				
				179		
			179	179		
			179	179		
76						
76						
76						
76						
76						
76						
	231					
76		59	179	179		
		59				
		59				
		59				
		59				
	231					
76			179	179	186	189
76			179	179	186	189

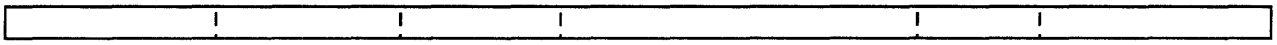


DEKAPON	LIME	GRAPEFRUIT	PLUM	CASHEW APPLE	PEAR	APPLE	MELON DE OLOR
Shiranuhi mandarin Suppl. J	Citrus aurantifolia	Citrus paradisi	d'Agen	Anacardium occidentale var. nanum	P. communis L.	?, Juice, Discovery, Anna, Delicious, Fuji, Braeburn, Granny Smith	Sicana odorifera
196					146	170	
					146		
196					146	174, 170, 15	184
196					146		
196			162		146	174, 152	184
196							
196						152	
196							184
196						152	
196		147			146		184
		147					
		s					
							184
							184
196				163			184
196				163			184
				163			184
196				163			184
196				163			
				163			
196		147			146	170	
196					146	174	
					92, 146	174, 152, 170	
					146	176, 152	
					92, 146	174, 176, 152	
196	186	75, 147					
196	186						

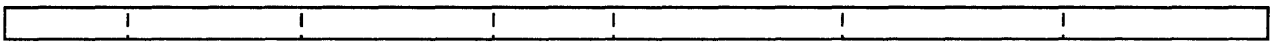
CANTALOUPE	GOOSEBERRY	NECTARINE		TAPEREBA	CAJA	UMBU-CAJA	OLIVE
Charentais (Cucumis melo L., var cantalupensis Naud., var. reticulatus)	Cape (Physalis peruviana)	Springbright	Vermeil	Spondias mombin	Spondias mombin	Spondias citheria	Olea europaea cv. Olivastra seggianese
157							
155	154						
157							
155	154		167				
157							
157							
	154		167				
	154						
	154						
	154			168	168		
	154						
157	154						
157							
157							
157						193	
157							
155, 157				168	168	193	
157				168			
155, 157				168	168	193	169
				168	168	193	
					168	193	



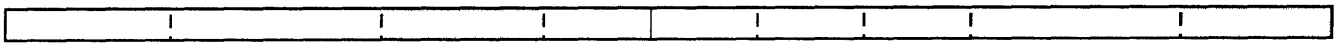
CAMU-CAMU	ARACA-BOI	CUPUACU	GUAYABITA DEL PINAR	GRAPE	PASSIONFRUIT
Myrciaria dubia	*Eugenia stipitata*	*Theobroma grandiflorum*	*Psidium salutare* (HBK)	Mystery	*Passiflora edulis*
			195		
			195		
		193	195		
			195		
	193		195	151	159
			195		70, 159
		193	195		70
			195		
			195		
			195		
					70
					70
			195		
		193	195		
193			195		70
		193			
	193				70, 159
193	193		195		159
193	193		195		



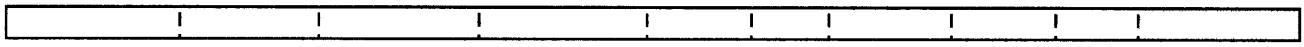
PEACH	DOGWOOD	HAWTHORN	MURICI	STRAWBERRY	BLACKBERRY	RASPBERRY
	Flowering, fruit of, Cornus florida	Crataegus sp.	Byrsonima crassifolia			
			126	62		
			126	62		
			126			
			126	62, 197	63	63
					63	63
				66	63	63
69			126	197	63	63
				197		
69			126	197	63	63
			126	197	63	63
			126	197		
			126			
	120	124	126	62	63	63
				66		
				62, 197	63	
				62		
				62	63	
					63	63
				68		



		TOTAL	FUNGI					TOTAL
BABY KIWI	SNAKE FRUIT	FRUIT	TRUFFLE		MUSHROOM		ASPERGILLIS	FUNGI
		35	Tuber aestivum	Tuber melanosporum	Agaricus bisporus	Coprinus comatus, fruit bodies	Stachybotrys chartarum	4
	85	0.02857143						0
86		0.34285714	194		207			0.5
86	85	0.2						0
86	85	0.42857143					153	0.25
86		0.28571429						0
86		0.62857143	132, 194	132				0.25
86		0.2	132					0.25
86		0.37142857				228		0.25
86		0.08571429						0
86		0.11428571						0
		0						0
86	85	0.05714286				228		0.25
		0						0
86	85	0.54285714	132, 194					0.25
		0.08571429						0
86	85	0.31428571			229	228		0.5
	85	0.05714286						0
	85	0.34285714						0
	85	0.11428571				228		0.25
	85	0.31428571						0
		0.11428571						0
	85	0.22857143						0
		0.05714286						0
		0.17142857						0
		0.17142857						0
		0.05714286						0
	85	0.28571429						0
		0.02857143						0
		0.05714286						0
	85	0.05714286						0
86		0.11428571						0
86	85	0.57142857	132, 194	132	207			0.5
		0.22857143						0
86		0.37142857						0
		0.22857143						0
		0.4						0
		0.08571429	132, 194	132				0.25
86		0.37142857						0
86		0.31428571						0

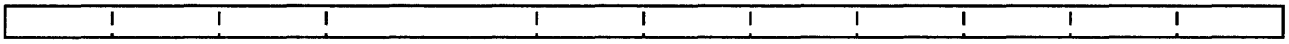


NUT/GRAIN									TOTAL
HAZELNUT	WALNUT	SOYBEAN	SORGHUM	CORN	RYE	WHEAT	HOPS	OAT	GRAIN
					type 1150		Humulus lupulus		9
									0
		52							0.11111111
									0
									0
51		52	148			149		53	0.55555556
51	161	52	148			149		53	0.66666667
51			148					53	0.33333333
51							198		0.22222222
								53	0.11111111
									0
									0
									0
									0
51	161		148			149, 172			0.44444444
									0
					173	149, 172			0.22222222
					173	172			0.22222222
	161					172			0.22222222
	161						198		0.22222222
	161						198		0.22222222
	161						198		0.22222222
							198		0.11111111
									0
									0
									0
									0
									0
									0
									0
									0
		52, 149		149	149		149		0.44444444
		52			149				0.22222222
									0
									0
									0
									0
						149			0.11111111
51	125, 161						198		0.33333333
	125, 161						198		0.22222222



ARTHROPODA

ANT		MOTH	STICK INSECT	BEE	WASP			FLY	BUG	
Diacamma spp. Venom	Camponotus spp. Mandibular gland	Cranberry blossom worm, Epiglaea apiata	Sipyloidea sipylus	Bumble, Bombus pomorum	Polistes dominulus, mandibular gland	Polybia sericea swarming trail pheromone	Decorator, Eucerceris spp.	Wheatstem sawfly Cephus cinctus Norton	Milkweed, Oncopeltus fasciatus	Plant, Phytocorus difficilis
									212	212
	217									
			214		201	202				
		213			201					
		213								
		213			201					
200		213			201					
	217	213		220	201	202	211, 216	209		
		213			201			209		
		213		220	201	202		209		
				220	201					
				220	201					
					201					
					201		216			
					201					
					201		211, 216			
									212	212



								TOTAL
			LOCUST		ROACH	VINEGAROON		ARTHROPODA
Bloodsucker, Rhodnius prolixus	Assasin, Triatoma infestans	Stink, Cosmopepla bimaculata	African migratory, Locusta migratoria migratorioides	Desert, Schistocerca gregaria	Therea petiveriana defense secretion	Mastigoproctus giganteus pygidial gland secretion	18
							0
							0
							0
							0
	219						0.055555556
							0.111111111
							0
							0
							0
							0
							0
							0.055555556
							0
233	221					204	0.277777778
	221						0.055555556
	221						0.166666667
	221						0.111111111
233			210	210		204	0.333333333
						204	0.222222222
	221			210		204	0.555555556
			210	210	203		0.333333333
				210			0.333333333
							0
							0.111111111
							0.111111111
							0.055555556
							0.111111111
							0.055555556
							0.111111111
							0
							0
							0
							0
							0
							0
		215					0.166666667
							0
							0
							0