Title

Cutoff in detection of eye irritation from vapors of homologous carboxylic acids and aliphatic aldehydes.

Journal

Neuroscience, 145(3)

ISSN

0306-4522

Authors

Cometto-Muñiz, J. Enrique
Cain, William S.
Abraham, Michael H.
et al.

Publication Date

2007

Data Availability

The data associated with this publication are within the manuscript.

Neuroscience **145**:1130-1137, 2007

Cut-off in detection of eye irritation from vapors of homologous carboxylic acids and aliphatic aldehydes

J. Enrique Cometto-Muñiz[a], William S. Cain[a], Michael H. Abraham[b] and Ricardo Sánchez-Moreno[b]

[a]Chemosensory Perception Laboratory, Department of Surgery (Otolaryngology), University of California, San Diego, La Jolla, CA 92093-0957

[b]Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK

Address for correspondence:

J. Enrique Cometto-Muñiz, Ph.D.
Chemosensory Perception Laboratory,
Department of Surgery (Otolaryngology),
9500 Gilman Dr. - Mail Code 0957
University of California, San Diego
La Jolla, CA 92093-0957

Phone: (858) 622-5832
FAX: (858) 458-9417
e-mail: ecometto@ucsd.edu

Section Editor: Dr. Richard J. Weinberg

Running head: Eye Irritation cut-off

Abstract

Using neat vapors of selected homologous aldehydes (decanal, undecanal, dodecanal) and carboxylic acids (pentanoic, hexanoic, heptanoic, octanoic, nonanoic), we explored the point where a certain homolog (and all larger ones) becomes undetectable by eye irritation (i.e., by ocular chemesthesis). This phenomenon has been observed in other homologous series that also reach a break-point, or cut-off, in chemesthetic detection. Participants ($11 \leq n \leq 32$) were tested using a three-alternative, forced-choice procedure. Flowrate to the eye equaled 4 or 8 L/min and time of exposure was 6 sec. The outcome showed that dodecanal and heptanoic acid were the shortest undetectable homologs. When the vapor concentration of the stimuli was increased by heating the liquid source to 37°C, homologs located before the cut-off point (e.g., hexanoic acid) became readily detected by all subjects, whereas homologs located at the cut off remained largely undetected. In addition, a comparison of calculated values of eye irritation thresholds for aldehydes and acids (from a successful model of ocular chemesthetic potency) with values of saturated vapor concentration at 23 and 37 °C indicated that the vapor concentration of dodecanal and heptanoic acid should have been enough to produce detection. The outcome suggests that the cut-off observed does not result from a low vapor concentration but from limitations in the structure or dimension(s) of the molecules that render them unsuitable to interact effectively with chemesthetic receptors.

Key words: Ocular chemesthesis – Homologous n-Aldehydes – Homologous Carboxylic Acids – Eye irritation detection – Molecular cut-off – Ocular Trigeminal Chemosensitivity

Introduction

Eye irritation from volatile organic compounds (VOCs) is a sensory endpoint of behavioral relevance in a variety of common everyday situations, for example, indoor environments of poor air quality (Wolkoff *et al.*, 2005). Airborne chemicals impinging upon exposed mucosae can produce pungent sensations, including stinging, freshness, prickling, piquancy, tingling, burning, irritation and the like. In the case of the ocular and nasal mucosa, a wide variety of vapors can activate trigeminal nerve endings and produce these sensations (Bryant and Silver, 2000, Doty and Cometto-Muñiz, 2003). This broad form of chemical sensitivity, originally known as the common chemical sense (Parker, 1912), is now referred to as chemesthesis, a term that captures the concept of chemically-induced somesthesis (Green et al., 1990, Green and Lawless, 1991). It is also known as chemical nociception (Lee et al., 2005), although low levels of stimulation might not produce pain. The present study addresses the importance of chemical structure-activity on ocular chemesthesis from vapors.

Ocular chemesthetic sensitivity results from stimulation of free nerve endings from C- and A-delta trigeminal fibers, called polymodal nociceptors, that innervate the cornea and conjunctiva (Belmonte *et al.*, 2004). Chemical vapors, e.g. VOCs, could directly stimulate these nociceptors via the large family of transient receptor potential (TRP) channels, for example (Numazaki and Tominaga, 2004, Nilius and Voets, 2005). VOCs that are reactive towards tissue may also produce nociception by damaging cells and producing secondary release of endogenous chemical mediators activating various

ion channels, such as acid sensing ion channels (ASIC), purinergic receptor subtype X channels (P2X), and serotonin ionotropic receptors (Rang et al., 1991, Wood and Docherty, 1997, McCleskey and Gold, 1999, Lee et al., 2005). Pharmacological and molecular-biology studies have identified a number of likely chemesthetic receptors such as the nicotine (Thuerauf et al., 1999, Alimohammadi and Silver, 2000, Thuerauf et al., 2006), capsaicin (Walpole *et al.*, 1996), and menthol (Eccles, 1994, Peier et al., 2002) receptors. The last two receptors are also thermoreceptors responding to warm/hot and cool/cold temperatures, respectively (Caterina et al., 1997, McKemy et al., 2002). The capsaicin receptor not only responds to chemically-related vanilloids (Szallasi and Blumberg, 1999) but also to unrelated VOCs (Trevisani et al., 2002, Silver et al., 2006), to other pungent compounds (Macpherson et al., 2005, McNamara et al., 2005) and even inorganic volatiles (Trevisani et al., 2005). Similarly, the nicotine receptor is modulated by VOCs such as homologous alcohols (Godden et al., 2001). Conversely, menthol (Macpherson et al., 2006) and other pungent substances (Jordt et al., 2004, Bautista et al., 2005) may activate a number of receptors. VOCs also stimulate trigeminal neurons insensitive to capsaicin and cooling, suggesting they activate other mechanism(s) and receptors (Inoue and Bryant, 2005). Most likely, chemesthetic activation results from the integrated response of a number of receptors and pathways (Tominaga *et al.*, 1998).

Studies of VOCs from various homologous chemical series have shown that chemesthetic detection thresholds decrease, indicating that potency increases, with increasing carbon chain length (Cometto-Muñiz, 2001). Interestingly, this trend reached a break point in each series where the homolog failed to produce chemesthesis, even at

vapor saturation. All ensuing larger homologs failed too. A quantitative structure-activity relationship (QSAR) model has revealed that chemesthetic potency rests largely on "selective" effects governing the transport of VOCs from the air to, ultimately, the receptor biophase (Abraham et al., 1998, Abraham et al., 2001, Abraham et al., 2003). The QSAR does not include a term that accounts for the break-point observed in chemesthesis. We have begun to explore the basis for this effect, and the location where it occurs within each series, with the aim of incorporating a parameter to account for it in the QSAR (Cometto-Muñiz et al., 2005b, Cometto-Muñiz et al., 2006).

Testing the chemesthetic effectiveness of increasing members of homologous series can help to characterize the chemical and structural boundaries that make a vapor an effective or ineffective chemesthetic stimulus, as illustrated by the break-point phenomenon. This phenomenon bears resemblance to the cut-off effect observed for the aqueous anesthetic potency of homologous VOCs (Franks and Lieb, 1985, Franks and Lieb, 1990, Franks and Lieb, 1994). In that case, the investigators concluded that molecules could exceed the size of binding pockets in protein receptors. We have investigated both ocular and nasal chemesthetic detection cut-offs along diverse VOCs and found experimental evidence of a chemical-structural limitation in the molecule, not overcome by increasing the vapor concentration (Cometto-Muñiz et al., 2004, Cometto-Muñiz et al., 2005b, Cometto-Muñiz et al., 2005a, Cain et al., 2006, Cometto-Muñiz et al., 2006). In the present study we explore this issue for homologous aliphatic aldehydes and carboxylic acids.

Experiment 1: Eye irritation detectability of saturated vapors at room temperature (23°C)

Materials and Methods

The protocol for all experiments was approved by an institutional review board at the University of California, San Diego. All participants provided written informed consent.

Subjects. We recruited a pool of 32 participants (17 female) with an average age of 25 (±9, SD) years, ranging from 18 to 56. Most of them (27, 16 female) performed in the normosmic range of a clinical olfactory test (Cain, 1989). Four subjects (1 female) performed in the mildly hyposmic range, and only one man in the moderately hyposmic range. Six subjects were smokers (2 female). There were 3 contact lens wearers (all women) but none wore them on testing days. Seven participants (4 female) self-reported seasonal allergies that were not active at the time of testing. Individual data from the few hyposmics, smokers, contact users, and allergics did not fall out of range from that of the rest of the participants.

A subset of 21 subjects (10 female) completed at least 20 trials per condition (i.e., chemical and flowrate, see below). When analyzing individual data, only the results from this more intensively tested group were included. The characteristics of the subset were comparable to that of the larger pool described above.

Stimuli. We tested the following neat three aliphatic aldehydes and three carboxylic acids (purity in brackets): decanal (95+%, Food Chemical Codex, i.e., FCC, quality), undecanal (96+%, FCC), dodecanal (95+%, FCC), heptanoic (97+%), octanoic (98+%), and nonanoic (96+%) acid. Previous work on nasal pungency had suggested that chemesthesis would fail to be evoked starting from one of these homologs (Cometto-Muñiz *et al.*, 1998). Stimuli were presented from the headspace (i.e., vapor phase) of a closed glass vessel system (1,900 ml) containing 200 ml of the neat chemical. The system, adapted for human eye irritation testing, has been described and illustrated in previous studies (Cometto-Muñiz et al., 2005b). Vapor concentrations at 23°C were measured by gas chromatography (flame ionization detector, FID) using a calibration curve for mass, specific for each chemical (Cometto-Muñiz et al., 2003). In the case of nonanoic acid, analytical sensitivity had to be increased by collecting vapor samples (100 ml) into adsorption tubes (Sorbent Tubes, 4.5 in L x 4 mm ID, packed with 20-35 mesh Tenax-TA/Carboxen1000/CarbosieveSIII) and desorbing the samples via a thermal desorption unit (ACEM Model 900, CDS Analytical, Inc.) directly into the gas chromatograph (Hewlett-Packard 5890) for quantification. Average measured concentrations in ppm by volume (±standard deviation, SD) were as follows: 59 (±0.9) for decanal, 32 (±2.5) for undecanal, 8.7 (±1.0) for dodecanal, 28 (±2.7) for heptanoic acid, 6.1 (±0.08) for octanoic acid, and 0.40 (±0.046) for nonanoic acid.

Procedure. To quantify detectability, we employed a three-alternative forced-choice procedure, using the headspace form vessels containing mineral oil (light, FCC) as blanks. Time of exposure equaled 6 sec. Flowrate to the eye equaled 4 and 8 L/min with nitrogen as carrier gas. Thus, there were 12 different stimuli (6 chemicals X 2 flowrates). To avoid depletion of headspace in stimulus vessels, each chemical was prepared in duplicate and presentation was alternated between duplicates. Subjects wore noseclips to avoid odor cues. The testing sequence of chemical and blanks within a trial (i.e., a "triad") and the order of presentation of the different chemical vapors and flow rates across trials were randomized. Participants were instructed not to proceed with the next exposure until all previous sensations (if any) had completely disappeared. We stress that all stimuli presented were at the very border of detection/no detection. Such brief and barely detectable stimulation levels do not produce clinical ocular signs (Podlekareva *et al.*, 2002). After testing each triad, subjects were required to select the presentation that felt "different" (typically stronger) from the other two, guessing if necessary, and to rate the confidence in their decision on a scale from "1" (not confident at all) to "5" (extremely confident). Participants took part in 3 to 4 sessions of 2-3 hours each.

Data analysis. Results are summarized as detection probability (i.e., detectability) and detection confidence of chemical stimulus. Detection probability was corrected for chance according to:

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

where P = detectability corrected for chance, m = number of choices per trial (in our case, three), and p(c) = proportion correct (i.e., number of correct trials / total number of trials) (Macmillan and Creelman, 1991). Statistical significance was established by analysis of variance (ANOVA) for repeated measurements (Software: SuperANOVA v.1.11, Abacus Concepts, Inc., Berkeley, CA).

Results

Figure 1 illustrates detectability and confidence of detection among homologous aldehydes and acids. As expected, detectability of the aldehydes decreased with carbon chain length such that dodecanal was practically undetectable at either flowrate. A higher flowrate produced a noticeable increase in detectability of an already quite detectable stimulus, i.e., decanal, but seemed ineffective for stimuli close to being undetectable, i.e., undecanal and dodecanal. In contrast, all three acids were undetectable, irrespective of flowrate, and confidence of their detection remained relatively flat and low. Using the

data on detectability from the 21 subjects that completed all conditions providing at least 20 trials per chemical and flowrate, we ran an analysis of variance (ANOVA) for repeated measurements using the factors flowrate (2 levels) and chemical (6 levels). Both factors were significant according to $F(1,20) = 10.951$, $p = 0.0035$, and $F(5,100) = 36.171$, $p < 0.0001$, respectively for flowrate and chemical. Their interaction also reached significance: $F(5,100) = 2.526$, $p = 0.05$ (including the Greenhouse-Geisser correction). Contrasts for the interaction revealed that the effect of flow was: a) not significant across the acids, and b) significant across the aldehydes ($p = 0.0005$) due exclusively to the effect on decanal ($p = 0.0001$). Thus, the ANOVA provided statistical support to the observed trends in Figure 1.

Insert Figure 1 about here

A look at the individual data from the subset of 21 intensively tested subjects revealed that most subjects followed the trends seen for the group as a whole, including a decreased detectability of aldehydes as carbon chain length increased, and a lack of effect of carbon chain length on detectability of the acids (Figure 2). For most participants, the acids were undetectable or close to undetectable.

Insert Figure 2 about here

Experiment 2: Eye irritation detectability of pentanoic and hexanoic acids

Experiment 1 established that neat homologous aliphatic aldehydes decrease in detectability with increasing carbon chain length such that dodecanal becomes practically undetectable. The same trend could not be shown among the acids given that none of them could be detected. This outcome suggested that lower members of the series needed to be tested to find the first undetectable homolog. In Experiment 2 we tested the eye irritation detectability of two additional, lower, acids, pentanoic and hexanoic. For comparison, we repeated the testing of dodecanal.

Materials and Methods

Subjects. Eleven participants (6 female) were recruited. Their average age was 30 (±13, SD) years, ranging from 19 to 52. All tested normosmic (Cain, 1989). One female was a smoker and two females used contact lenses but did not wear them on testing days. Four subjects (1 female) self-reported seasonal allergies that were not active at the time of testing. Five subjects (4 female) had participated in Experiment 1.

Stimuli. The stimuli included neat pentanoic (99+%, FCC) and hexanoic (98+%, FCC) acids, and dodecanal (95+%, FCC). Their respective concentrations (±SD) as measured by gas chromatography (FID detector) were: 136 (±3.0), 54 (±3.9), and 8.7 (±1.0) ppm.

Procedure. Same as in Experiment 1, with flowrate to the eye at 4 L/min.

Data analysis. Same as Experiment 1.

Results

Figure 3 illustrates the level of detectability of the three chemicals. It is quite clear that pentanoic acid was easily detected (P=0.93) whereas hexanoic acid was detected only at a low level (P=0.31). The detectability of dodecanal was almost at chance level (P=0.11), practically identical to that in Experiment 1 (P=0.09, see Figure 1).

Insert Figure 3 about here

Experiment 3: Eye irritation detectability of dodecanal, hexanoic acid, and heptanoic acid at 23 and 37 °C

The combined results of Experiments 1 and 2 pointed towards dodecanal and heptanoic acid as the cut-off homologs within their respective series. The relatively low level of detection of hexanoic acid (P=0.31) led us to include it too for further testing. In Experiment 3 we set out to test if an increase in vapor concentration of these three chemicals, achieved by heating their sources to 37°C, could precipitate detection.

Materials and Methods

Subjects. We tested 23 participants (13 female). Their average age was 27 (±12, SD) years, ranging from 18 to 56. All of them tested normosmic (Cain, 1989) and were nonsmokers. Four subjects used contact lenses but did not wear them on testing days. Three subjects (2 female) reported respiratory and/or seasonal allergies that were not active at the time of testing. Twenty participants (11 female) completed at least 20 trials per condition (chemical and temperature, i.e., concentration, see below) and the analysis of individual data, including the repeated measures ANOVA, was done on this group.

Stimuli. The concentrations (at 23°C) of dodecanal, hexanoic acid, and heptanoic acid were as in Experiments 1 and 2. Their concentrations (ppm±SD) at 37°C as determined by gas chromatography (FID detector) were 39 (±3.7) for dodecanal, 330 (±31) for hexanoic acid, and 82 (±8.7) for heptanoic acid. These values represent increments of 4.5, 6.1, and 2.9 times, respectively, compared to their concentrations at 23°C.

Procedure. Same as in Experiment 1, with flowrate to the eye at 4 L/min. In Experiment 3 each chemical was tested at 23°C and at 37°C, in the same session, against blanks at the corresponding temperature. Heated chemicals and blanks were presented from bottles resting in two calibrated water baths that delivered the vapor at approximately 37°C. Water baths and bottles were covered by a sheet of lab surface protector (made of cellulose fibers on top and polyethylene backing on bottom) perforated to allow only the top of the bottle to show. This sheet served, firstly, to hide

the content of the bottles from view, and, secondly, to minimize temperature loss from the water bath to the room. The testing room was well ventilated and contained an activated carbon-based air purifier.

Data analysis. Same as in Experiment 1.

Results

Figure 4 illustrates the outcome. The detectability of hexanoic acid jumped dramatically from virtually chance detection (P=0.04) to almost perfect detection (P=0.95) when its vapor concentration was increased by heating its liquid source to 37°C. In contrast, the detectability of heptanoic acid remained close to chance level (from about P≈0.0 to P=0.11) and that of dodecanal only reached P = 0.25. These trends were confirmed by the results of an ANOVA for repeated measurements on the factors chemical (three levels) and concentration, i.e., temperature, (two levels) that showed significant differences for both chemical ($F(2,38)=68.69$, $p<0.0001$) and concentration ($F(1,19)=168.66$, $p<0.0001$), as well as for their interaction ($F(2,38)=35.56$, $p<0.0001$).

Insert Figure 4 about here

A look at the individual data revealed that whereas every single subject increased the detectability of hexanoic acid at the higher concentration (H), less than half the subjects increased their detection of heptanoic acid and/or dodecanal (Figure 5). For these two chemicals, participants failed to show a uniform trend as seen for hexanoic acid

(Figure 6). Considering the range of individual variability observed even when all subjects follow a uniform trend (e.g., Figure 5, hexanoic acid), we note that, out of 23 subjects, roughly 17 (8 females) in the case of heptanoic acid and 15 (8 females) in that of dodecanal failed to increase their detection of eye irritation with an increase in vapor concentration (Figure 6). In fact, most subjects who failed to increase detection did so for both chemicals (n=13, 7 females), and half or more of the subjects that increased detection did so for both chemicals (n=4, 3 females). Only four subjects increased detection for dodecanal but not for heptanoic acid, and only two increased it for heptanoic acid but not for dodecanal. The relative performance of the concentration-responsive group compared to the concentration-unresponsive group is illustrated in Figure 7 for both heptanoic acid and dodecanal.

Insert Figures 5, 6, and 7 about here

Discussion

The present results fall into line with those from previous studies on cut-off points for ocular chemesthesis along homologous esters, alcohols, alkylbenzenes, and ketones. Among acetate esters and n-alcohols, decyl acetate and 1-undecanol, respectively, were the shortest homologs that failed to elicit eye irritation (Cometto-Muñiz et al., 2005b). Among alkylbenzenes and 2-ketones, heptyl benzene and 2-tridecanone, respectively, were the shortest homologs that failed to elicit eye irritation (Cometto-Muñiz *et al.*, 2006). When the saturated vapor concentration of these four cut-off homologs was

increased (via heating to 37°C) at least 2.7 times (case of decyl acetate) and up to 8.2 times (case of 1-undecanol), the increase in detectability by eye irritation was always minimal ($\Delta P<0.16$) whether reaching statistical significance or not. To help put these values in perspective we mention that chemesthetic concentration-detection (i.e., psychometric) functions often grow from chance detection to almost perfect detection (i.e., $\Delta P\geq0.90$) within an increase in vapor concentration of less than 10 times (Cometto-Muñiz et al., 2002, Cain et al., 2006). In the work reported here, the saturated vapor concentration of the cut-off homologs dodecanal and heptanoic acid (Figure 1) was increased (via heating to 37°C) 4.5 and 2.9 times, respectively. Still, detectability barely increased by $\Delta P=0.21$ for dodecanal, and remained within 2 X SE (standard error) of chance level (i.e., $P=0.0\pm0.1$) for heptanoic acid (Figure 4). In contrast, a concentration increase of 6.1 times for hexanoic acid, the homolog just before the cut-off point, could bring its detectability from virtually chance ($P<0.05$) to virtually perfect detection ($P>0.95$) (Figure 4).

Additional information can be gathered by looking at the performance of individual subjects (Figures 2, 5, and 6), where the results also agree closely with those obtained previously for other series (Cometto-Muñiz et al., 2005b, Cometto-Muñiz et al., 2006). For a homolog situated before the cut-off, such as hexanoic acid, increasing its vapor concentration produces a dramatic increase in detectability, as observed in Figure 5 for every subject (n=23). (Many individuals even went from chance to almost perfect detection.) In contrast, for homologs situated at the cut-off, such as heptanoic acid and dodecanal, increasing its vapor concentration only produces a modest increase in

detection for some participants whereas for most participants no increase occurs (Figure 6). Importantly, we note that among the subjects that failed to increase detection with concentration, a number of them (3 to 6) already showed, at the lower concentration, a modest but above chance level of detectability, i.e., $0.2<P<0.5$ (Figure 6, Left). Still, these subjects roughly maintained the same (above chance) detectability after the concentration has been increased about 3 times (heptanoic acid) or 4.5 times (dodecanal). In the context of the relative steep nature of chemesthetic functions, as discussed above, this suggests that, for these individuals, concentration is no longer driving detection. An alternative limiting factor for the lack of chemesthetic potency of these vapors is molecular dimension(s). This alternative is consistent with the protein nature of described chemesthetic receptors (Pedersen et al., 2005, Owsianik et al., 2006) whose suspected, but largely unknown, binding site(s) (Gunthorpe et al., 2002, Voets et al., 2005) would fail to accommodate molecules beyond a critical dimension, a phenomenon that has already been observed and described for cut-offs in anesthetic potency (Eger and Laster, 2001).

We have put forward a QSAR model based on a solvation equation that has successfully described and predicted ocular and nasal chemesthetic potency of vapors (Abraham et al., 1998, Abraham et al., 2003). We note that calculations from the model of ocular chemesthetic potency for aldehydes indicate that detection should have occurred at 37°C vapor saturation for dodecanal and, perhaps, beyond it (Figure 8). In turn, calculations for acids indicate that detection should even have occurred at 23°C

vapor saturation for heptanoic acid and well beyond it (Figure 8). The calculations further support the role of molecular dimension as the basis for the cut-offs.

Insert Figure 8 about here

The QSAR model has mechanistic significance and implies that chemesthesis, as assessed psychophysically, relies very heavily on selective processes of transfer of the irritant VOC from the air to the receptive biophase, and relies to a much lower extent on specific processes of ligand-receptor interactions. In contrast, olfactory potency, although also relying on selective processes (Katada et al., 2005), provides considerably more leverage to specific interactions (Abraham et al., 2002). These findings are very compatible with the information on the contrasting number and diversity of putative receptors for the two chemosensory systems in humans: a few tens (30-50) for chemesthesis (Lee et al., 2005, Nilius and Voets, 2005, Pedersen et al., 2005) versus a few hundreds (350-400) for olfaction (Buck, 2004, Niimura and Nei, 2006), despite both modalities responding to roughly the same range of VOCs, albeit at different concentration ranges (Cometto-Muñiz, 2001). As reports of particular cut-off homologs for ocular and nasal chemesthesis accumulate across a growing number of chemically-diverse series, it may become possible to apply molecular modeling approaches to elucidate critical common dimensions that all cut-off molecules are likely to share. The identification and quantification of these critical dimensions will produce a new parameter(s) that, added to the present QSAR equation for chemesthetic potency of

vapors toward humans, will enhance its applicability by including a term that addresses the cut-off effect (Abraham et al., 2001).

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, and, in part, by Philip Morris USA Inc. and Philip Morris International. Thanks are due to T. Chang, E. Kertowidjojo, and W.-C. Wu for technical assistance.

References

Abraham, M. H., Gola, J. M., Cometto-Muñiz, J. E. and Cain, W. S., 2002. A model for odour thresholds. Chem Senses 27: 95-104.

Abraham, M. H., Gola, J. M. R., Cometto-Muñiz, J. E. and Cain, W. S., 2001. The correlation and prediction of VOC thresholds for nasal pungency, eye irritation and odour in humans. Indoor Built Environ 10: 252-257.

Abraham, M. H., Hassanisadi, M., Jalali-Heravi, M., Ghafourian, T., Cain, W. S. and Cometto-Muñiz, J. E., 2003. Draize rabbit eye test compatibility with eye irritation thresholds in humans: A quantitative structure-activity relationship analysis. Toxicol Sci 76: 384-391.

Abraham, M. H., Kumarsingh, R., Cometto-Muñiz, J. E. and Cain, W. S., 1998. An algorithm for nasal pungency thresholds in man. Arch Toxicol 72: 227-232.

Alimohammadi, H. and Silver, W. L., 2000. Evidence for nicotinic acetylcholine receptors on nasal trigeminal nerve endings of the rat. Chem Senses 25: 61-66.

Bautista, D. M., Movahed, P., Hinman, A., Axelsson, H. E., Sterner, O., Hogestatt, E. D., Julius, D., Jordt, S. E. and Zygmunt, P. M., 2005. Pungent products from garlic

activate the sensory ion channel TRPA1. Proc Natl Acad Sci U S A 102: 12248-12252.

Belmonte, C., Acosta, M. C. and Gallar, J., 2004. Neural basis of sensation in intact and injured corneas. Exp Eye Res 78: 513-525.

Bryant, B. and Silver, W. L., 2000. Chemesthesis: The Common Chemical Sense. In: Finger, T. E. et al. (Eds.), The Neurobiology of Taste and Smell. 2nd Edition. Wiley-Liss, New York, pp. 73-100.

Buck, L. B., 2004. Olfactory receptors and odor coding in mammals. Nutr Rev 62: S184-188; discussion S224-141.

Cain, W. S., 1989. Testing olfaction in a clinical setting. Ear Nose Throat J 68: 316, 322-318.

Cain, W. S., Lee, N. S., Wise, P. M., Schmidt, R., Ahn, B. H., Cometto-Muñiz, J. E. and Abraham, M. H., 2006. Chemesthesis from volatile organic compounds: Psychophysical and neural responses. Physiol Behav 88: 317-324.

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D. and Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816-824.

Cometto-Muñiz, J. E., 2001. Physicochemical basis for odor and irritation potency of VOCs. In: Spengler, J. D. et al. (Eds.), Indoor Air Quality Handbook. McGraw-Hill, New York, pp. 20.21—20.21.

Cometto-Muñiz, J. E., Cain, W. S. and Abraham, M. H., 1998. Nasal pungency and odor of homologous aldehydes and carboxylic acids. Exp Brain Res 118: 180-188.

Cometto-Muñiz, J. E., Cain, W. S. and Abraham, M. H., 2003. Quantification of chemical vapors in chemosensory research. Chem Senses 28: 467-477.

Cometto-Muñiz, J. E., Cain, W. S. and Abraham, M. H., 2004. Detection of single and mixed VOCs by smell and by sensory irritation. Indoor Air 14 Suppl 8: 108-117.

Cometto-Muñiz, J. E., Cain, W. S. and Abraham, M. H., 2005a. Determinants for nasal trigeminal detection of volatile organic compounds. Chem Senses 30: 627-642.

Cometto-Muñiz, J. E., Cain, W. S. and Abraham, M. H., 2005b. Molecular restrictions for human eye irritation by chemical vapors. Toxicol Appl Pharmacol 207: 232-243.

Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H. and Gola, J. M. R., 2002. Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene. J Appl Toxicol 22: 25-30.

Cometto-Muñiz, J. E., Cain, W. S., Abraham, M. H. and Sanchez-Moreno, R., 2006. Chemical boundaries for detection of eye irritation in humans from homologous vapors. Toxicol Sci 91: 600-609.

de Kruif, C. G., Schaake, R. C. F., van Miltenburg, J. C., van der Klauw, K. and Blok, J. G., 1982. Thermodynamic properties of the normal alkanoic acids. III. Enthalpies of vaporization and vapor pressures of 13 normal alkanoic acids. J Chem Thermodyn 14: 791-798.

Doty, R. L. and Cometto-Muñiz, J. E., 2003. Trigeminal chemosensation. In: Doty, R. L. (Ed.), Handbook of Olfaction and Gustation (2nd Edition). Marcel Dekker, New York, pp. 981-1000.

Eccles, R., 1994. Menthol and related cooling compounds. J Pharm Pharmacol 46: 618-630.

Eger, E. I., 2nd and Laster, M. J., 2001. The effect of rigidity, shape, unsaturation, and length on the anesthetic potency of hydrocarbons. Anesth Analg 92: 1477-1482.

Franks, N. P. and Lieb, W. R., 1985. Mapping of general anesthetic target sites provides a molecular basis for cutoff effects. Nature 316: 349-351.

Franks, N. P. and Lieb, W. R., 1990. Mechanisms of general anesthesia. Environ Health Perspect 87: 199-205.

Franks, N. P. and Lieb, W. R., 1994. Molecular and cellular mechanisms of general anaesthesia. Nature 367: 607-614.

Godden, E. L., Harris, R. A. and Dunwiddie, T. V., 2001. Correlation between molecular volume and effects of n-alcohols on human neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. J Pharmacol Exp Ther 296: 716-722.

Green, B. G. and Lawless, H. T., 1991. The psychophysics of somatosensory chemoreception in the nose and mouth. In: Getchell, T. V. et al. (Eds.), Smell and Taste in Health and Disease. Raven Press, New York, pp. 235-253.

Green, B. G., Mason, J. R. and Kare, M. R., 1990. Preface. In: Green, B. G. et al. (Eds.), Chemical Senses. Vol. 2: Irritation. Marcel Dekker, Inc., New York, pp. v-vii.

Gunthorpe, M. J., Benham, C. D., Randall, A. and Davis, J. B., 2002. The diversity in the vanilloid (TRPV) receptor family of ion channels. Trends Pharmacol Sci 23: 183-191.

Inoue, T. and Bryant, B. P., 2005. Multiple types of sensory neurons respond to irritating volatile organic compounds (VOCs): calcium fluorimetry of trigeminal ganglion neurons. Pain 117: 193-203.

Jordt, S. E., Bautista, D. M., Chuang, H. H., McKemy, D. D., Zygmunt, P. M., Hogestatt, E. D., Meng, I. D. and Julius, D., 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427: 260-265.

Katada, S., Hirokawa, T., Oka, Y., Suwa, M. and Touhara, K., 2005. Structural basis for a broad but selective ligand spectrum of a mouse olfactory receptor: mapping the odorant-binding site. J Neurosci 25: 1806-1815.

Lee, Y., Lee, C. H. and Oh, U., 2005. Painful channels in sensory neurons. Mol Cells 20: 315-324.

Macmillan, N. A. and Creelman, C. D., 1991. Detection theory: A user's guide. Cambridge University Press, Cambridge.

Macpherson, L. J., Geierstanger, B. H., Viswanath, V., Bandell, M., Eid, S. R., Hwang, S. and Patapoutian, A., 2005. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15: 929-934.

Macpherson, L. J., Hwang, S. W., Miyamoto, T., Dubin, A. E., Patapoutian, A. and Story, G. M., 2006. More than cool: promiscuous relationships of menthol and other sensory compounds. Mol Cell Neurosci 32: 335-343.

McCleskey, E. W. and Gold, M. S., 1999. Ion channels of nociception. Annu Rev Physiol 61: 835-856.

McKemy, D. D., Neuhausser, W. M. and Julius, D., 2002. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416: 52-58.

McNamara, F. N., Randall, A. and Gunthorpe, M. J., 2005. Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). Br J Pharmacol 144: 781-790.

Niimura, Y. and Nei, M., 2006. Evolutionary dynamics of olfactory and other chemosensory receptor genes in vertebrates. J Hum Genet 51: 505-517.

Nilius, B. and Voets, T., 2005. TRP channels: a TR(I)P through a world of multifunctional cation channels. Pflugers Arch 451: 1-10.

Numazaki, M. and Tominaga, M., 2004. Nociception and TRP Channels. Curr Drug Targets CNS Neurol Disord 3: 479-485.

Owsianik, G., D'Hoedt, D., Voets, T. and Nilius, B., 2006. Structure-function relationship of the TRP channel superfamily. Rev Physiol Biochem Pharmacol 156: 61-90.

Parker, G. H., 1912. The relation of smell, taste, and the common chemical sense in vertebrates. Journal of the Academy of Natural Sciences Philadelphia 15: 219-234.

Pedersen, S. F., Owsianik, G. and Nilius, B., 2005. TRP channels: an overview. Cell Calcium 38: 233-252.

Peier, A. M., Moqrich, A., Hergarden, A. C., Reeve, A. J., Andersson, D. A., Story, G. M., Earley, T. J., Dragoni, I., McIntyre, P., Bevan, S. and Patapoutian, A., 2002. A TRP channel that senses cold stimuli and menthol. Cell 108: 705-715.

Podlekareva, D., Pan, Z., Kjærgaard, S. and Mølhave, L., 2002. Irritation of the human eye mucous membrane caused by airborne pollutants. Int Arch Occup Environ Health 75: 359-364.

Rang, H. P., Bevan, S. and Dray, A., 1991. Chemical activation of nociceptive peripheral neurones. Br Med Bull 47: 534-548.

Riddick, J. A. and Bunger, W. B., 1970. Organic solvents. In: Weissberger, A. (Ed.), Techniques of Chemistry, vol.2*,* vol.2. Wiley-Interscience, New York.

Silver, W. L., Clapp, T. R., Stone, L. M. and Kinnamon, S. C., 2006. TRPV1 Receptors and Nasal Trigeminal Chemesthesis. Chem Senses.

Stevenson, R. M. and Malinowski, S., 1987. Handbook of the Thermodynamics of Organic Compounds. Elsevier, New York.

Szallasi, A. and Blumberg, P. M., 1999. Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol Rev 51: 159-212.

Thuerauf, N., Kaegler, M., Dietz, R., Barocka, A. and Kobal, G., 1999. Dose-dependent stereoselective activation of the trigeminal sensory system by nicotine in man. Psychopharmacology (Berl) 142: 236-243.

Thuerauf, N., Markovic, K., Braun, G., Bleich, S., Reulbach, U., Kornhuber, J. and Lunkenheimer, J., 2006. The influence of mecamylamine on trigeminal and olfactory chemoreception of nicotine. Neuropsychopharmacology 31: 450-461.

Tominaga, M., Caterina, M. J., Malmberg, A. B., Rosen, T. A., Gilbert, H., Skinner, K., Raumann, B. E., Basbaum, A. I. and Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21: 531-543.

Trevisani, M., Patacchini, R., Nicoletti, P., Gatti, R., Gazzieri, D., Lissi, N., Zagli, G., Creminon, C., Geppetti, P. and Harrison, S., 2005. Hydrogen sulfide causes vanilloid receptor 1-mediated neurogenic inflammation in the airways. Br J Pharmacol 145: 1123-1131.

Trevisani, M., Smart, D., Gunthorpe, M. J., Tognetto, M., Barbieri, M., Campi, B., Amadesi, S., Gray, J., Jerman, J. C., Brough, S. J., Owen, D., Smith, G. D., Randall, A. D., Harrison, S., Bianchi, A., Davis, J. B. and Geppetti, P., 2002. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5: 546-551.

Verevkin, S. P., Krasnykh, E. L., Vasiltsova, T. V., Koutek, B., Doubsky, J. and Heintz, A., 2003. Vapor pressures and enthalpies of vaporization of a series of the linear aliphatic aldehydes. Fluid Phase Equilib 206: 331-339.

Voets, T., Talavera, K., Owsianik, G. and Nilius, B., 2005. Sensing with TRP channels. Nat Chem Biol 1: 85-92.

Walpole, C. S., Bevan, S., Bloomfield, G., Breckenridge, R., James, I. F., Ritchie, T., Szallasi, A., Winter, J. and Wrigglesworth, R., 1996. Similarities and differences in the structure-activity relationships of capsaicin and resiniferatoxin analogues. J Med Chem 39: 2939-2952.

Wolkoff, P., Nojgaard, J. K., Troiano, P. and Piccoli, B., 2005. Eye complaints in the office environment: precorneal tear film integrity influenced by eye blinking efficiency. Occup Environ Med 62: 4-12.

Wood, J. N. and Docherty, R., 1997. Chemical activators of sensory neurons. Annu Rev Physiol 59: 457-482.

Figure Legends

Figure 1. Average detection of eye irritation (left *y*-axis) and confidence of detection (right *y*-axis) for the three aldehydes and three acids tested at two flowrates. Each point represents the outcome of at least 500 trials from 32 subjects. Bars indicate standard error of the mean.

Figure 2. Individual detection of eye irritation for each stimulus, averaged across the two flowrates, by 21 subjects. Each symbol represents one subject. Single data points comprise the outcome of 40 trials made by a subject. Thick lines and symbols depict the average data for the group.

Figure 3. Average detection of eye irritation for pentanoic acid, hexanoic acid and decanal. Each value represents the outcome of 220 trials from 11 subjects. Bars indicate standard error of the mean.

Figure 4. Group detection of eye irritation from hexanoic acid, heptanoic acid, and dodecanal presented at vapor saturation at 23°C and at vapor saturation at 37°C (i.e., Heated, H). Each column represents an average of 430 trials from 23 subjects. Bars indicate standard error of the mean.

Figure 5. Individual detection of eye irritation from hexanoic acid, heptanoic acid, and dodecanal presented at vapor saturation at 23°C and at vapor saturation at 37°C (i.e.,

heated, H). Each symbol (joined by a dashed line) represents one subject and reflects the outcome of 20 trials from the subject. Large, filled symbols (joined by a thick, continuous line) represent the group (n=20) average data.

Figure 6. Left. Individual data for subjects that failed to increase their detection of heptanoic acid and dodecanal with an increase in concentration, i.e., chemicals heated to 37°C (H). Right. Individual data for subjects that did increase their detection of the stimuli with increased concentration.

Figure 7. Comparison between a group of concentration-unresponsive subjects, for which an increase in concentration of the vapor failed to precipitate or increase detection of eye irritation, and a group of concentration-responsive subjects, for which the increase in concentration did precipitate or increase detection. The left panel shows results for heptanoic acid and the right panel those for dodecanal. Data for the unresponsive groups represent the average of 320 trials (for heptanoic acid) or 280 trials (for dodecanal) per stimulus; data for the responsive groups represent the average of 110 trials (for heptanoic acid) or 150 trials (for dodecanal) per stimulus. Bars indicate standard error.

Figure 8. Vapor concentration of: a) QSAR-calculated eye irritation thresholds (EIT) (Abraham et al., 2003), b) saturated vapor concentration (SVC) at 23°C, and c) SVC at 37°C as a function of carbon chain length of homologous aldehydes and carboxylic acids. The outcome shows how the vapor concentration achieved at 37°C, in the case of aldehydes, and even at 23°C, in the case of acids, should have been enough to produce

detection of dodecanal, heptanoic acid, and larger homologs. Values of SVC for aldehydes and acids were taken from the literature (Riddick and Bunger, 1970, de Kruif et al., 1982, Stevenson and Malinowski, 1987, Verevkin et al., 2003) or, if unavailable, from a linear plot of SVC vs. carbon chain length for the particular homologous series.

FIGURE 1

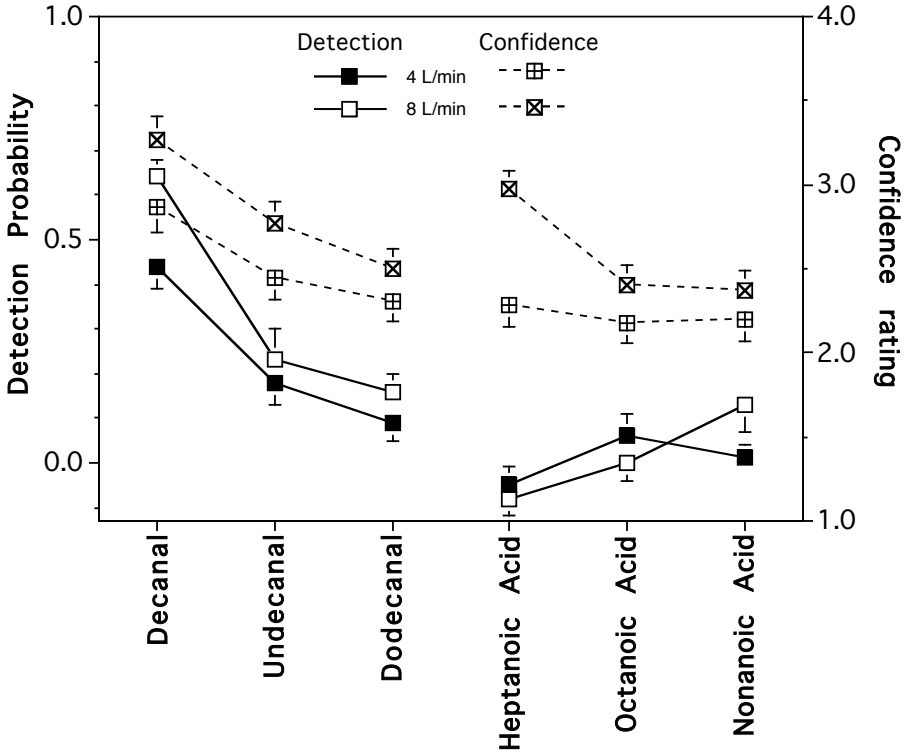


FIGURE 2

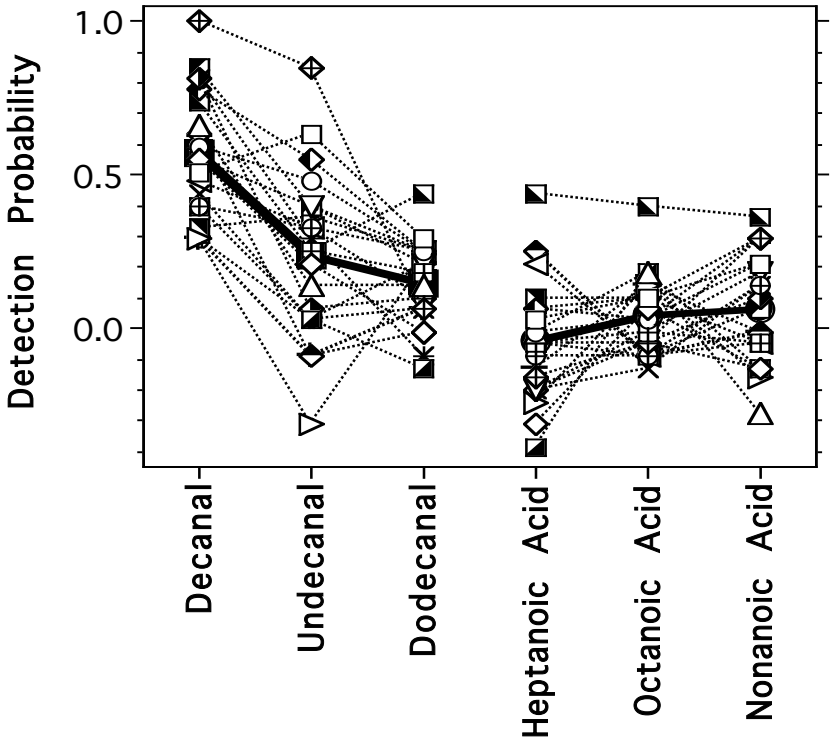


FIGURE 3

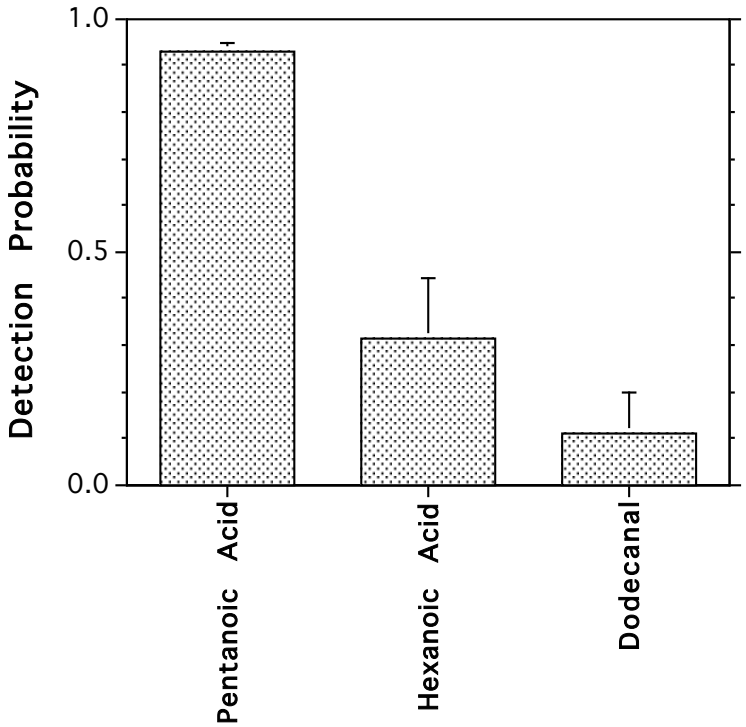


FIGURE 4

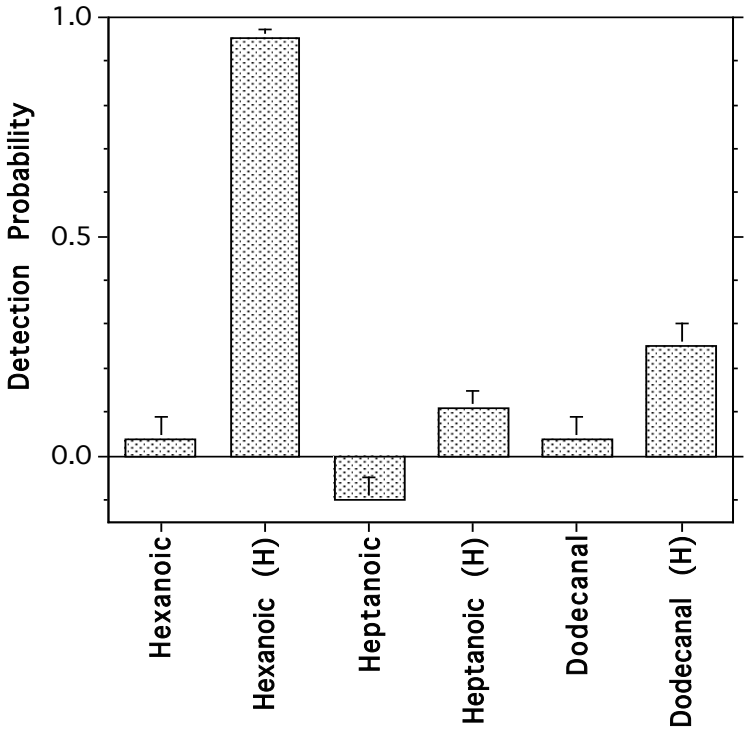


FIGURE 5

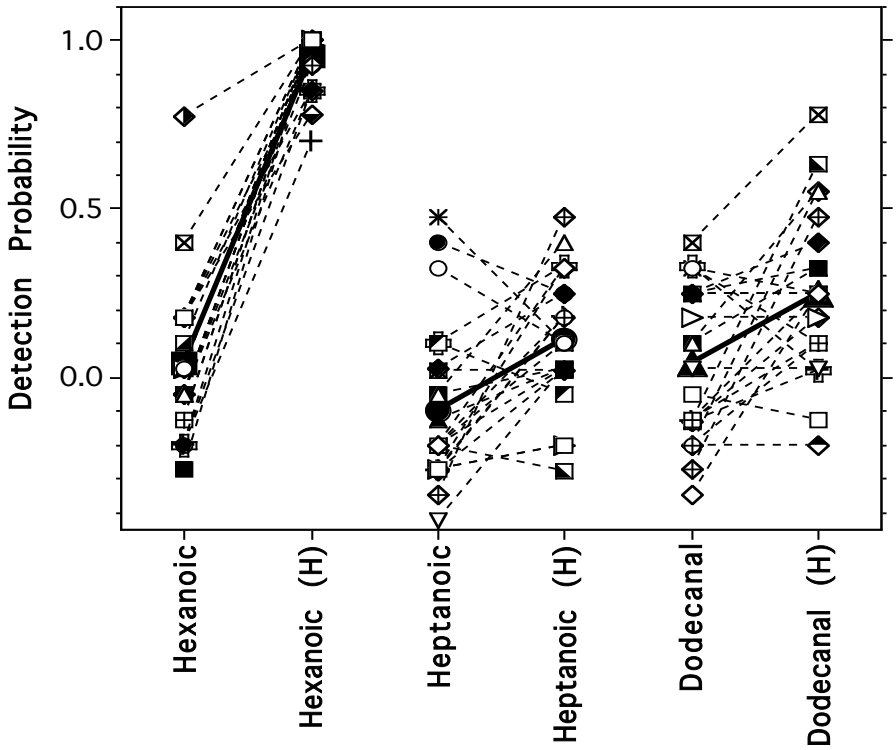


FIGURE 6

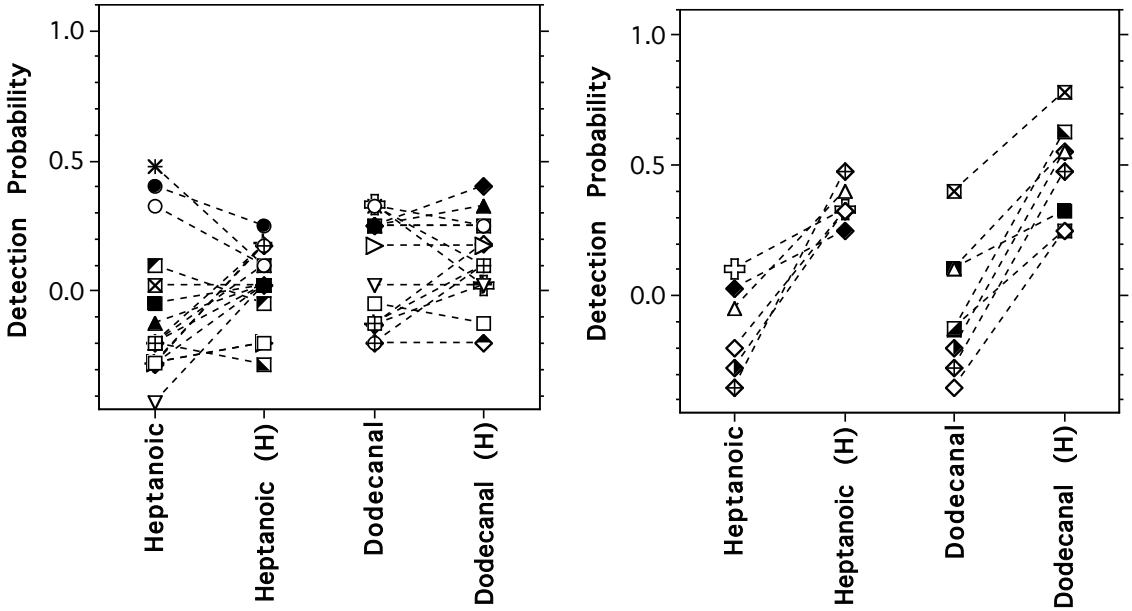


FIGURE 7

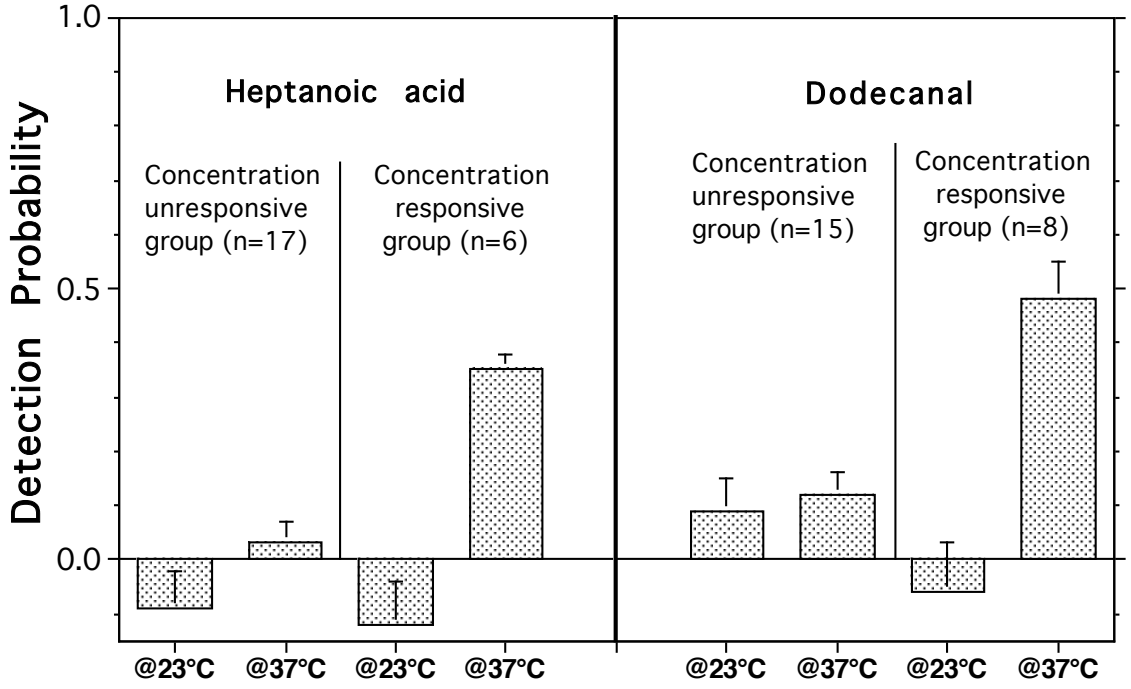
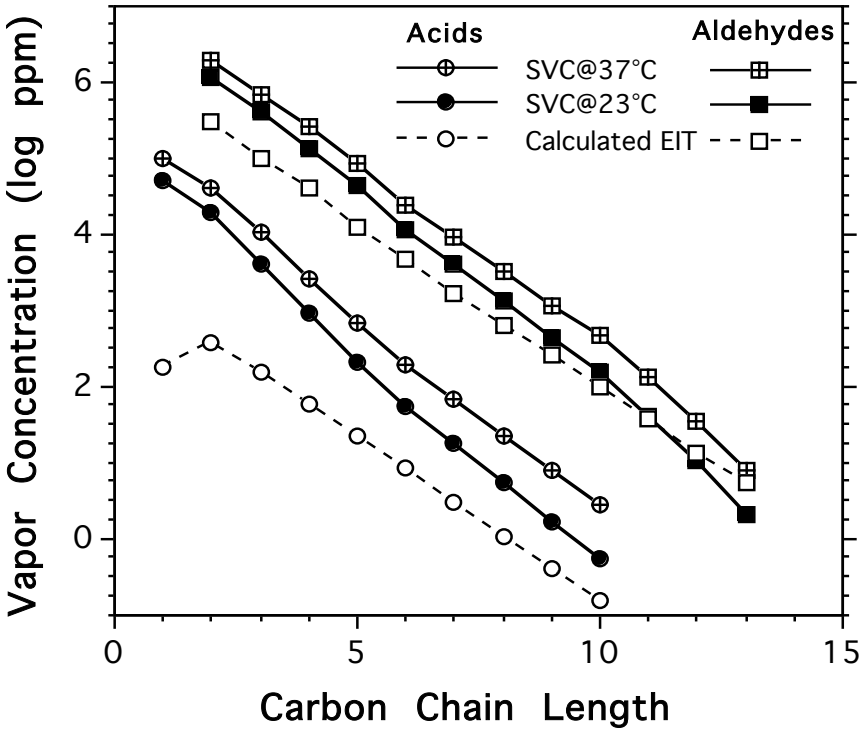


FIGURE 8



This is a pre-copyedited, author-produced version of an article accepted for publication in Neuroscience following peer review. The version of record Neuroscience **145**:1130-1137, 2007 is available online at: http://www.sciencedirect.com/science/article/pii/S0306452206017477?via%3Dihub - DOI: 10.1016/j.neuroscience.2006.12.032