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Trigeminal and Olfactory Chemosensory Impact of Selected Terpenes

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Running head: Chemosensory thresholds for terpenes

Abstract

COMETTO-MUÑIZ, J. E., W. S. CAIN, M. H. ABRAHAM AND R. KUMARSINGH. *Trigeminal and olfactory chemosensory impact of selected terpenes.* PHARMACOL BIOCHEM BEHAV. In Experiment 1, four normosmics and four anosmics (3 congenital, 1 idiopathic) provided odor and nasal pungency thresholds, respectively, for the following terpenes: Δ^3-carene, p-cymene, linalool, 1,8-cineole, and geraniol, plus the structurally-related compound cumene. Additionally, all subjects provided nasal localization (i.e., right/left) and eye irritation thresholds. Trigeminally-mediated thresholds (i.e., nasal pungency, nasal localization, and eye irritation) lay about three orders of magnitude above odor thresholds — which ranged between 0.1 and 1.7 ppm. The results implied uniform chemesthetic sensitivity across tasks and sites of impact. In Experiment 2, normosmics and anosmics provided odor and nasal pungency thresholds, respectively, for three pairs of isomeric terpenes: α- and γ-terpinene, α- and ß-pinene, and R(+)- and S(-)-limonene. Odor thresholds ranged between 1.4 and 19 ppm — that is, about an order of magnitude higher than those of the previous terpenes — with no substantial differences between odor thresholds of members of a pair. Regarding chemesthetic impact, only α-terpinene evoked nasal pungency. The overall outcome suggests comparable trigeminal chemosensitivity between nose and eyes and between normosmics and anosmics, as shown before for homologous n-alcohols. It also lends support to a previously-derived solvation model of the chemesthetic potency of airborne substances, and indicates the likely importance of certain molecular-size restrictions for effective trigeminal impact.

Keywords: Chemosensory thresholds - Odor - Nasal lateralization - Nasal irritation - Eye irritation - Anosmia - Terpenes - Olfaction - Trigeminal nerve

Introduction

The sensory impact of volatile organic compounds (VOCs) in humans rests principally on stimulation of the olfactory nerve (cranial nerve I) and the trigeminal nerve (cranial nerve V). The first gives rise to odor sensations and the second to pungent sensations such as prickling, piquancy, tingling, irritation, burning, freshness, stinging and the like. Pungency from VOCs is experienced in all exposed mucosae (28), but the present study focuses on the nasal mucosa, where sensations of nasal pungency arise, and on the ocular mucosa, where sensations of eye irritation arise.

The pharmacological and toxicological characterization of the senses of smell and chemical irritation or chemesthesis (26) includes the study of the breadth and sensitivity of responses towards the spectrum of chemicals. Recent studies on the molecular biology of smell (35) have provided additional support to the long-held view of the existence of a large number of different odorant receptors, probably in the order of 1,000. This brings the number of possible odorants into the tens of thousands (24, 34) or perhaps even hundreds of thousands (31). Within this context, a systematic strategy to study the breadth of chemical tuning and sensitivity in olfaction and chemesthesis has considerable merit.

One strategy to uncover the physicochemical basis for odor and pungency of VOCs consists of measuring chemosensory thresholds for homologous series of compounds. Previous work has addressed alcohols, acetates, ketones, alkylbenzenes, aliphatic aldehydes and carboxylic acids (9–13, 15–17). Along homologous series, physicochemical properties change systematically, and carbon chain length represents a convenient “unit of change” against which to analyze sensory results.

Most chemicals that evoke odor can also evoke pungency, though odor thresholds invariably lie below pungency thresholds (9–13, 15–17). Thus, measurement of nasal pungency thresholds in participants with normal olfaction, i.e., normosmics, can only be done against the background of an often strong odor. Such an odorous background precludes the use of a proper "blank" stimulus to implement, in normosmics, a forced-choice procedure to measure nasal pungency thresholds. We have found that the criterion for calling a sensation "barely" pungent, within an odorous background, varies widely from subject to subject. Under these conditions it has been shown that reports of nasal irritation are strongly influenced by response bias (18, 19). To solve the problem we have measured nasal pungency thresholds in participants lacking olfaction, i.e., anosmics, for whom odors do not interfere. Recently we have reported that, for homologous alcohols, alternative trigeminal chemesthetic thresholds such as eye irritation and nasal localization — a chemesthetically mediated ability, see (29) — are similar in normosmics and anosmics, indicating that nasal pungency thresholds in anosmics can serve as surrogates for "odor unbiased" nasal pungency thresholds in normosmics (15).

In the present investigation, we have extended the study of coherently measured olfactory and chemesthetic thresholds to a group of terpenes. This diverse family of substances, commonly present in essential oils of plants, constitute key ingredients in aromas and fragances for both their chemosensory and pharmacological effects. With the terpenes, we revisited the issue of whether additional indices of trigeminal sensitivity, i.e., thresholds for eye irritation and nasal localization, produce a similar outcome in normosmics and anosmics. In addition, from the point of view of structure-activity relationships, the terpenes provided an opportunity to look at the effects of structural isomerism — e.g., linalool ($C_{10}H_{18}O$) vs. geraniol ($C_{10}H_{18}O$) — and optical

isomerism — e.g., R(+)limonene vs. S(–)limonene — on olfactory and chemesthetic thresholds.

Materials and Methods

Experiment 1

Stimuli. The following substances were used: cumene (99%), p-cymene (98%), delta-3-carene (90% plus ≈5% 2-carene), linalool (97%), 1-8 cineole (eucalyptol) (99%), and geraniol (98%). Mineral oil (light, USP) served as solvent for all stimuli. Their structural formulas appear in Figure 1.

Insert Figure 1 about here

Threefold-step dilution-series of each compound were prepared in quadruplicate. The series started with undiluted chemical, 100% v/v, (labeled dilution step 0) and continued with 33% (dilution step 1), 11% (dilution step 2), 3.7% (dilution step 3), etc.

Stimuli were presented via 270-ml polypropylene squeeze-bottles (7), containing 30 ml of solution. For nasal testing, the bottles had caps with pop-out spouts that could fit inside one nostril and therefore allowed separate testing of each nostril. For ocular testing, the bottles had caps of the sort used in variable volume dispensers which allowed exposure of one eye at a time to an expelled aliquot from the headspace within the bottle, see (10).

Just after preparation of a series, concentration in the headspace of each bottle was measured via gas chromatography (FID detector) using a gas-sampling valve (1 ml loop). From then on, concentration was measured in alternatively selected even and odd dilution steps three or four times throughout the duration of the study to check for

stability. Bottles containing undiluted chemical (100% v/v) were assumed to have headspace saturated with chemical at room temperature (≈ 23°C). Such saturated vapor concentration (in ppm) was derived from handbooks or databases on vapor pressure. Vapor concentration in all other bottles was referred to that of the saturated vapor. The coefficient of variation (37) for FID readings across concentrations and terpenes averaged 18% ±7% (SD).

Subjects. The anosmic group comprised four subjects (two males, two females), all nonsmokers. The two females, 37 and 39 years old, and one male, 58 years old, were congenital anosmics. The other male, 41 years old, was an idiopathic anosmic.

The normosmic group also comprised four subjects (two males, two females), all nonsmokers. The females were 23 and 36 years old, and the males 41 and 53 years old.

The subjects were given a standardized olfactory test to classify them as anosmics or normosmics (7). This test probes into overall olfactory function via the combined approach of measuring odor thresholds and odor identification, each nostril tested separately. The protocol for the study was approved by the Human Subjects Committee of the University of California, San Diego. Subjects gave written consent before participation.

Procedure. Odor, nasal pungency, and eye irritation thresholds. All three thresholds were measured via a two-alternative forced-choice procedure with presentation of progressively higher concentrations. Starting from the lowest concentration, each trial entailed the presentation of a blank (mineral oil) and a stimulus. The subject's task was to choose the stronger stimulus. If the participant was correct, the same concentration was presented next, paired with a blank. If the participant was incorrect, the next higher concentration was presented next, also paired with a blank.

The first concentration chosen correctly five times in a row was taken as the threshold. Each nostril and eye was tested separately. Each type of threshold was measured eight times per subject-stimulus combination.

Nasal localization thresholds. A similar type of two-alternative forced-choice procedure with presentation of ascending concentrations was employed to measure these thresholds. In this case, the experimenter operated a mechanical squeezer that simultaneously sent to each nostril matched volumes of headspace from one of two bottles, see (15). One bottle contained stimulus, the other a blank. A trial consisted of two successive presentations. On one of them the stimulus was presented to the right (left) nostril, and on the other it was presented to the left (right) nostril. When the experimenter was testing localization in the right nostril, he would ask the subject which presentation led to a stronger perception in the right nostril. When the experimenter was testing localization in the left nostril, he would ask the subject which presentation led to a stronger perception in the left nostril. Correct choices led to presentation of the same concentration and incorrect choices led to presentation of the next higher concentration. The interval between trials was at least 45 sec. Five correct choices in a row for a given nostril was the criterion for the localization threshold. Nasal localization thresholds were also measured eight times per subject-stimulus combination.

Subjects participated in 10 to 12 sessions over a period of weeks. Sessions lasted between 1 and 3 hours.

Data analysis. The geometric mean summarized results across measurements for the same individual and across individuals in the same group (i.e., anosmic or normosmic). In the figures, results are expresed as averages ± standard deviations of the logs. Cases of indeterminate thresholds (i.e., those were threshold criterion was not

achieved) were excluded from the average. No significant differences in thresholds were observed between nostrils or between eyes for either anosmics or normosmics.

Experiment 2

Stimuli. The following terpenes were used: α-terpinene (85%), γ-terpinene (97%), (1S)-(-)-α-pinene (99+%, 87+% enantiomeric excess by Gas Liquid Chromatography), (1S)-(-)-ß-pinene (99%), (R)-(+)-limonene (97%, 98% enantiomeric excess by Gas Liquid Chromatography), (S)-(-)-limonene (95+%, FCC). Their structural formulas appear in Figure 2. Mineral oil served as solvent. Preparation of concentration series followed the protocol of Experiment 1.

Insert Figure 2 about here

Subjects. The anosmic group comprised three nonsmoking congenital anosmics, two females aged 39 and 42, and one male aged 59. The normosmic group comprised four nonsmokers, two females aged 25 and 37 and two males aged 31 and 58.

Procedure. Only odor and nasal pungency thresholds were measured for the terpenes tested in Experiment 2. Procedural details for their testing matched those of Experiment 1.

Results

Experiment 1

Figure 3 shows average odor and nasal pungency thresholds measured in normosmics and anosmics, respectively. Odor thresholds among terpenes ranged

between about 0.1 ppm for geraniol and 1.7 ppm for carene. Nasal pungency thresholds ranged between 235 ppm for cineole and 2,777 ppm for carene. Every terpene reached odor threshold in all normosmics on all repetitions by the criterion of five correct choices in a row. In contrast, not every terpene reached nasal pungency threshold in all anosmics on all repetitions by that same criterion: On one extreme, carene and cineole virtually always reached pungency threshold; on the other extreme, geraniol failed to reach pungency threshold in 88% of instances. Between these extreme cases, cumene, linalool, and p-cymene failed to reach a pungency threshold in 22%, 31%, and 56% of instances, respectively, across 32 opportunities.

Insert Figure 3 about here

Figure 4 depicts nasal localization thresholds obtained in normosmics and anosmics. Although no terpene was localized on every run in either group, the same general trend of efficiency to elicit a trigeminal response was apparent here; cineole, cumene, and carene were localized more readily than the other terpenes. Geraniol and p-cymene were unlocalizable in either group. Linalool, unlocalizable for anosmics, was seldom localized by normosmics. No notable differences were found between normosmics and anosmics in localization thresholds of any terpenes, although normosmics achieved localization in a higher percentage of instances than anosmics for every terpene except carene, where the percentages differed only nominally in favor of the anosmics. Figure 4 also shows that nasal localization thresholds lay at or above nasal pungency thresholds.

Insert Figure 4 about here

Figure 5 shows that eye irritation thresholds in normosmics and anosmics fell nicely into register with one another and with nasal pungency thresholds, except for

geraniol which elicited no pungency. For this ocular index of trigeminal chemosensitivity, carene and cineole were again the most efficient stimuli, reaching threshold on all repetitions for both groups. Geraniol was the least efficient, although it did reach threshold in about 20% of instances across both groups. Between these extremes lay cumene, linalool, and p-cymene, in increasing order of failure to reach threshold. This is the exact same order as that obtained for nasal pungency.

Insert Figure 5 about here

Experiment 2

Every terpene in this experiment reached odor threshold in all normosmics on all repetitions by the criterion of five correct choices in a row. In contrast, none of the three isomeric pairs of terpenes reached nasal pungency threshold in all anosmics on every repetition. Neither the pinenes nor the limonenes reached pungency threshold in the anosmics in most instances. α-Terpinene reached nasal pungency threshold in all eight instances in one anosmic and in five and three out of eight instances, respectively, in the other two. Typically, threshold was reached at dilution step 0, i.e., undiluted chemical. γ-Terpinene consistently reached nasal pungency threshold in only one anosmic — the same person most responsive to α-terpinene — and did it in all eight instances at dilution step 0. In the other two anosmics, γ-terpinene reached pungency threshold in 3 out of 8 instances, always at dilution step 0.

Figure 6 depicts the odor thresholds for the three pairs of terpenes and the nasal pungency threshold for α-terpinene. These terpenes produced odor thresholds over the range 1.4 ppm for α-terpinene to 19 ppm for α-pinene. Overall, these odor thresholds lie about one order of magnitude above those obtained in Experiment 1. Odor threshold

differences between the two members of each of the three pairs of isomers — two structural, one optical — were relatively small, typically close to or below half an order of magnitude.

Insert Figure 6 about here

Discussion

The odor thresholds for the terpenes studied fell in a range of approximately two orders of magnitude, between 0.1 ppm for geraniol and 19 ppm for α-pinene. No radical differences in odor thresholds occurred among structural isomers of $C_{10}H_{18}O$, i.e., linalool, cineole, and geraniol, or among structural isomers of $C_{10}H_{16}$, i.e., δ-3-carene, the two pinenes, the two limonenes, and the two terpinenes.

Differences in odor quality between some enantiomers, i.e., optical isomers, have been known for a long time. Reputed differences in quality were sometimes questioned as attributable to the presence of impurities. In 1971, three independent investigations using the enantiomers R(-) and S(+)-carvone showed conclusively that enantiomers can have different odor qualities (25, 30, 36). These studies and more recent work on odor thresholds for enantiomers of carvone and α-ionone (32) suggest the existence of olfactory receptors with chiral selectivity. Regarding differences for odor thresholds of enantiomers, we found quite similar odor thresholds for R(+) and S(-)-limonene. An ANOVA on the odor thresholds (log ppm) for terpinenes and pinenes revealed a significant difference ($p<0.05$) between α and γ terpinenes but no difference between α and β pinenes. It also revealed that the terpinenes produced significantly lower thresholds ($p<0.05$) than the pinenes, a result in agreement with previous work (8). It has been noted that, even when study-to-study variability in reported absolute odor

thresholds for the same substances can be very large (38), there is surprisingly good agreement in the relative values of odor thresholds across substances (33). In other words, given a group of compounds A,B,C, etc. common to a number of studies measuring odor thresholds, it is quite usual to find that the reported thresholds for any chemical varies widely, but it is also likely to find that the various studies agree on the relative order of the compounds when ranked, for example, from higher to lower thresholds. In terms of comparing the variability of olfactory vs. trigeminal thresholds, the present study, as well as all our previous ones, e.g., (15), points to considerably less variability in the trigeminal responses.

In terms of the chemesthetic impact of the terpenes, three of those tested here, 3-carene, α-pinene, and R(+)-limonene, were studied in 2-hour, whole-body exposures by Falk and collaborators for their toxicokinetics, effects on pulmonary function, and sensory irritative and CNS symptoms (21-23). Each terpene was presented at 1.8, 40 and 81 ppm. Although the radically different time and extent of exposure precludes a close comparison with our exposures of 1 to 3 seconds via a single nostril, it is interesting to note that only 3-carene consistently produced reports of sensory irritation, as also found here. A previous study of a group of of 15 anosmics of various etiologies found that six of them could detect limonene (presumably the racemic) in most or all of six to ten detection trials (20).

The results of Experiment 1 show similarity among three indices of chemesthetic sensitivity of human trigeminal functioning: nasal pungency (in anosmics), eye irritation, and nasal localization. The outcome indicates similar sensitivity between anosmics and normosmics for eye irritation and nasal localization thresholds. Comparable results between groups were obtained previously for homologous n-alcohols (15) confirming that for all these compounds trigeminal chemosensory function in anosmics and

normosmics shows essential agreement, with a tendency for normosmics to produce sligthly lower thresholds. A study of chemo-somatosensory event-related potentials to the predominantly pungent stimulus carbon dioxide revealed a marginally (but significantly) increased response in normosmics as compared to a group of anosmics and hyposmics (27). The question of whether normosmics can detect pungency at lower levels than anosmics remains open and probably needs to be addressed using a larger group of subjects. The difference, if real, seems in any event small.

A number of issues regarding structure-activity for chemesthetic potency deserve attention. In an aromatic series, viz., homologous alkylbenzenes, the capacity to elicit a nasal pungency threshold at any concentration faded early in the series, i.e., showed a cut-off effect (16). No pungency could be detected by anosmics for homologs above propyl benzene (12). In contrast, aliphatic series have reached a cut-off for members with approximately eight carbons in the principal chain (14, 16). These results were confirmed here, where the aromatic substances cumene and p-cymene failed to evoke pungency in a number of instances while the comparable cyclic aliphatic counterpart 3-carene did evoke it. In fact, cyclization of aliphatic carbon-chains helps to maintain their pungency-evoking properties as shown by 1,8-cineole vs. the structural isomers linalool and geraniol. The pungency of these two last terpenes begins to fade (linalool) or has already faded (geraniol) as would be expected from previous results with similar carbon-chain length counterparts, 1-octanol (9) and octyl acetate (10), though admittedly the comparison is not straightforward due to the presence of two double bonds in the terpenes. In any case, cyclization alone is not necessarily enough to maintain pungency. The spatial arrangement of the cycled molecule is also important, as shown by the lack of chemesthetic impact of α-pinene, ß-pinene, and the limonenes compared to their structural isomers α- and γ-terpinene, and, principally, δ-3-carene.

In summary, the presence of a benzene — that is, aromatic — moiety may reduce the chemesthetic potency of VOCs, but the cyclization of lineal aliphatic compounds of about 8 carbons in a chain may enhance such potency.

Nasal pungency thresholds for a wide variety of VOCs have been successfully described and predicted (3, 4, 16) by a quantitative structure–activity relationship (QSAR) based on a solvation model (1, 2). The solvation equation applied to nasal pungency employs four physicochemical parameters: polarity/dipolarizability, overall hydrogen-bond acidity, overall hydrogen-bond basicity, and lipophilicity. With the exception of linalool — with a predicted threshold of 1.58 log ppm and an observed threshold of 2.55 log ppm — the equation predicts the nasal pungency thresholds for the terpenes reasonably well (Figure 7). In terms of relative potency to evoke nasal pungency, and insofar as the solvation model applies, the structural changes discussed above are relevant only with respect to the mentioned physicochemical parameters. Nevertheless — as discussed elsewhere (16) — in terms of pungency cut-offs, the solvation approach cannnot make predictions if such cut-offs rest on molecular size and shape, factors often relevant to so-called “biological” cut-offs. In these cases, considerations of molecular structure such as those discussed above may take an importance of their own.

Insert Figure 7 about here

Altogether, the results obtained so far allow optimism about the possibility of a comprehensive modeling of human chemesthetic responses — be they nasal pungency, eye irritation (5, 6), or nasal localization — via the solvation approach. Perhaps the relative simplicity of the chemesthetic sensory system, compared to olfaction, makes it a suitable initial step from which to build a comparable physicochemical model for the olfactory system.

Acknowledgments

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Figure Legends

Figure 1. Structural formulas of the six compounds tested in Experiment 1.

Figure 2. Structural formulas of the six compounds tested in Experiment 2.

Figure 3. Thresholds for odor and nasal pungency (±SD) for compounds tested in Experiment 1. The percentage of instances across sessions in which some compounds failed to evoke a pungency threshold, i.e., unresponsiveness, is indicated. Bars indicating SD are sometimes hidden by the points.

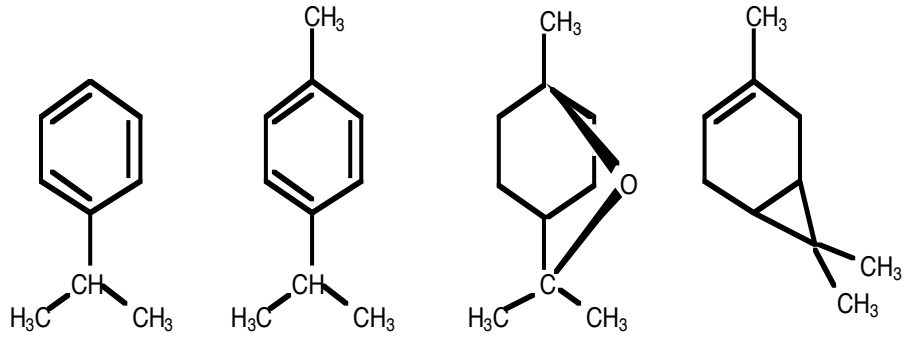
Figure 4. Thresholds for nasal localization (±SD) for compounds tested in Experiment 1. The percentage of instances in which some compounds failed to evoke a localization threshold in normosmics and anosmics is indicated. Nasal pungency thresholds (broken line) are depicted for comparison. Bars indicating SD are sometimes hidden by the points.

Figure 5. Thresholds for eye irritation (±SD) for compounds tested in Experiment 1. The percentage of instances in which some compounds failed to evoke an eye irritation threshold in normosmics and anosmics is indicated. Nasal pungency thresholds (broken line) are depicted for comparison. Bars indicating SD are sometimes hidden by the points.

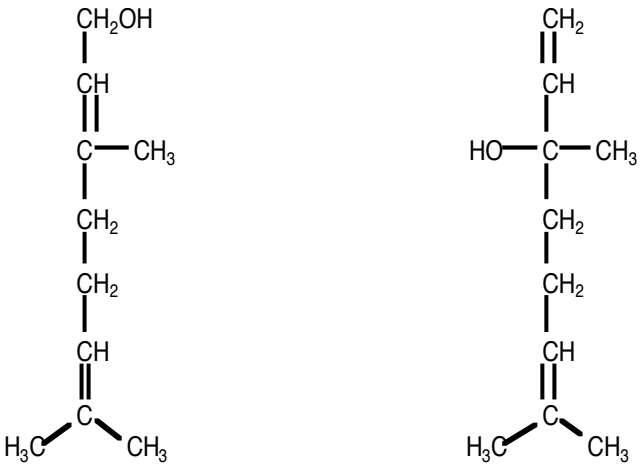
Figure 6. Thresholds for odor and nasal pungency (±SD) for compounds tested in Experiment 2. Only γ-terpinene consistently evoked nasal pungency. Bars indicating SD are sometimes hidden by the points.

Figure 7. Observed (±SD) vs. predicted (±SD) nasal pungency thresholds for terpenes tested here (δ-3-carene, cumene, p-cymene, linalool, 1,8-cineole, and α-terpinene) plus one (L-menthol) tested previously (9). Predicted values calculated according to (4). The dotted line represents the line of identity. Correlation coefficient (r) is 0.89. Bars indicating SD are sometimes hidden by the points.

FIGURE 1

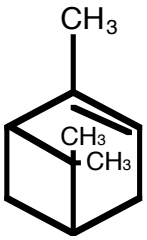


Cumene (C_9H_{12}) p-Cymene ($C_{10}H_{14}$) 1,8-Cineole ($C_{10}H_{18}O$) δ-3-Carene ($C_{10}H_{16}$)

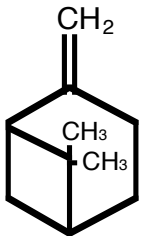


Geraniol ($C_{10}H_{18}O$) Linalool ($C_{10}H_{18}O$)

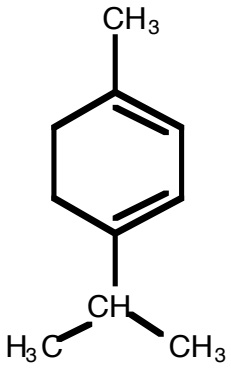
FIGURE 2



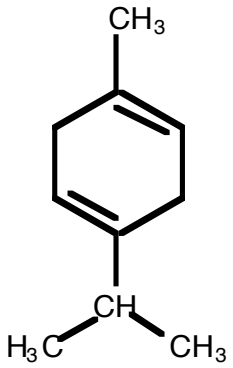
α-Pinene ($C_{10}H_{16}$)



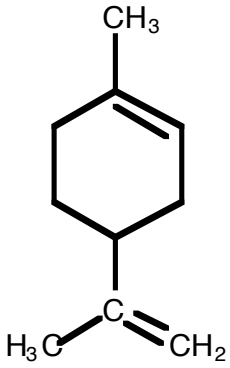
β-Pinene ($C_{10}H_{16}$)



α-Terpinene ($C_{10}H_{16}$)



γ-Terpinene ($C_{10}H_{16}$)



Limonene ($C_{10}H_{16}$)

FIGURE 3

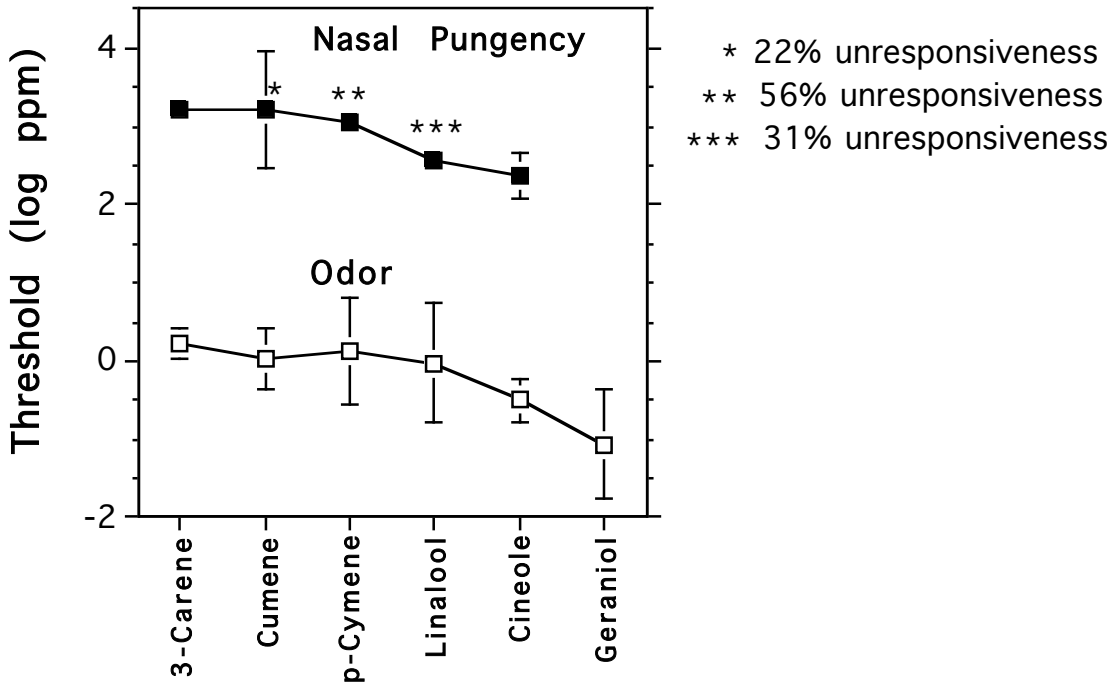

Nasal Pungency
Odor
Threshold (log ppm)
4
2
0
-2
*
**
***
3-Carene
Cumene
p-Cymene
Linalool
Cineole
Geraniol
* 22% unresponsiveness
** 56% unresponsiveness
*** 31% unresponsiveness

FIGURA 4

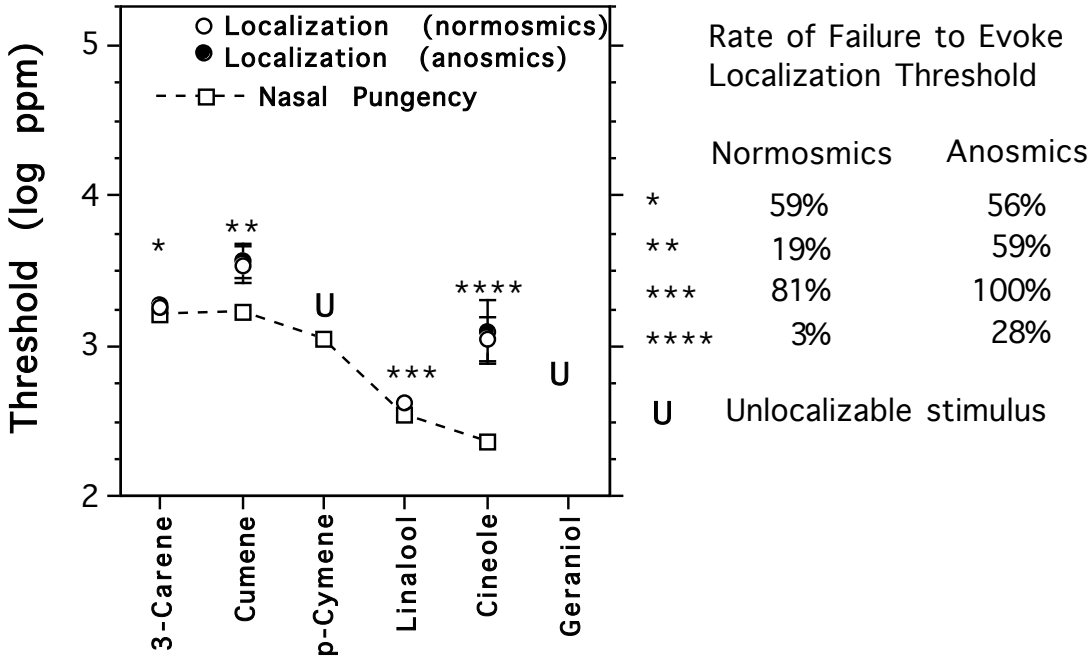

Threshold (log ppm)
5
4
3
2
Localization (normosmics)
Localization (anosmics)
Nasal Pungency
*
**
U
***
****
U
3-Carene
Cumene
p-Cymene
Linalool
Cineole
Geraniol
Rate of Failure to Evoke Localization Threshold
Normosmics
Anosmics
* 59% 56%
** 19% 59%
*** 81% 100%
**** 3% 28%
U Unlocalizable stimulus

FIGURE 5

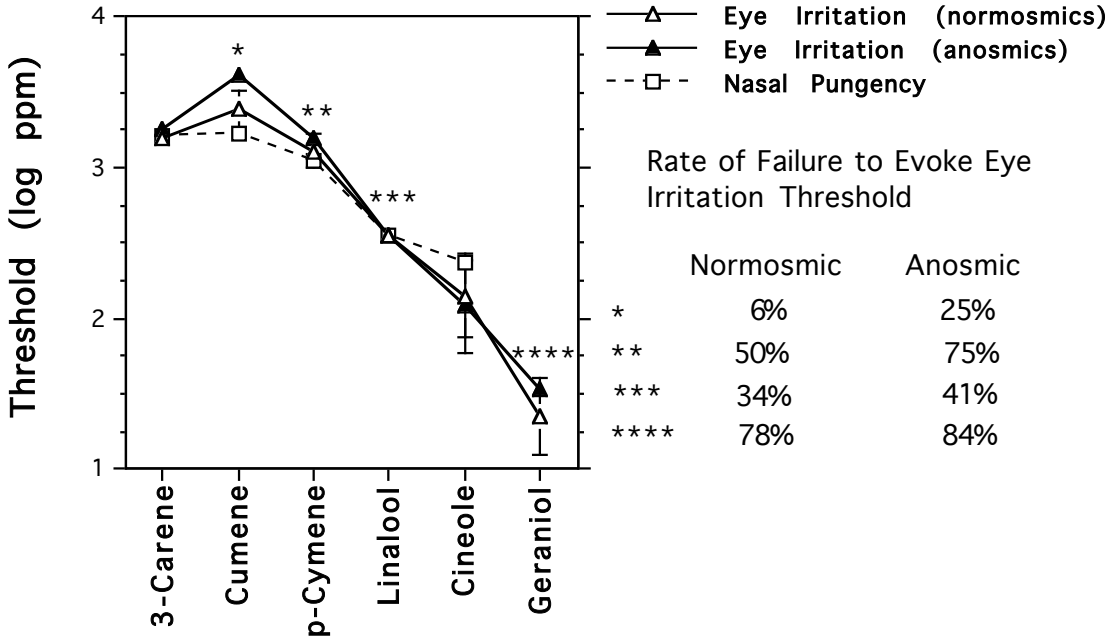

Threshold (log ppm)
4
3
2
1
*
**
***
****
3-Carene
Cumene
p-Cymene
Linalool
Cineole
Geraniol
Eye Irritation (normosmics)
Eye Irritation (anosmics)
Nasal Pungency
Rate of Failure to Evoke Eye Irritation Threshold
Normosmic
Anosmic
* 6% 25%
** 50% 75%
*** 34% 41%
**** 78% 84%

FIGURE 6

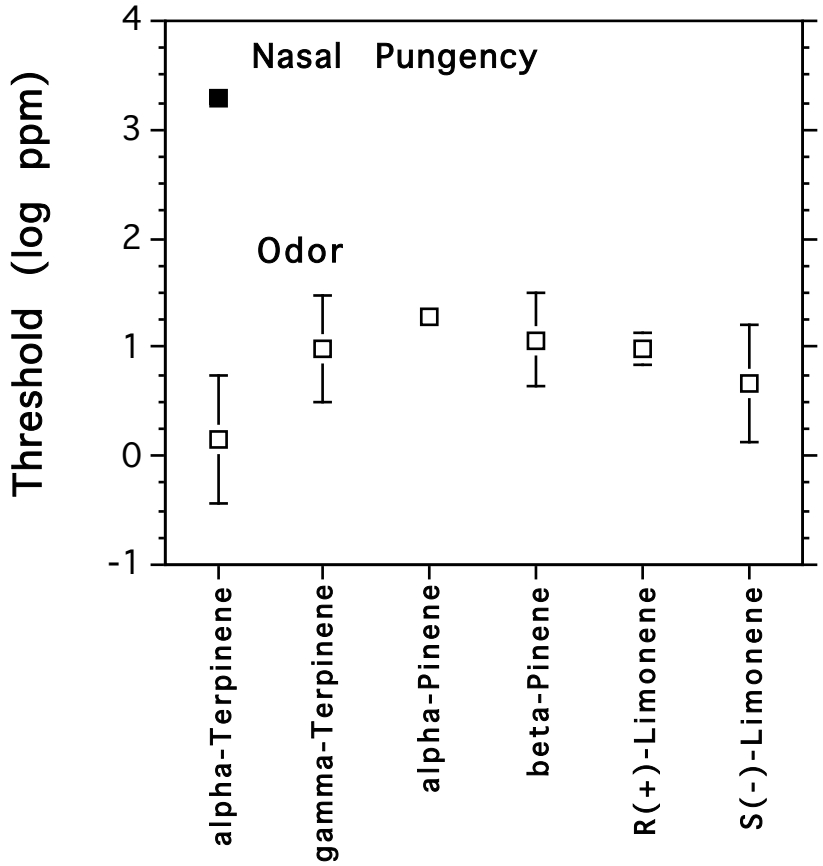
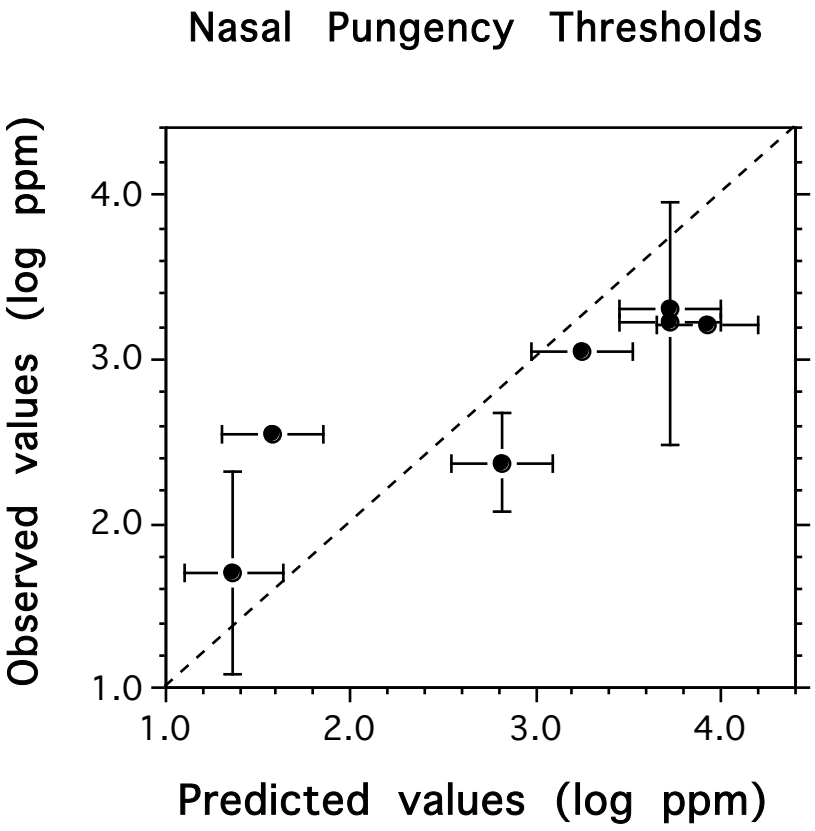

Threshold (log ppm)
4
3
2
1
0
-1
Nasal Pungency
Odor
alpha-Terpinene
gamma-Terpinene
alpha-Pinene
beta-Pinene
R(+)-Limonene
S(-)-Limonene

FIGURE 7


Nasal Pungency Thresholds
Observed values (log ppm)
Predicted values (log ppm)
1.0
2.0
3.0
4.0

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