Title

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Journal

Chemical senses, 30(8)

ISSN

0379-864X

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Publication Date

2005

Data Availability

The data associated with this publication are within the manuscript.

Peer reviewed

Chemical Senses **30**:627-642, 2005

Determinants for Nasal Trigeminal Detection of Volatile Organic Compounds

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Running head: Nasal Trigeminal Detection of Vapors

Abstract

We explored the influence of methodological and chemical parameters on the detection of nasal chemesthesis (i.e., trigeminal stimulation) evoked by volatile organic compounds (VOCs). To avoid odor biases, chemesthesis was probed via nasal pungency detection in anosmics and via nasal localization (i.e., lateralization) in normosmics, in both cases using forced-choice procedures. In the experiments with anosmics, 12 neat VOCs were selected based on previous reports of lack of chemesthetic response. Although none of the VOCs reached 100% detection, detectability and confidence of detection were higher when using a glass vessel system adapted with nosepieces to fit the nostrils tightly than when using wide-mouth glass jars. Half the stimuli were detected well above chance and half were not. When the latter were tested again heated to 37°C, i.e., body temperature (from room temperature, 23°C) to increase their vapor concentration, only one, octane, significantly increased its detectability. Chemesthesis gauged with normosmics mirrored that with anosmics. Gas chromatography measurements showed that, even at 23°C, the saturated vapor concentrations of the undetected stimuli, except vanillin, were well above the respective calculated nasal pungency threshold (NPT) from an equation that, in the past, had accurately described and predicted NPTs. We conclude that, except for octane and perhaps vanillin, the failure of the other four VOCs to precipitate nasal chemesthesis rests on a chemical-structural limitation, e.g., the molecules lack a key property to fit a receptor pocket, rather than on a concentration limitation, e.g., the vapor concentration is too low to reach a threshold value.

Keywords: Structure-Activity in Chemesthesis; Trigeminal Nerve; Anosmics; Nasal Pungency; Nasal localization or lateralization; Chemical Irritation Cut-Off.

Introduction

Chemosensory detection of volatile organic compounds (VOCs) by humans rests on the senses of smell and chemesthesis. Whereas odors are detected in the olfactory mucosa that covers the upper back portion of the nasal cavity via the olfactory nerve (cranial nerve I), chemesthetic sensations are primarily detected in all three face mucosae: ocular, nasal, and oral, primarily via the trigeminal nerve (cranial nerve V) (Bryant and Silver, 2000). A recurrent topic in the study of olfaction involves whether some chemicals could produce only "pure" olfactory responses, that is, could not elicit chemesthesis at any concentration. At present, the evidence supports the conclusion that most VOCs can elicit both olfactory and trigeminal activity albeit to a different degree depending on the conditions of stimulation and, very importantly, depending on the concentration tested (Doty and Cometto-Muñiz, 2003). We have revisited this issue by selecting a dozen VOCs that either failed to evoke nasal detection in anosmic subjects (e.g., (Doty, 1975; Doty et al., 1978) and are often used as prototypical "odor-only" stimuli (e.g., (Leopold et al., 2000; Welge-Lussen et al., 2003), or were found in previous studies of homologous chemical series to have reached a cut-off in carbon chain length such that even their neat vapor fails to produce nasal chemesthesis (see review in (Cometto-Muñiz, 2001). Using these stimuli, the present study explores methodological and chemical boundaries for detection of nasal chemesthesis.

One of the factors explored relates to a strategy to secure a response devoid of odor biases. Previous research showed that olfaction is more sensitive than chemesthesis to detect VOCs (Cometto-Muñiz and Cain, 1990; Doty *et al.*, 1978). In other words, as the concentration of a chemical vapor increases, it begins to be detected only by smell, and, as it increases further, it reaches a point where chemesthesis joins in. Thus, there is a concentration gap between a purely olfactory response and an olfactory-plus-trigeminal response. Unfortunately,

psychophysical procedures that control for response-biases, for example, forced-choice paradigms against blanks, cannot be directly implemented for nasal chemesthetic detection of VOCs since the target stimulus would be obvious to participants through its odor before it elicits any nasal pungency. One approach to avoid the influence of smell is to probe nasal chemosensory detection in participants lacking olfaction, i.e., anosmics (Cometto-Muñiz and Cain, 1990; Doty, 1975). Another approach consists in testing participants with an intact olfaction, i.e., normosmics, but in terms of nasal localization or lateralization rather than detection. This technique tests the ability of subjects to identify the nostril receiving the VOC when the contralateral nostril simultaneously receives plain air (see (Cometto-Muñiz and Cain, 1998; Dalton et al., 2000; Wysocki et al., 1997). Success in such task rests on trigeminal but not on olfactory activation (Kobal et al., 1989; Schneider and Schmidt, 1967).

A second factor explored relates to the features of stimulus presentation. Within the "static" vapor delivery approach (Cain et al., 1992) we used two types of containers: a relatively small one (237 ml) with a wide-mouth from which subjects sampled the vapor, and a larger one (1,900 ml) adapted with nosepieces and specifically designed to optimize nasal chemosensory sampling of vapors (Cometto-Muñiz et al., 2000). Gas chromatography served to secure an experimental knowledge of the vapor concentration of each VOC in the headspace of both types of containers.

A third factor explored entailed comparing various molecular dimensions and properties among the 12 chemicals tested to identify those that might have played a critical role in making a VOC an effective or ineffective nasal chemesthetic stimulus. In particular, we were interested in determining whether a failure to produce chemesthesis could be overcome by an increase in the vapor concentration available from the neat VOC. The vapor concentration increase was achieved by heating the liquid VOC source from room temperature (i.e., 23°C) to body

temperature (i.e., 37°C). Although heating the VOCs further would have achieved even higher vapor concentrations, we decided not to surpass body temperature to avoid engaging heat and, particularly, nociceptive-heat (above 43°C) receptors in nasal trigeminal endings. Interactions between these receptors and chemesthetic receptors are complex, not completely understood and could not be discounted (cf. Green, 2004).

Experiment 1: Detection of Vapors at Room Temperature

A Committee from the Human Research Protections Program of the University of California, San Diego approved the study protocol covering all experiments. All subjects gave written informed consent on forms approved by the Committee.

Materials and Methods

Subjects

Six anosmic subjects (4 females, 2 males) participated in the experiments. Anosmia was confirmed by administering a standardized clinical olfactory test (Cain, 1989). All participants were nonsmokers. The females were 31, 36, 47, and 50 years old, and the males were 48 and 66 years old. The 31, 47, and 50 year old females, and the 66 year old male were congenital anosmics. The remaining female and male were classified as idiopathic anosmics.

Stimuli and Equipment

We tested the following neat 12 chemicals (with purity defined by gas-liquid or high-pressure-liquid chromatography given in parenthesis, and the label “FCC” indicating that the chemical met Food Chemical Codex specifications): butyl benzene (>99%), coumarin (99.9%), decyl acetate (98.9%), eugenol (99.72%, FCC), geraniol (98.6%, FCC), nonanal (99.3%, FCC), octane (98%), octanoic acid (99.7%, FCC), 1-octanol (99.8%, FCC), ß-phenyl ethyl alcohol or

phenethyl alcohol (99.9%, FCC), 2-undecanone (99.4%, FCC), and vanillin (99.49%, FCC). Mineral oil (light, FCC) served as blank in all trials.

Two types of containers were employed to present the stimuli to the anosmics: 1) 237-ml, 5 cm-wide mouth, glass jars (hereafter referred to as small jars) and 2) 1,900-ml glass vessels, specially adapted with nose pieces for nasal stimulation with vapors (Cometto-Muñiz et al., 2000) (hereafter referred to as large vessels) (see Figure 1).

Insert Figure 1 about here

The small jars were filled with either 15 ml (liquids) or 15 g (solids) of the chemical. The large vessels were filled with 200 ml (liquids) or 50 g (solids) of the chemical. For both types of containers stimuli were prepared in triplicate and used alternatively within a testing session.

The concentration of the stimulus in the headspace of each container was measured by gas chromatography (GC) (flame ionization detector) via either direct sampling with a gas-tight syringe or by concentrating the samples in adsorption tubes (Sorbent tube, 4.5 in. L x 4 mm ID, packed with 20:35 mesh Tenax-TA/Carboxen 1000/Carbosieve SIII) and thermally desorbing them via a thermal desorption unit connected to the GC (Table 1). A calibration curve for mass measured for each compound served to convert GC readings into concentration units (ppm by volume) (see (Cometto-Muñiz et al., 2003). In the case of small jars, two approaches to sampling were followed: In the first one, closely matching the conditions in which the anosmics sampled the stimulus, the jar was opened and a sample was taken from the headspace of the jar with a gas-tight syringe. In the second one, aimed at minimizing dilution of stimulus by room air, a metallic foil remained covering the jar after the cap was open and the needle of the gas-tight syringe punctured the foil, penetrating into the headspace and sampling it with minimum exposure to room air. The values obtained for small jars using both approaches are presented

in Table 1. In the case of large vessels, since the anosmics sampled the headspace via tightly fitting nosepieces (see Figure 1) and analytical sampling was done via septum-lined perforated caps that preserved the integrity of the headspace, dilution by room air was not a significant concern.

Insert Table 1 about here

Procedure

Small jars. In order to replicate the testing conditions from previous studies that employed similar small jars (Doty, 1975; Doty et al., 1978), we employed a two-alternative forced choice procedure (2AFC). The order of presentation of chemicals and the order of presentation of stimulus and blank on each trial were randomized. Subjects were blindfolded and the experimenter held the open jar close to the nose of the subject. The participant held a clean paper towel covering upper lip and mouth to avoid any contact with the container (see (Doty et al., 1978). After deciding which presentation (first or second) produced the stronger sensation, the subject rated the confidence in the decision on a scale ranging from “1” (not confident at all) to “5” (extremely confident).

Large vessels. We employed a three-alternative forced choice procedure (3AFC). The order of presentation of chemicals and the order of presentation of stimulus and blanks on each trial were randomized. Subjects were blindfolded on those trials that entailed presentation of a solid chemical or a colored liquid chemical. After each triad, subjects rated their confidence in the decision on the scale mentioned above.

To reduce the possibility of uncomfortably strong sensations and minimize repeated exposures when detection was evident, two precautions were followed: 1) anosmics were instructed to sniff cautiously at the beginning and, if no clear sensation was present, to increase

the vigor of their sniff. 2) If a stimulus in the same type of container was correctly detected with the highest confidence (i.e., “5”) the first three times it was presented, further presentations were limited to six additional trials. One anosmic (female, 49 years old, congenital) achieved such performance when tested via the large vessels with butyl benzene, eugenol, nonanal, and 1-octanol. Anosmics participated in 4 to 6 sessions, each lasting between 1.5 and 3 hours, until 20 judgments per stimulus and type of container (except as noted above) were collected. In a typical session all 12 stimuli would be presented from each type of container at least 3 times.

Data Analysis

The results are presented as plots of detection probability and/or confidence rating as a function of chemical stimulus. Detection probability was corrected for chance according to (Macmillan and Creelman, 1991):

$P = (m \cdot p(c) - 1)/(m - 1)$

where P = probability of detection corrected for chance, m = number of choices (i.e., two or three), and p(c) = proportion correct (i.e., number of correct trials / total number of trials). The scale ranges from P = 0.0, that is, chance detection, to P = 1.0, that is, perfect detection. Statistical significance of trends was established by an analysis of variance (ANOVA) for repeated measurements (software: SuperANOVA v.1.11, Abacus Concepts, Inc., Berkeley, CA). Corrections for nonsphericity using either the conservative Greenhouse-Geisser (G-G) epsilon value or the liberal Hunyh-Feldt (H-F) epsilon value, left all reported significant factors and interactions still significant at $p \leq 0.05$.

Results

Analytical data

Figure 2 shows the headspace concentration measured for each stimulus from the large vessels and from the small jars sampled with and without an aluminum foil. The figure also shows values of vapor concentration taken from the literature (i.e., saturated vapor concentration at room temperature, 23 °C). Given the ample variability of saturated vapor concentration data reported for a particular chemical and temperature across references (see (Cometto-Muñiz *et al.*, 2003), the values cited here were selected based on the reliability and coherence of the source. Chemicals in Figure 2 are listed in decreasing order of concentration as measured in the large vessels. In most cases, the concentration from the large vessels is higher than, or at least equal, than that from small jars (nonanal is an exception). Use of a foil to sample from the small jars prevented dilution by room air, keeping the headspace concentration closer to the levels measured in large vessels. Notice that the variability of measurements is always highest for the small jars sampled without the foil (Table 1 and Figure 2). Finally, except for the two solid stimuli, coumarin and vanillin, differences between selected values from the literature and measured values were no larger than 25%, an amount that compares favorably to the much larger differences known to occur among alternative sources of vapor saturation data for a given compound and temperature (see (Cometto-Muñiz *et al.*, 2003).

Insert Figure 2 about here

Psychophysical data

Figure 3 shows the average nasal pungency detectability measured from the anosmic group for each of the 12 stimuli presented via the large vessels and via the small jars. The chemicals are plotted in decreasing order of detectability, as gauged using the large vessels. The outcome indicates that the levels of detectability obtained via large vessels, particularly for the most detectable stimuli, are higher than those obtained via small jars. Figure 4 includes a

plot of the average confidence that the anosmic group had in the detection choice made, using one or the other type of container. Use of the large vessels not only produced a higher level of nasal pungency detectability for the stimuli but also increased the confidence with which the anosmics made the detection decision.

Insert Figures 3 and 4 about here

An analysis of variance (ANOVA) for repeated measurements including the factors "container" (2 levels: large vessel and small jar) and chemical (12 levels) gave statistical support to the trends discussed above. The factor "chemical" was significant ($F(11,55) = 9.142$, $p < 0.0001$) and, although the factor "container" did not achieve significance, the interaction "container" times "chemical" was significant ($F(11,55) = 2.990$, $p = 0.0044$, indicating that detectability across chemicals varied with container. If we exclude the last four chemicals in Figure 3, for which detectability is close to chance for both delivery containers, and run an identical ANOVA with the remaining 8 chemicals, for which detectability is clearly above chance (e.g., $P \geq 0.2$) for at least one container, the factor "container" closely approaches the brink of significance ($F(1,5) = 5.221$, $p = 0.07$). The Appendix shows data for individual anosmics.

Experiment 2: Detection of Vapors Heated to 37 °C

Materials and Methods

Subjects

Four of the anosmics (3 females, 1 male) tested in Experiment 1 continued to be available for further testing. All were congenital anosmics, aged 31, 47, 50, and 66 (the male) years.

Stimuli and Equipment

We tested the 6 neat chemicals that in the previous experiment proved to be the least detectable by the anosmics (see Figure 3). In order of decreasing detectability, they were: octane, decyl acetate, phenethyl alcohol, vanillin, octanoic acid, and coumarin. Their purity and specifications were as in Experiment 1. As before, mineral oil served as blank.

Only large vessels were used in Experiment 2 and the amount of stimulus in each vessel was as in Experiment 1. Again, stimuli were prepared in triplicate and used alternatively within a session. In contrast with Experiment 1, the stimuli were heated in calibrated water baths to 37°C. Measurements taken with a thermocouple (Omega Instruments, Stanford, CT) confirmed that the temperature in the headspace of the vessels averaged (±SD) 37.1 (±1.3) °C. Under these conditions we measured the headspace concentration of each chemical by gas chromatography, as described in Experiment 1. We also performed additional measurements of headspace concentration for the stimuli in Experiment 2 but unheated. Table 2 incorporates these additional measurements and compares the headspace concentration of unheated and heated stimuli. All chemicals increased their headspace concentration at 37 °C. The increase, in terms of ppm by volume, amounted to a factor of 1.2 for octane (10,715 to 12,882 ppm), 1.6 for octanoic acid (9.5 to 15 ppm), 1.7 for ß-phenyl ethyl alcohol (from 102 to 170 ppm) and decyl acetate (from 33 to 56 ppm), 4.2 for coumarin (from 8.5 to 36 ppm), and 8.5 for vanillin (from 0.071 to 0.60 ppm).

Insert Table 2 about here

Procedure

The procedure was analogous to that employed in Experiment 1 when using large vessels. In the present experiment, subjects did not need to be blindfolded since the bottles resting in the water baths were covered by a sheet of lab bench surface protector (made of

cellulose fibers on top and polyethylene backing on bottom), perforated to allow only the cap of the vessel and its protruding tubes and nosepieces (see Figure 1) to be seen by the participant. The covering sheet also served to minimize temperature loss from the water bath to the testing room. Anosmics participated in 4 to 7 sessions, each lasting between 1.5 and 2.5 hours, until an average of 39 judgments per stimulus per subject were collected. This provided a group total of 156 judgments per stimulus. In a single session the 6 stimuli would be presented at least 6 times but more typically 9 times.

Data Analysis

Same as in Experiment 1.

Results

Figure 5 presents the average nasal pungency detectability of the six stimuli, heated to 37°C, to the anosmic group. For comparison, the graph also shows the average detectability obtained with the same anosmics and stimuli, but when chemicals were presented at room temperature, i.e., 23°C. The trend in detectability across VOCs is essentially the same at both temperatures. Only octane substantially gained in detectability, rising from P≈0.3 to P≈0.7, as a result of increasing its vapor concentration by heating. Detectability for all other compounds remained close to chance ($-0.20<P<0.20$), as in Experiment 1. These results are reflected in the ratings of confidence where octane was detected with a higher confidence than the rest (Figure 5). Statistical confirmation of the outcome came from an ANOVA for repeated measurements including the factors “temperature of the stimulus” (2 levels: 23 and 37 °C) and “chemical” (6 levels). Although neither the interaction between both factors nor the factor “temperature” reached significance, the factor “chemical’ did ($F(5,15) = 8.186$, $p = 0.0007$). A further contrast comparison within the factor “chemical” showed a significant difference between the

detectability of octane and that of all other VOCs ($p < 0.0001$). The Appendix shows data for individual anosmics.

Insert Figure 5 about here

Experiment 3: Nasal Localization of Vapors at Room Temperature

Materials and Methods

Subjects

Five normosmics (3 females) participated. They were 20, 21, 21, 24 (male) and 50 (male) years old. Four of them were nonsmokers. One female (21 years-old) was a light smoker (2 cigarettes/day).

Stimuli and Equipment

From the 12 VOCs tested with anosmics (Experiment 1), we selected a sample of four compounds representative of the complete range of detectabilities observed: butyl acetate ($P \approx 0.75$), 2-undecanone ($P \approx 0.40$), decyl acetate ($P \leq 0.20$), and octanoic acid ($P \approx 0.00$). We chose to select VOCs that belong to typical homologous series: alkylbenzenes, 2-ketones, acetate esters, and carboxylic acids. Purity of chemicals and blanks (mineral oil) were as in Experiment 1. They were presented neat (200 ml) from the large vessels (Figure 1) at room temperature (23°C).

Procedure

Nasal localization (or lateralization) consists in a two-alternative forced-choice procedure whereby subjects sniff simultaneously form two nosepieces (as those shown in Figure 1), one fitted to the right and the other to the left nostril. One nosepiece connects to the headspace of a vessel with blank and the other to that of a vessel with stimulus. Both vessels are covered with an opaque plastic sleeve so the participant only sees two identical tubes ending in a nosepiece.

Upon sniffing, the subject must decide which nostril experienced the stronger sensation and rate the confidence in the decision as described above. The order of presentation of chemicals and the nostril receiving the stimulus were randomized across trials. To avoid the possibility of uncomfortably strong sensations, the precautions mentioned under Procedure for Experiment 1 (large vessels) were followed. Subjects participated in 1 to 3 sessions, each lasting about 2 hours, until 20 to 60 judgments per stimulus and subject were collected.

Data Analysis

Same as in Experiment 1.

Results

Figure 6 presents both the group and individual subject results. Butyl benzene showed the highest probability of localization ($P \approx 0.74$), followed by 2-undecanone ($P \approx 0.39$), and, finally, decyl acetate and octanoic acid ($P \approx 0.20$). With minor variations, all subjects followed this general trend. The outcome for nasal localization in normosmics was remarkably similar to that of nasal pungency detection in anosmics (both tested at room temperature) for the common set of compounds (Figure 7).

Insert Figures 6 and 7 about here

Experiment 4: Nasal Localization of Vapors Heated to 37°C

Materials and Methods

Subjects

Same as in Experiment 3.

Stimuli and Equipment

Analogous to Experiment 3, except that the glass vessels containing stimuli or blanks were heated in calibrated water baths such that the temperature in the headspace averaged (±SD) 37.8 (±0.5) °C. As described in Experiment 2 (Procedure), a sheet of lab bench surface protector, covering each water bath, served to hide the vessels from the view of the participants, and to minimize temperature loss from the bath.

Procedure

Same as in Experiment 3.

Data Analysis

Same as in Experiment 1.

Results

Figure 8 illustrates the results for the group and for each individual participant. Again, with minor variations, all subjects showed a similar trend. A comparison between the data obtained at 23°C and at 37°C indicates that the clearly detectable stimuli (butyl benzene and 2-undecanone) increased their detectability at the higher temperature whereas the almost undetectable stimuli (decyl acetate and octanoic acid) did not (Figure 9). A two-way ANOVA for repeated measurements including the factors “VOC” (five levels) and “temperature” (2 levels) gave statistical support to the results. The main factor VOC was significant ($F(3,12) = 37.508$, $p < 0.0001$), the main factor temperature was not, but their interaction was significant ($F(3,12) = 6.814$, $p = 0.0062$), indicating that butyl benzene and 2-undecanone significantly increased their detectability at 37°C whereas decyl acetate and octanoic acid failed to do so. Specific contrast comparisons within the factor VOC showed that the detectability of butyl benzene and 2-

undecanone combined was significantly higher than that of decyl acetate and octanoic acid combined ($p < 0.0001$); also that the detectability of butyl benzene was significantly higher than that of 2-undecanone ($p = 0.0024$), but the detectability of decyl acetate was not significantly different form that of octanoic acid. Trends for confidence of detection across chemicals tested at 23 and at 37 °C followed closely the trends in detectability at the respective temperature, including the outcome that confidence of detection was higher at 37 than at 23 °C but only for the clearly detectable stimuli: butyl benzene and 2-undecanone (Figure 9).

Insert Figures 8 and 9 about here

Discussion

Psychophysical considerations

The present study provides a more sensitive quantification of the trigeminal detectability of neat VOCs than previously available (Doty, 1975; Doty et al., 1978). It also provides further evidence of the close comparability of results obtained when chemesthetic detection is assessed by anosmics via nasal pungency and when it is assessed by normosmics via nasal localization (Figure 7). The outcome confirmed the lack of nasal chemesthetic impact from some substances typically regarded as non-trigeminal such as vanillin, coumarin, and phenyl ethyl alcohol, but also revealed that other such compounds do possess chemesthetic impact, e.g., eugenol and geraniol, or can acquire it upon an increase in vapor concentration, e.g., octane. In terms of a cut-off for chemesthetic effectiveness reported along homologous series (see review in (Cometto-Muñiz, 2001), the present results agree with previous observations that decyl acetate reaches the cut-off point among acetates (Cometto-Muñiz and Cain, 1991; Cometto-Muñiz et al., 2005), 1-octanol lies before the cut-off point among n-alcohols (Cometto-Muñiz et al., 2005; Cometto-Muñiz et al., 2000) and octanoic acid lies at or just beyond the cut-off point

among carboxylic acids (Cometto-Muñiz et al., 1998a). In the case of butyl benzene, among the alkylbenzenes, and 2-undecanone, among the 2-ketones, the results found here indicate that they both lie before the cut-off point in their respective series since they were detected above chance level by anosmics and normosmics (Figure 7). Research is underway to determine the exact position of the cut-off along the last mentioned two series, as well as whether the effect can be overcome by increasing the headspace concentration via heating the VOCs to 37°C.

Use of the large vessels with nosepieces improved the detectability of most stimuli over that obtained with the open small jars (see Figure 3) (cf. (Doty et al., 1986). Such improvement was also reflected on the higher ratings of confidence of detection given by the anosmics when they were tested using the large vessels (Figure 4). In a previous investigation exploring nasal pungency thresholds in anosmics, the glass vessels also showed improved performance compared to plastic squeeze bottles, another simple and widely used static delivery system for testing human chemoreception (Cometto-Muñiz et al., 2000). Thus, optimization of stimulus delivery by use of the large vessel system led to the conclusion that only 5 of the 12 chemicals tested cannot be detected above chance by the anosmic group even at the higher vapor concentrations achieved at 37°C. These chemicals are: decyl acetate, phenethyl alcohol, octanoic acid, vanillin, and coumarin.

Structure-activity relationships for nasal chemesthesis

An important step in furthering our understanding of trigeminal chemesthesis consists in establishing the physicochemical determinants of chemesthetic potency from VOCs. A recent approach has used a solvation equation defined by four physicochemical parameters, or descriptors (Abraham, 1993), to model and predict the human nasal pungency potency of non-reactive VOCs (Abraham et al., 1998). This equation applies to selective or transfer processes, i.e., those that govern the biological effect of chemicals that exert their action principally by

reaching and distributing themselves across biological matrices (Abraham and Weathersby, 1994). The most recent update of the equation applied to nasal pungency (Abraham et al., 2001) reads as follows:

$$\text{Log}(1/\text{NPT}) = -8.080 + 1.767\,\mathbf{S} + 3.298\,\mathbf{A} + 1.076\,\mathbf{B} + 0.857\,\mathbf{L} \quad (1)$$

where NPT stands for nasal pungency threshold in units of ppm by volume. The descriptors (capital letters in bold) in equation (1) are physicochemical properties of the stimuli **(i.e., VOCs)**. They represent the following: **S** is the VOC dipolarity-polarizability, **A** and **B** are respectively the VOC effective hydrogen bond acidity and basicity, and **L** (log L^{16}) is defined through L^{16}, the VOC gas-hexadecane partition coefficient at 298K. The constant and the coefficients in the equation are found by multiple linear regression analysis. However, they are not merely fitted coefficients since they reflect the complementary physicochemical properties of the biophase that would be most receptive to the VOCs, i.e., they provide a description of the chemical environment of the **receptor(s**) (Abraham et al., 1998). The statistics of equation (1) are: N = 48 (total number of VOCs), R^2 = 0.950 (proportion of variance explained), SD = 0.27 (standard deviation), and F = 211 (Fischer statistic).

The success of equation (1) applied to nasal pungency thresholds indicated that trigeminal chemesthetic potency as represented by the 48 VOCs tested indeed rested very heavily in selective or transfer processes rather than on chemically- and structurally-restricted specific interactions. Nevertheless, as discussed above, investigations of nasal pungency thresholds along and across homologous series (reviewed in (Cometto-Muñiz, 2001) have found a cut-off effect in chemesthetic potency. That is, they have found a homolog that failed to evoke nasal chemesthesis, even at vapor saturation. All larger homologs failed as well. Other biological processes, for example, anesthesia, have also shown cut-offs (Franks and Lieb, 1985). Equation (1) can only predict a cut-off if the calculated threshold exceeds the saturated vapor of the VOC at room temperature (≈23°C). If the cut-off rested on a structural/size

molecular limitation, none of the descriptors on equation (1) could capture the effect. Nevertheless, it is possible to add a new descriptor to the equation to account for such limitation. This has been done for olfaction where two additional descriptors, one based on maximum molecular length and another on chemical functionality, were needed to improve the descriptive power of the corresponding equation for odor thresholds (Abraham et al., 2002).

The present results indicate that decyl acetate, phenethyl alcohol, octanoic acid, vanillin, and coumarin cannot evoke significant nasal pungency at the concentration of vapor saturation for 23°C. For all stimuli except vanillin, Table 3 shows that the measured vapor saturation concentration at 23°C is between 3.8 times (octanoic acid) and 25 times (coumarin) higher than the predicted nasal pungency threshold from equation (1). Of course, at 37°C these factors are larger, and even for vanillin the available vapor concentration is now 4.3 times higher than the predicted threshold. Still, the VOCs continue to fail to evoke pungency. With the only possible exception of vanillin, the outcome strongly suggests that the failure, or cut-off, is not due to a limitation in reaching a high enough concentration for threshold. Rather, the cut-off could come from a limitation related to molecular size, structure, or dimensions. For example, the VOCs could be too large or bulky to interact effectively with a target site or to fit into the binding pocket of a receptor.

Insert Table 3 about here

A revealing strategy to estimate the molecular dimensions that begin to produce a cut-off in a biological response is to look into homologous series. Two of the VOCs tested in Experiment 2 belong to such series: decyl acetate and octanoic acid. Figure 10 plots values of: a) saturated vapor at 23°C, b) saturated vapor at 37°C, c) measured nasal pungency thresholds (NPTs) (Cometto-Muñiz and Cain, 1991), and d) predicted NPTs (from equation (1)) as a function of the carbon chain length of the variable alkyl group of acetates. The figure illustrates

how the trend for NPTs, either experimental or predicted, never reaches the value of saturated vapor concentration at 23°C, least of all that at 37°C. The outcome strongly suggests that the cut-off in NPT rests not on a purely concentration-related restriction but on a chemical-structural restriction. At present, there are not enough experimental data points on NPTs from homologous carboxylic acids (cf. (Cometto-Muñiz et al., 1998a) to look for clear trends in this series as done with the acetates.

Insert Figure 10 about here

In agreement with the failure of decyl acetate, even when heated to 37°C, to elicit nasal chemesthesis, a recent study of ocular chemesthesis, i.e., eye irritation, from homologous acetates and n-alcohols found a cut-off at the level of decyl acetate and 1-undecanol (Cometto-Muñiz et al., 2005). Based on an analysis in terms of the longest unfolded length of homologous acetates and alcohols, the study suggested that an eye irritation cut-off occurs when the molecular length of the stimulus reaches a value between 18 and 19 Å. The observation agrees with the present results for decyl acetate though the maximum length for octanoic acid, taken as the cut-off for homologous carboxylic acids, is shorter than expected from the eye irritation study, being 14 Å (Table 4). Two factors likely influence the exact position of the cut-off: 1) Assuming that mucosal chemesthesis rests on a receptor-based process, genetic variability among subjects could account for differences in the exact length/size of the cut off homolog (Cometto-Muñiz et al., 2005). A strong indication for this is the relatively high detectability (P=0.45) of the heated octanoic acid (and of heated decyl acetate and phenethyl alcohol) for Anosmic 1 but not for the other anosmics (Figure 3-Appendix). 2) The exact cut-off might vary between nasal and ocular mucosa. Although previous results suggests general overall similarity in nasal and ocular chemesthetic sensitivity (Cometto-Muñiz and Cain, 1995), there are cases where, under identical conditions, the eye tended to show higher sensitivity than the nose (Cometto-Muñiz, 2001; Cometto-Muñiz et al., 1998b). Table 4 presents the longest unfolded

length (D) for all stimuli tested here. All stimuli detected above chance level, either at room temperature or heated to 37°C, have a length below 18 Å. Apart from octanoic acid discussed above, three stimuli failed to be detected, even when heated, despite having a length below 18 Å: ß-phenyl ethyl alcohol (10.2 Å), vanillin (10.1 Å), and coumarin (9.8 Å). A simple chemical commonality among these VOCs is that they are cyclic aromatic chemicals whereas the others, except butyl benzene and eugenol, are lineal aliphatic. Given that butyl benzene (11.7 Å) and eugenol (12.7 Å) have longer molecular lengths than the three undetected aromatics, it is possible that in bulkier, cyclic molecules, the simple parameter unfolded length is no longer a determining factor for chemesthetic effectiveness.

Insert Table 4 about here

To explore if alternative molecular features might help explain the results, we used a software program (HyperChem, 2003) to calculate the dimensions (width, depth, length, and volume) of the smallest box that each VOC would fit into (Table 4). Unfortunately, we found no simple molecular size effect that might contribute to a biological cut-off. Note that the longest unfolded molecular length (D) is larger than the corresponding length of the box. This is partly because D refers to an unfolded length whereas the VOC in the box may be folded to some extent. In any event, we can consider that the different lengths result simply from a scaling factor: For the 12 VOCs we have that:

D = 3.10 + 0.956 (box length)

The statistics are: $R^2 = 0.989$, s = 0.31 and F = 861.

Particularly striking is the loss of trigeminal potency in vanillin compared to eugenol, where the only difference is the replacement of the carbonyl group, in position para to the hydroxyl group, for an allyl (2-propene) group. At first sight, it is therefore a surprise that eugenol evokes nasal pungency at 23°C whereas vanillin fails to evoke nasal pungency even at

37°C. However, although this small structural change leads to a moderate change in the calculated NPT of about half an order of magnitude (vanillin 0.14 and eugenol 0.73 ppm) it results in a marked change in melting point: eugenol is liquid at room temperature and vanillin melts only at 82°C. A compound that is solid at room temperature will have a saturated vapor pressure lower than that of the corresponding supercooled liquid. Indeed, the saturated vapor pressure of vanillin is three orders of magnitude lower than that of eugenol (Table 1) and only reaches the order of magnitude of the calculated NPT at 37°C (Table 3). On the other hand, the saturated vapor pressure of eugenol is 70 times the calculated NPT already at 23°C.

Through optimization of stimulus delivery and psychophysical procedure, this study has improved the precision in the quantification of nasal chemesthetic detectability for a dozen VOCs of common use in chemosensory research. Half of them turned out to be detected above chance level to one or another degree. An additional substance, octane, became detectable upon heating the liquid stimulus source to 37°C and, thus, increasing the saturated vapor concentration, indicating that the restriction to trigeminal detection was purely concentration-related. The remaining five compounds failed to elicit trigeminal detection, even when heated, despite the fact that their measured vapor concentrations were well above the expected threshold for nasal chemesthesis. This suggests that these VOCs lack other kind of chemical/structural features, necessary for trigeminal stimulation. Our initial attempt to characterize such features using straightforward molecular measurements, as those shown in Table 4, has not been successful, perhaps due to the many simultaneous dimensions in which these assorted variety of substances differ. One strategy to avoid this problem is to focus scrutiny on closely related chemicals varying systematically along a simple dimension, for example, members of homologous series. This approach has had initial success in the study of ocular chemesthesis (Cometto-Muñiz et al., 2005). As a practical outcome, the present results

serve as a convenient reference guide for investigators who need to select stimuli according to varying degrees of effectiveness in nasal trigeminal stimulation of humans.

Acknowledgments

The work described in this article was supported by research grant number R01 DC 005003 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health. Thanks are due to S. Snyder, K. Stutz, K. Sheets, E. Violette, and R. Sanchez for technical assistance. Thanks are also due to A. Borboa, T. Chai, and E. Lu for their help in running some of the experiments.

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APPENDIX

Experiment 1. Individual data: Nasal pungency detection of vapors at 23°C

Since each anosmic was intensively tested, it is illustrative to look at the individual data. Figure 1-Appendix shows individual plots of detection probability per stimulus when using either large vessels or small jars. Notice that the same order of chemicals is used in all plots of this figure as in Figures 3 and 4. This uniform order reveals that the detectability trend across all 12 stimuli observed for the group is also present, with minor variations, for each individual subject. The group results, then, are clearly not an artifact of averaging different trends. For anosmics 1 (A1) and 2 (A2), and, to a lesser extent, anosmics 5 (A5) and 6 (A6), use of the large vessels increased the detectability of most stimuli whereas for anosmics 3 (A3) and 4 (A4) type of container made no clear overall difference. Inspection of confidence ratings from individual anosmics can also prove informative. These ratings are presented in Figure 2-Appendix, where stimuli are shown in identical order as in previous graphs. Two effects can be noticed when comparing large vessels with small jars for individual anosmics in terms of confidence ratings: 1) Confidence from stimulus presentation in large vessels, in particular for highly detectable chemicals, is higher than that from presentation in small jars. 2) Confidence from stimulus presentation in large vessels closely follows the pattern of detectability (from large vessels) across chemicals, whereas confidence from stimulus presentation in small jars is low, flat, and either fails to follow or follows less closely the pattern of detectability (from small jars).

Insert Figures 1-Appendix and 2-Appendix about here

Experiment 2. Individual data: Nasal pungency detection of vapors at 37°C

Figure 3-Appendix shows that the trend in detectability across heated VOCs for each individual anosmic fell quite well into register with the average group trend. The trend for anosmic 6 followed the rest less closely but, even for this participant, octane was more detectable than the rest of the stimuli.

Insert Figure 3-Appendix about here

Table 1. Concentration (log ppm ± SD) measured by gas chromatography in the headspace of large vessels and small jars for the 12 chemicals tested.

Stimulus	Large vessels (log ppm ±SD)	Small jars (log ppm ±SD) (sampled through foil)	Small jars (log ppm ±SD) (sampled without foil)
Butyl benzene	3.22 ±0.12	3.01 ±0.02	2.70 ±0.17
Coumarin	0.93 ±0.08	0.94 ±0.06	No data
Decyl acetate	1.55 ±0.13	1.62 ±0.04	1.12 ±0.16
Eugenol	1.71 ±0.11	1.59 ±0.11	0.99 ±0.23
Geraniol	1.07 ±0.13	1.26 ±0.05	0.78 ±0.19
Nonanal	2.25 ±0.18	2.68±0.07	2.21±0.51
Octane	4.03 ±0.04	4.01 ±0.05	3.74 ±0.15
Octanoic acid	1.11 ±0.10	1.13 ±0.10	0.51 ±0.23
1-Octanol	2.31 ±0.08	1.86 ±0.06	1.72 ±0.12
ß-Phenyl ethyl alcohol	2.08 ±0.13	1.69 ±0.03	1.77±0.39
2-Undecanone	2.03 ±0.07	1.81 ±0.09	1.58 ±0.26
Vanillin	-1.35 ±0.20	-1.55 ±0.14	No data

Table 2. Comparison between the headspace concentration of stimuli in unheated (23 °C, room temperature) and in heated (37 °C) large vessels, as measured by gas chromatography.

Stimulus	Unheated (log ppm ±SD)	Heated (log ppm ±SD)
Coumarin	0.93 ±0.08	1.56 ±0.23
Decyl acetate	1.52 ±0.13	1.75 ±0.13
Octane	4.03 ±0.05	4.11 ±0.02
Octanoic acid	0.98 ±0.17	1.17 ±0.14
ß-Phenyl ethyl alcohol	2.01 ±0.15	2.23 ±0.05
Vanillin	-1.15 ±0.29	-0.22 ±0.33

Table 3. Values of saturated vapor concentration (SVC) at 23 and 37 °C, and of predicted NPTs (from equation (1)) for the five VOCs still unable to evoke nasal pungency even when heated to 37°C. Also shown for each compound is the ratio SVC / Predicted NPT at 23 and at 37 °C.

VOC	**SVC @ 23°C**	**SVC @ 37°C**	**Predicted NPT**	**Ratio SVC / Predicted NPT**	
	(ppm)	**(ppm)**	**(ppm)**	**@ 23°C**	**@ 37°C**
Decyl acetate	33	56	5.0	6.6	11.2
Phenethyl alcohol	102	170	7.5	13.6	22.7
Octanoic acid	9.5	15	2.5	3.8	6.0
Vanillin	0.071	0.60	0.14	0.51	4.3
Coumarin	8.5	36	0.34	25	106

Table 4. Longest unfolded length (D) and box dimensions, all in Å, of the VOCs tested.

Stimulus	D	Width	Depth	Length	Volume
Butyl benzene	11.68	4.88	3.50	8.86	151.17
Coumarin	9.81	5.34	2.97	7.11	112.74
Decyl acetate	19.02	2.86	1.83	16.58	86.62
Eugenol	12.72	5.07	3.11	9.58	150.90
Geraniol	12.67	5.07	1.83	9.72	90.26
Nonanal	14.71	2.67	1.81	12.03	58.10
Octane	12.91	2.41	1.81	10.54	45.94
Octanoic acid	14.03	2.66	1.81	11.52	55.47
1-Octanol	14.03	2.20	1.83	11.55	46.48
Phenethyl alcohol	10.22	4.74	1.83	8.13	70.87
2-Undecanone	16.69	2.71	1.82	14.30	70.36
Vanillin	10.12	5.40	1.84	7.02	69.69

Figure legends

Figure 1. Picture of the large vessels employed to test nasal trigeminal detection of VOCs.

Figure 2. Concentration measured for each chemical in the headspace of large vessels (filled circles), small jars with foil (squares), small jars without foil (triangles), and values of saturated vapor concentration at 23 °C reported in the literature, selected for reliability and coherence (see text) (diamonds). Bars on experimental data, often covered by the symbol, represent standard deviation (SD). The literature sources used were: octane and 1-octanol (Boublik et al., 1984); butyl benzene (Wilhoit and Zwolinski, 1971) (Thermodynamic Tables, Thermodynamics Research Center, Texas A & M University Systems, College Station, TX); nonanal, ß-phenyl ethyl alcohol, 2-undecanone, eugenol, decyl acetate, geraniol, coumarin, and vanillin (Stephenson and Malanowski, 1987); octanoic acid (de Kruif et al., 1982).

Figure 3. Detectability by the anosmic group (n=6) of the 12 stimuli when tested using the large vessels versus when using the small jars. Each point represents the average of 120 trials, except as noted under “Procedure”. Bars indicate standard error.

Figure 4. Left graph. Detectability (left *y*-axis) and confidence of detection (right *y*-axis) by the anosmic group (n=6) for the 12 stimuli when using the large vessels. Right graph. Same as left, but when using the small jars. The order of stimuli is the same as in Figure 3. Bars indicate standard error.

Figure 5. Group detectability (four anosmics) for the six stimuli tested in Experiment 2 at 37°C (filled circles) compared with the detectability by the same group for the same stimuli but presented at 23°C, Experiment 1 (empty circles). Bars indicate standard error. Average

confidence ratings obtained in Experiment 2 are also shown (triangles) and can be read in the right *y*-axis.

Figure 6. Probability of nasal localization (corrected for chance) for each of the four chemicals, tested at room temperature (23°C). Data are shown for the normosmic group (n=5) and for each individual participant. The group data represents a total of 160 trials per compound made by the five subjects. S1, S2, etc. stand for subject 1, subject 2. etc.

Figure 7. Similarity between two indices of nasal chemesthesis: Probability of nasal detection by anosmics (n=6) (Experiment 1) and probability of nasal localization by normosmics (n=5) (Experiment 3) for a common set of neat VOCs, presented at room temperature, ranging from highly detectable to non-detectable. Bars indicate standard error.

Figure 8. Analogous to Figure 6 but with stimuli tested at 37°C. Here, the group data represents a total of 140 trials per compound made by the five normosmic subjects. S1, S2, etc. stand for subject 1, subject 2. etc.

Figure 9. Comparison of detectability (filled symbols, left *y*-axis) by nasal localization at 23 and at 37 °C for the four VOCs by the same group of normosmics (n=5). Bars indicate standard error. Also included are ratings for confidence of detection (empty symbols, right *y*-axis) at each temperature, given by the group.

Figure 10. Values of saturated vapor concentration at 23 and at 37 °C along homologous acetates from methyl (1) to decyl (10) acetate. The graph illustrates how the trend for measured (Cometto-Muñiz and Cain, 1991) or predicted (equation (1)) NPTs never reaches vapor

saturation at either temperature, thus suggesting that the observed cut-off in nasal pungency at decyl acetate is unlikely to be based on a purely concentration-restriction (see text).

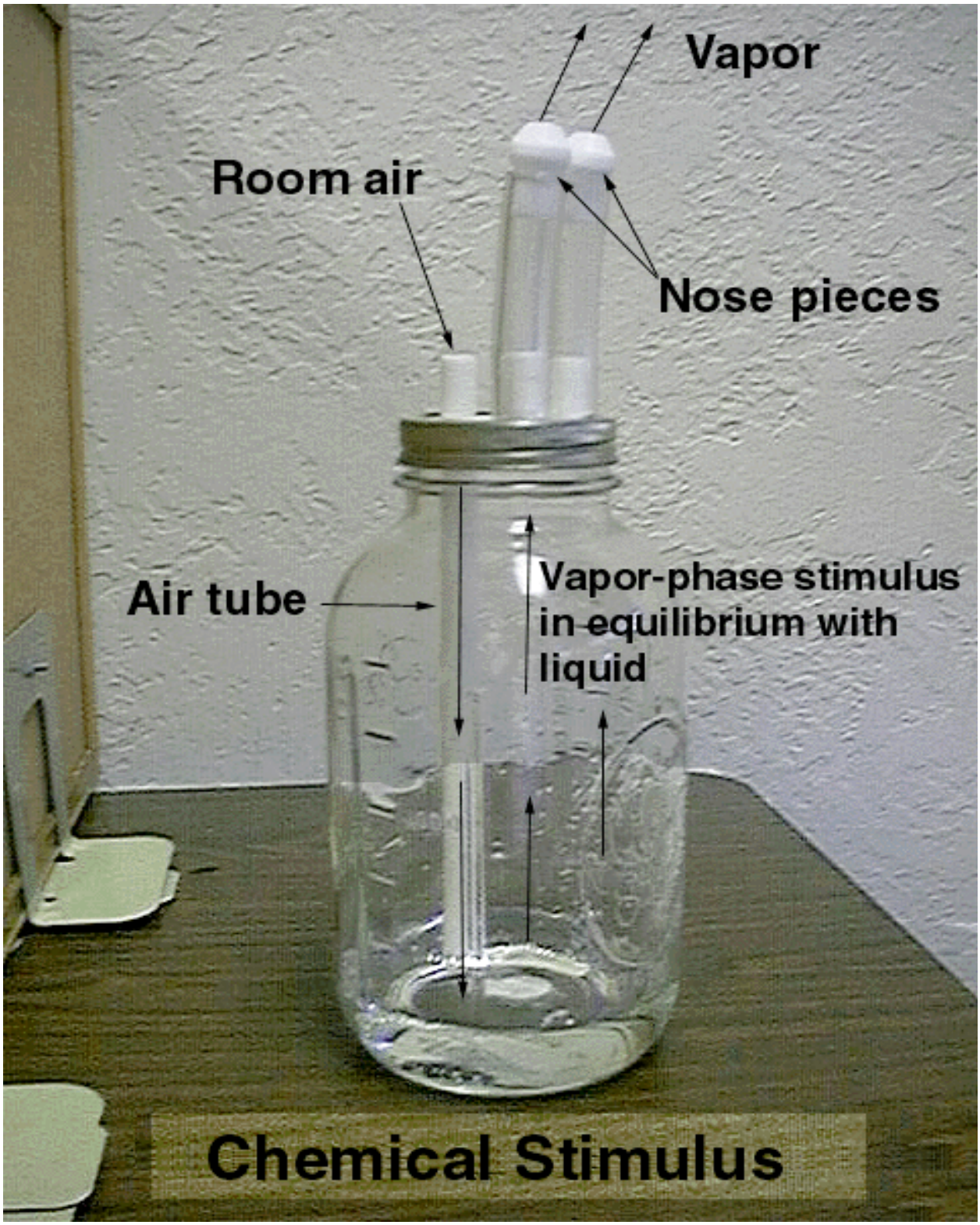
APPENDIX Figure Legends

Figure 1-Appendix. Detectability by individual anosmics for the 12 stimuli presented via the two containers. Each graph presents the data from one anosmic (i.e., 20 trials per stimulus except as noted under “Procedure”.) In all graphs stimuli are shown in the same order as in Figures 3 and 4. A1, A2, etc. stand for anosmic 1, anosmic 2, etc.

Figure 2a-Appendix. Detectability (left *y*-axis) and confidence of detection (right *y*-axis) by individual anosmics for the 12 stimuli when presented via the large vessels. Each graph presents the data from one anosmic. Figure 2b-Appendix. Same as in 2a, but when stimuli were presented via the small jars. Note that in all graphs stimuli are shown in the same order as in Figures 3, 4, and Figure 1-Appendix. A1, A2, etc. stand for anosmic 1, anosmic 2, etc.

Figure 3-Appendix. Detectability (left *y*-axis) (filled circles) and confidence of detection (right *y*-axis) (triangles) by individual anosmics for the six stimuli tested at 37°C in Experiment 2. Each individual graph also shows the average group detectability from Experiment 2 (empty circles) for comparison. The order of stimuli in each graph is the same as in Figure 5. A1, A2, etc. stand for anosmic 1, anosmic 2, etc.

FIGURE 1



Delivery system for Nasal Trigeminal testing

FIGURE 2

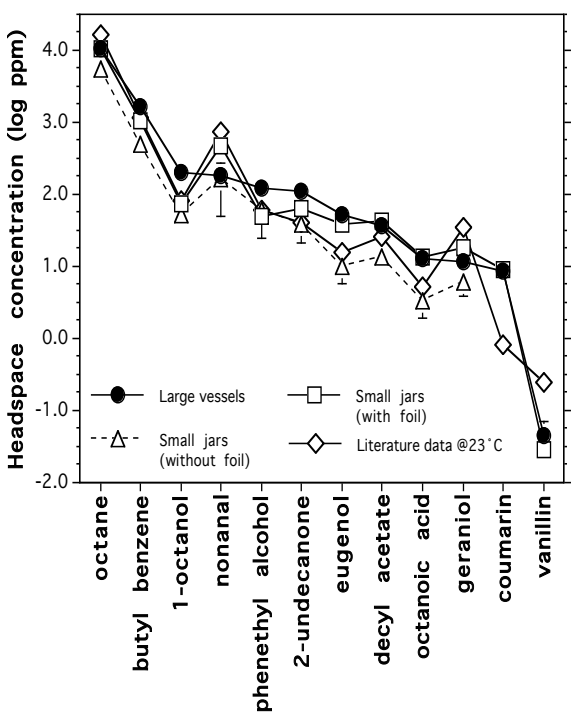


FIGURE 3

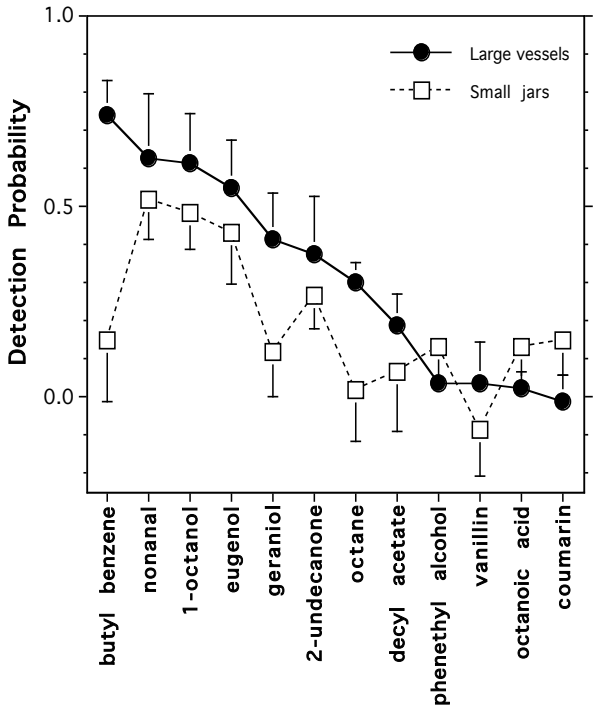


FIGURE 4

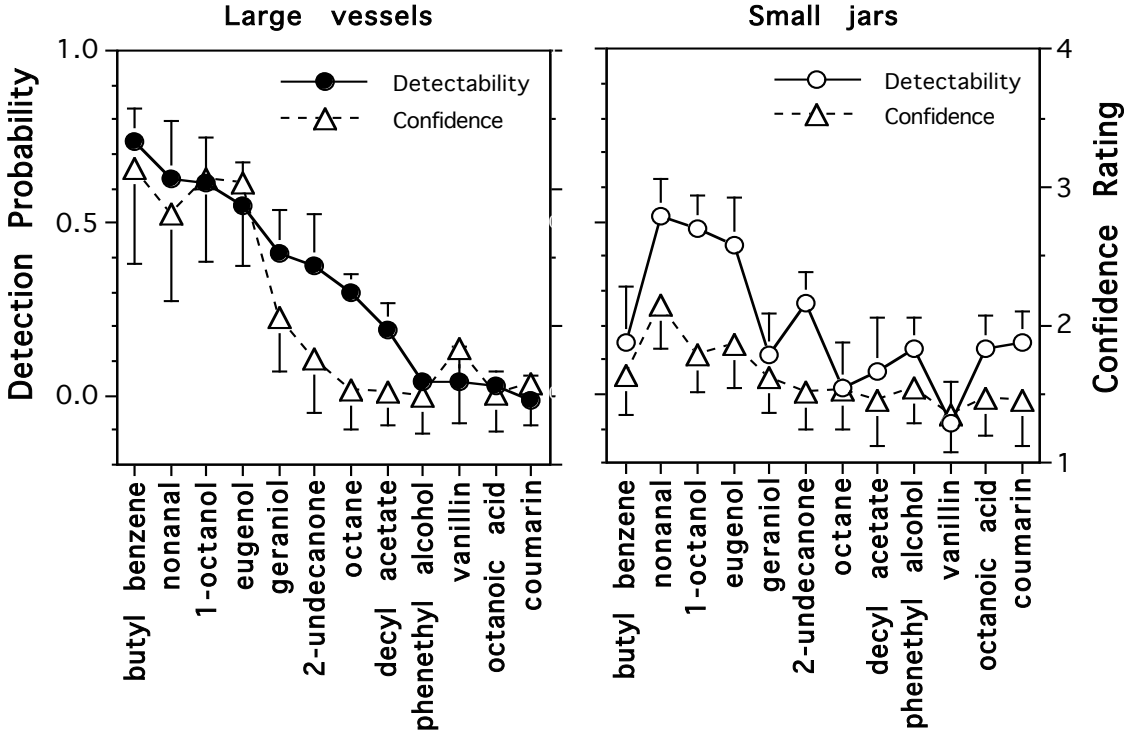


FIGURE 5

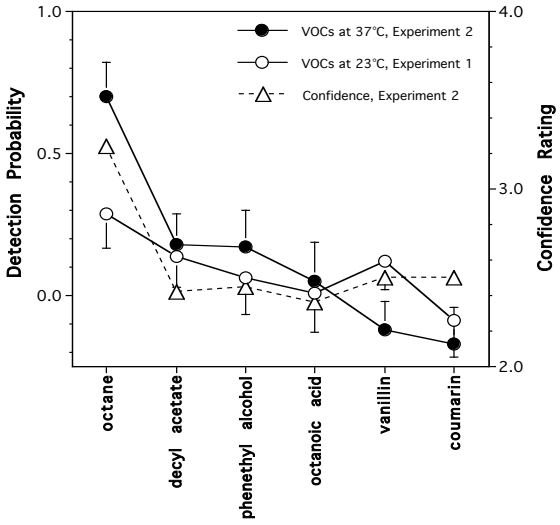


FIGURE 6

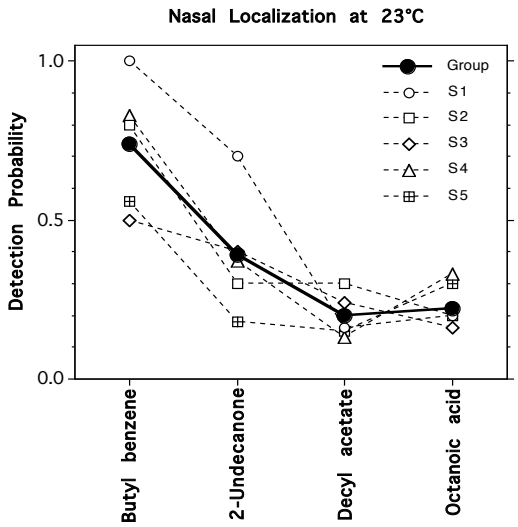


FIGURE 7

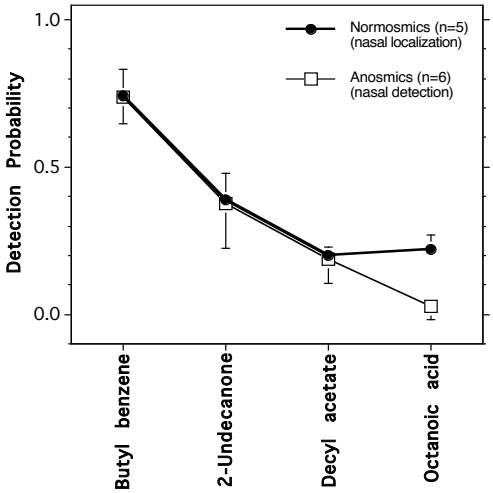


FIGURE 8

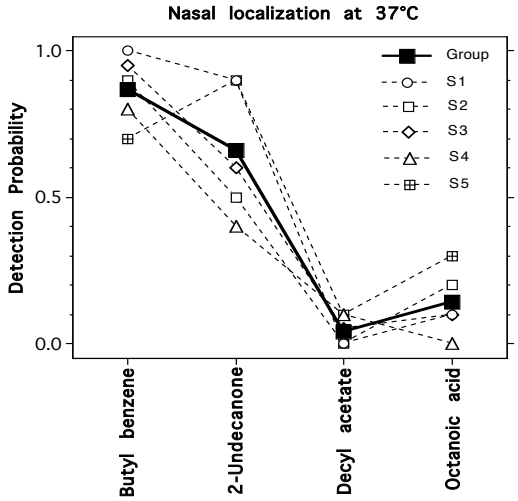


FIGURE 9

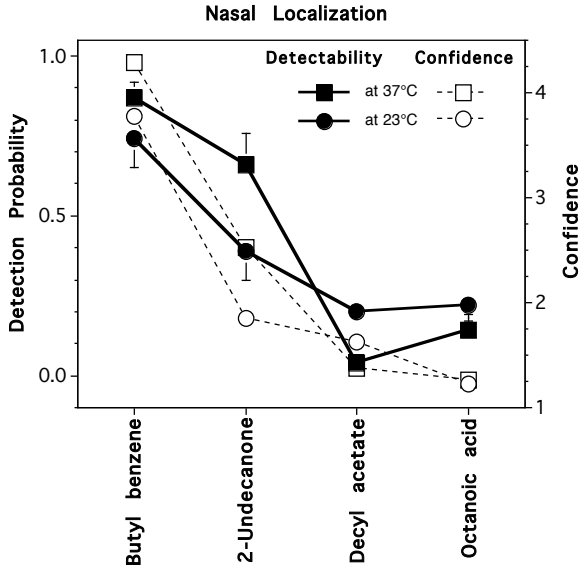
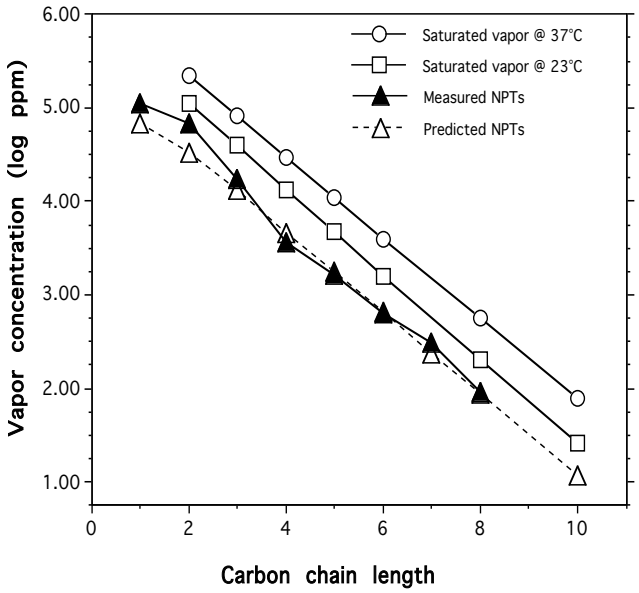


FIGURE 10


Saturated vapor @ 37°C
Saturated vapor @ 23°C
Measured NPTs
Predicted NPTs
Vapor concentration (log ppm)
Carbon chain length
6.00
5.00
4.00
3.00
2.00
1.00
0
2
4
6
8
10

APPENDIX FIGURES

FIGURE 1-APPENDIX

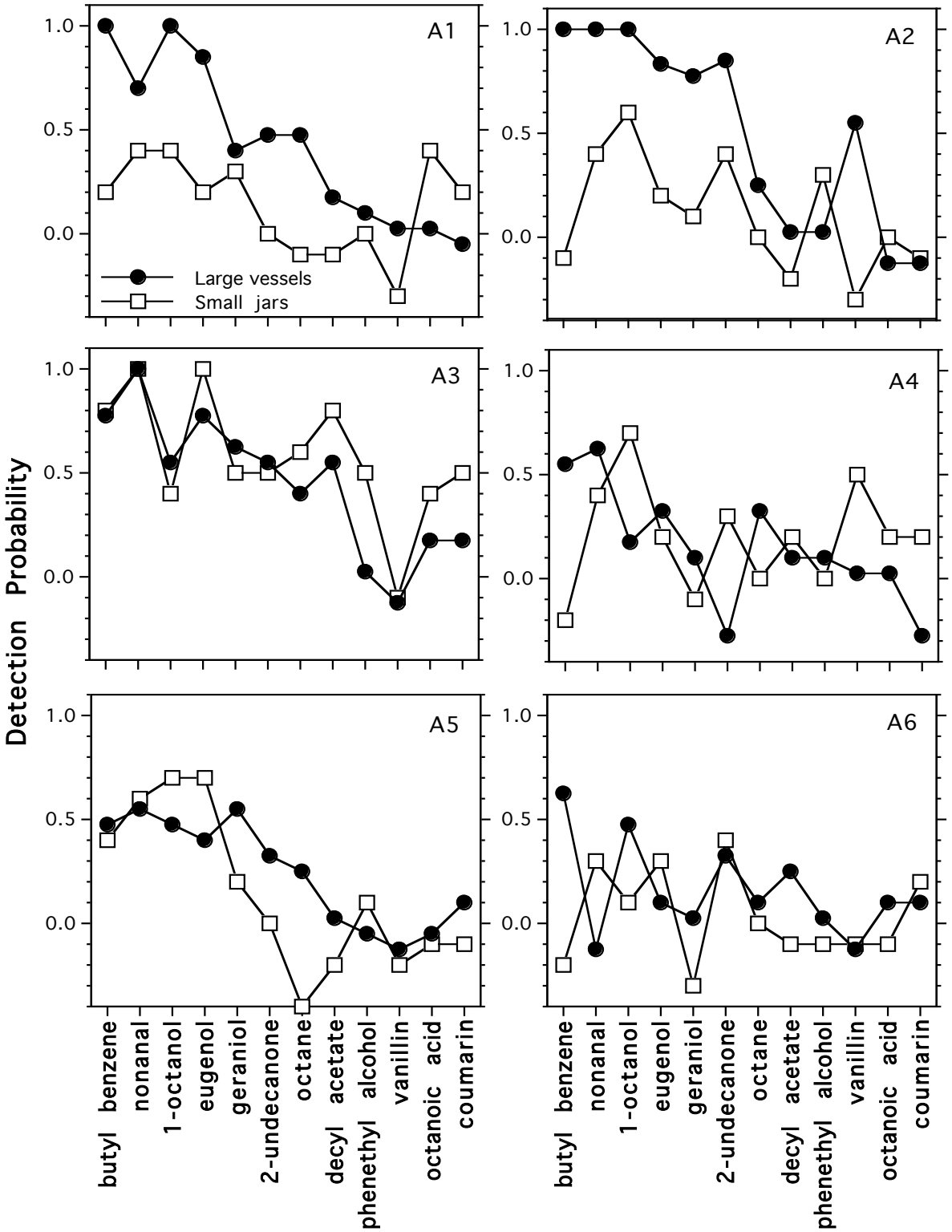


FIGURE 2a-APPENDIX – Large vessels

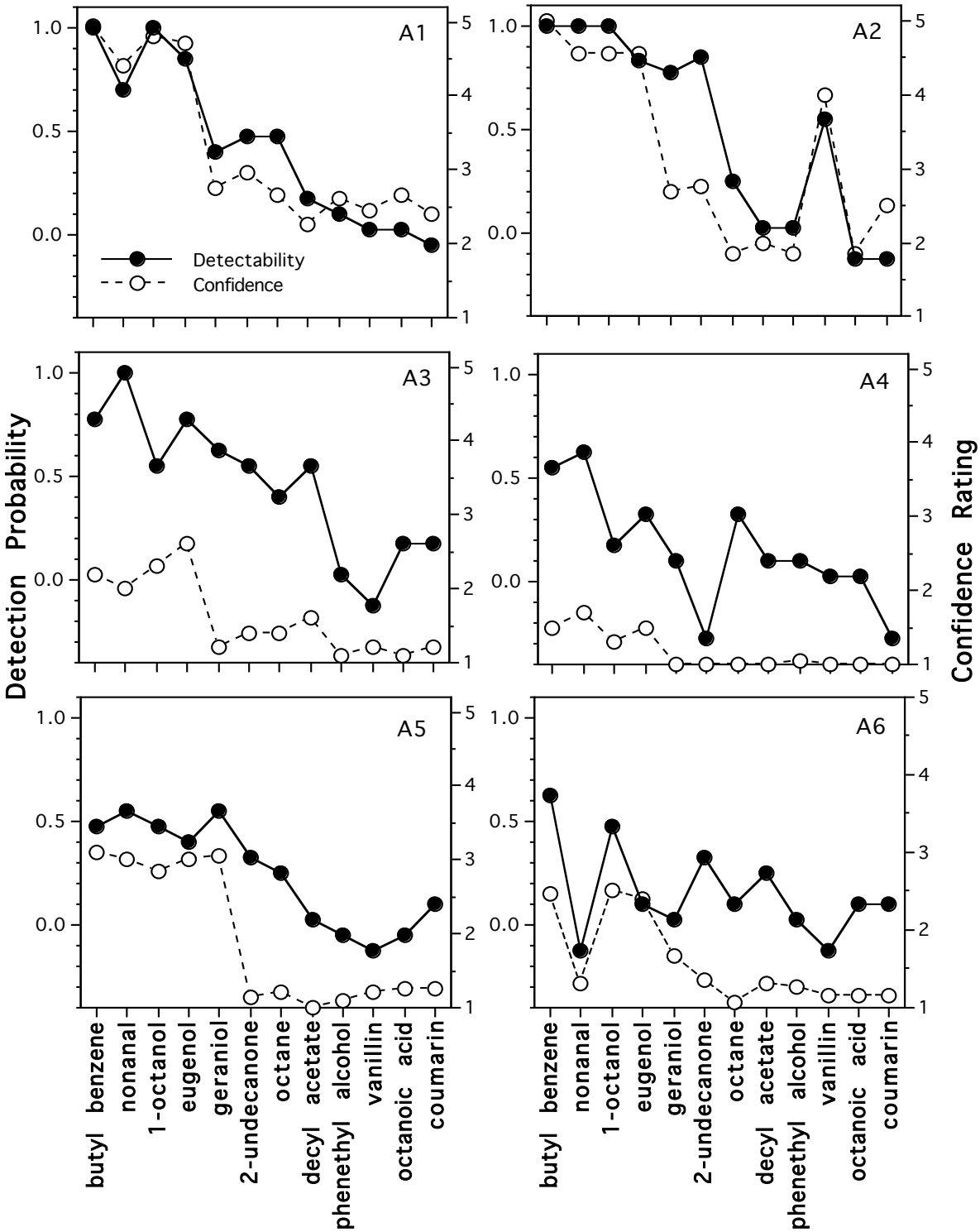


FIGURE 2b-APPENDIX – Small jars

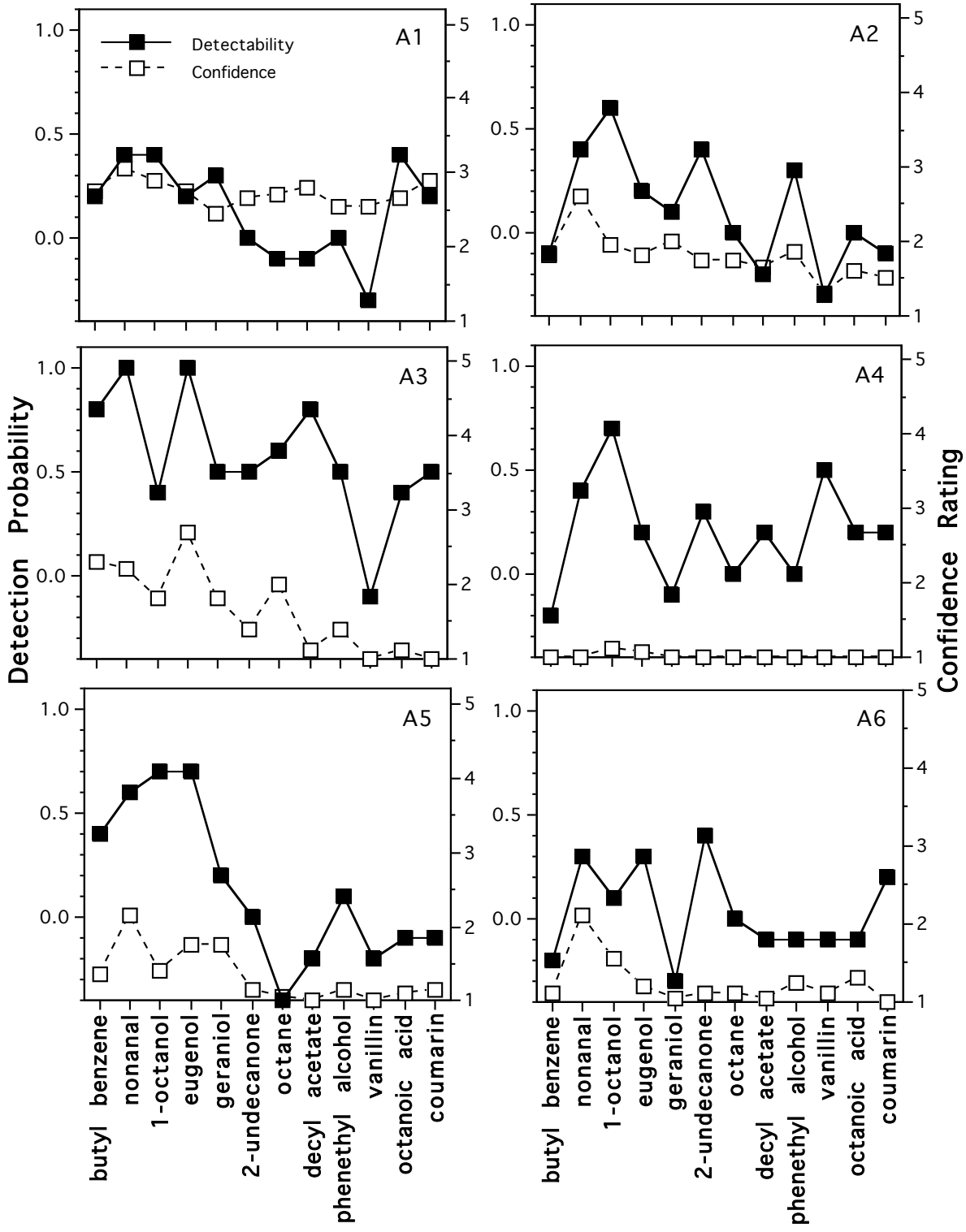
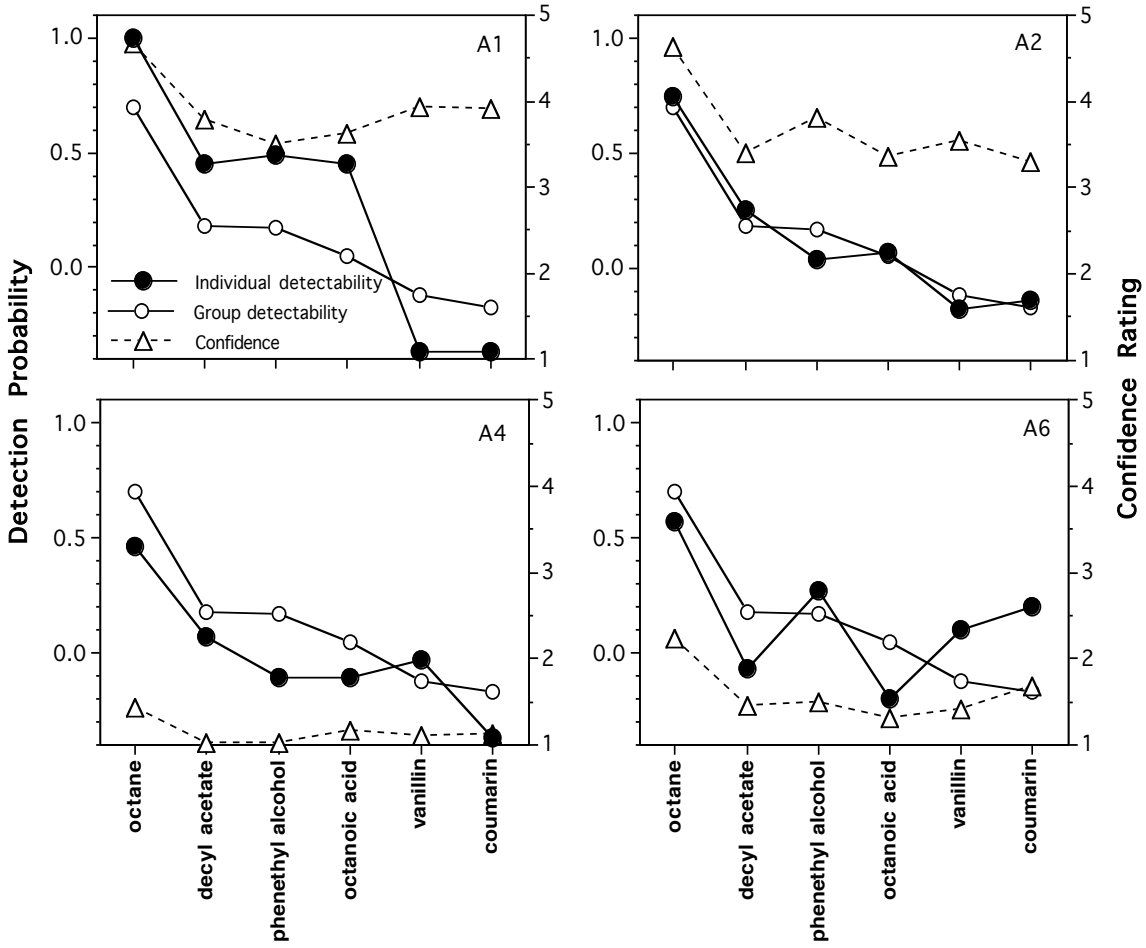


FIGURE 3-APPENDIX



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