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Odor detection of single chemicals and binary mixtures

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Running head: Odor detection of Mixtures

Abstract

The investigation explored the olfactory detectability of two chemically and structurally similar esters, ethyl propanoate and ethyl heptanoate, presented singly and in mixtures. Initially, we measured concentration-detection (i.e., psychometric) functions for the odor of ethyl propanoate and ethyl heptanoate presented singly. Using this information, we prepared binary mixtures of the two chemicals in varying complementary proportions and, also, selected concentrations of the single compounds, such that, if a rule of response-addition (i.e., independence of detection) were to hold, the stimuli (mixed and single) should approximate equal detection. Next, we measured the actual detectability of these stimuli within the same experiment. The results were analyzed in terms of response-addition (or -additivity) and of dose-addition (or -additivity). The outcome revealed that at low levels of detectability the mixtures approximate response-addition, that is, independence of detection, whereas at high levels of detectability they approximate dose-addition. In the light of previous findings for the olfactory detection of the more dissimilar chemical pairs 1-butanol/2-heptanone and butyl acetate/toluene, we conclude that the described outcome generalizes across a variety of chemical pairs.

Keywords: Olfactory nerve — Concentration-detection odor functions — Odor mixture detection — Odor response-addition — Odor dose-addition — Olfactory additivity

Introduction

Behavioral studies of odor mixtures in humans [37, 42, 43] and in animals, including monkeys [38], rats [34, 40, 56], catfish [54], spiny lobsters [41], and bees [22, 51], have focused on testing the discriminability between mixtures and components, or establishing whether single components in mixtures are perceived individually or as a whole. Neurophysiological studies in animals have addressed these questions, and also compared whether responses to mixtures are of the same "type" (i.e., excitatory, suppressive, or nonresponsive) or show the same temporal pattern as responses to the individual components [28, 33, 52].

Odor mixtures can also be studied at the detection level, comparing odor detection thresholds for single chemicals with those for their mixtures. A number of such studies have been performed in humans [8, 20, 30, 36, 49, 50]. A common finding in these investigations was the existence of some degree of cooperativity among the single constituents such that the mixtures gained in odor detectability. Nevertheless, none of the studies included measurement of odor concentration-detection (i.e., psychometric) functions for individual chemicals, a laborious but revealing strategy in the analysis of how the odor detectability of single chemicals relates to that of their mixtures. A chemosensory study of 1-butanol and 2-heptanone based on psychometric functions concluded that, as a first approximation and across the overall range spanning from chance to virtually perfect detection, odor detectability of mixtures followed a rule of dose-additivity of components [18]. A finer look at the topic including selection of pre-

determined levels of detectability (i.e., relatively low and relatively high) within the threshold range and an analysis of response-additivity found, for the structurally more diverse chemicals butyl acetate and toluene, a range-dependent effect [15]. At low detectability (but, still, above chance) the two components of the binary mixtures followed a response-addition model, whereas, at high detectability (but, still, below perfect detection), they fell strongly short of it. Inconsistency in the patterns at low and high levels of detectability also ruled out a simple dose-addition model.

The specific structure and properties of the chemicals mixed govern their interaction with the array of olfactory receptors, and shape the resulting neural message [32, 53]. They may also influence the amount of dose- and response- addition observed in mixtures. At near-threshold levels, we can safely disregard interactions among the mixed chemicals themselves as a factor in dose- and response- addition: Experiments and calculations made on model solvents mimicking the olfactory receptor biophase indicate that interactions between two esters in such a biophase are negligible at the vapor concentrations necessary for odor detection [29]. In fact, even at the much higher vapor concentrations necessary for nasal pungency or eye irritation (i.e., trigeminal) detection [17], the extent of interaction for an ester pair in a biophase is not likely to be more than 1 or 2 % [29]. Albeit the lack of an identical strategy and analysis between the 1-butanol/2-heptanone and the butyl acetate/toluene studies limits a direct comparison, the different chemical contrast between the alcohol and the ketone, compared to that of the acetate and the alkylbenzene, might have driven a dissimilar outcome (cf. [15, 19]). In this regard, studies of quantitative structure-activity relationships (QSARs) for the odor potency of

single chemicals (including the four compounds mentioned) have indicated that, in addition to parameters governing the transfer of the stimulus from the vapor phase to the olfactory receptor biophase, parameters involving molecular size and chemical functionality play a significant role in olfactory potency [2, 3]. In contrast, the chemesthetic (i.e., nasal pungency and eye irritation) potency of largely the same single chemicals rests almost exclusively on transfer processes [1, 4-6], at least until a critical molecular size is reached where chemesthesis fades away (we had called this a "cut-off" effect) [14].

In the present investigation we have tested the odor detectability, singly and in mixtures, of the esters ethyl propanoate and ethyl heptanoate, using both a response-addition and a dose-addition analysis. From the point of view of chemical structure and functionality, the members of the new pair are more similar than the members of the previous pairs, i.e., 1-butanol/2-heptanone and butyl acetate/toluene. The two ethyl esters chosen here have also been tested recently, among other ethyl ester homologs, in electrophysiological studies of the olfactory bulb of rats, where they were presented sequentially rather than simultaneously, and at levels of high stimulation [25, 26]. The outcome revealed that the odorant receptive fields (i.e., molecular receptive range expressed in terms of carbon chain length) of bulbar mitral-tufted cells changed as a result of previous exposure to one of the ethyl ester homologs. Interestingly, the investigations found that the width of the median odorant receptive field of these cells spanned 3-4 carbons, just the difference between our chosen stimuli.

Materials and Methods

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

Subjects

All participants performed in the normosmic range when tested on the Connecticut Chemosensory Clinical Research Center (CCCRC) test of olfaction [11].

Experiment 1. Psychometric functions for the odor of single chemicals. We tested 22 normosmics (10 females, 12 males) with an average age (±SD) of 26 (±10) years, and ranging from 18 to 50 years of age. All were nonsmokers.

Experiment 2. Odor detectability of selected mixtures and single chemicals. We tested 20 normosmics (9 females, 11 males) with an average age (±SD) of 26 (±10) years, and ranging from 18 to 50 years of age. All were nonsmokers. Seven of them (2 females, 5 males) had participated in Experiment 1.

Stimuli and equipment

Experiment 1. Psychometric functions for the odor of single chemicals. Stimuli comprised ethyl propanoate (97+%) and ethyl heptanoate (98+%). Mineral oil

(Light, Food Chemical Codex quality) was used as solvent and blank. Duplicate dilution series made in 3-fold dilution steps for both ethyl propanoate and ethyl heptanoate were prepared starting from 0.0033 and 0.01 % v/v, respectively. Vapor stimuli were stored and delivered from glass vessels (1,900 ml capacity) containing 200 ml of solution. The vessels have been described and used in previous studies of odor detectability of single chemicals and mixtures [15].

Vapor concentration in the headspace of the vessels was measured by gas chromatography (flame ionization detection, FID) via direct sampling with a gas-tight syringe. When required, analytical sensitivity was enhanced by concentrating headspace samples in an adsorption tube (Sorbent tube, 4.5 in. L x 4 mm ID, packed with 20:35 Tenax-TA/Carboxen 1000/CarbosieveSIII) and thermally desorbing them via a thermal desorption unit (ACEM Model 900, CDS Analytical, Inc.) connected to the GC. Measurements were taken periodically to ensure stability. The relationship between vapor- and liquid-phase concentration for the two chemicals was given by the following equations:

For ethyl propanoate: $y = 0.958x + 3.378$ with $r = 0.992$ (1)

For ethyl heptanoate: $y = 0.878x + 1.272$ with $r = 0.992$ (2)

where “y” represents log ppm (by volume) and “x” represents log %v/v. Chromatographic readings were converted into concentration units (ppm by volume) by means of a calibration curve for mass created by liquid injections of known masses of each chemical into the gas chromatograph [16].

Experiment 2. Odor detectability of selected mixtures and single chemicals. We employed the same chemicals (stimuli and blank) and glass vessels as in Experiment 1. Nevertheless, in Experiment 2, stimuli comprised binary mixtures (in different proportions) and single chemicals. Using the psychometric functions for ethyl propanoate (EP) and heptanoate (EH) measured in Experiment 1 and shown in Figure 1 (which range from chance detection, i.e., P=0.0, to perfect detection, i.e., P=1.0), we calculated the concentrations of EP and EH producing detection probabilities (P) of 0.20, 0.40, 0.60, and 0.80. Next, we prepared two sets of seven stimuli. Each set included four stimuli consisting of a single chemical (two were EP and two EH), and three stimuli consisting of binary mixtures of EP and EH in different proportions. The seven stimuli in the first set included the following: 1) the concentration of EP producing P=0.40, labeled $EP_{0.40}$; 2) a mixture of the concentration of EP producing P=0.30 and that of EH producing P=0.10, labeled $EP_{0.30}/EH_{0.10}$; 3) a mixture of the concentration of EP producing P=0.20 and that of EH also producing P=0.20, labeled $EP_{0.20}/EH_{0.20}$; 4) a mixture of the concentration of EP producing P=0.10 and that of EH producing P=0.30, labeled $EP_{0.10}/EH_{0.30}$; 5) the concentration of EH producing P=0.40, labeled $EH_{0.40}$; 6) the concentration of EP producing P=0.20, labeled $EP_{0.20}$; and 7) the concentration of EH producing P=0.20, labeled $EH_{0.20}$.

Following the same nomenclature as above, the seven stimuli in the second set included: 1) $EP_{0.80}$; 2) $EP_{0.60}/EH_{0.20}$; 3) $EP_{0.40}/EH_{0.40}$; 4) $EP_{0.20}/EH_{0.60}$; 5) $EH_{0.80}$; 6) $EP_{0.60}$; and 7) $EH_{0.60}$. Note that in the first set and in the second set, the detectability of each mixture — i.e., stimuli 2), 3), and 4) — approximates either P≈0.40 or P≈0.80,

respectively, under an assumption of independence of detection (see equation (3) in Data Analysis). In other words, independence of detection means that the detectability of a mixture simply results from the combined detectability of the components (equation (3)) and can be referred to as response-addition. Also, each set included stimuli that were single chemicals, including single EP and single EH at P=0.40 and P=0.20, within the first set, and single EP and single EH at P=0.80 and P=0.60, within the second set. Assuming independence of detection between mixed odorants, stimuli 1), 2), 3), 4), and 5) within each set should evoke a similar level of detectability (see Data Analysis). The strategy allows for a direct comparison of the odor detectability of mixtures and single chemicals by testing all stimuli in the same context, using a common procedure and the same subjects.

To minimize the possibility of temporary depletion of the headspace vapor in any vessel due to repetitive smell sampling, each stimulus described above was prepared in quintuplicate. Chromatographic samples were taken periodically from alternate stimuli and replicas to monitor stability.

Procedure

Experiment 1. Psychometric functions for the odor of single chemicals. To obtain concentration-detection (psychometric) functions for odor from the single chemicals, we employed a 3-alternative, forced-choice (3-AFC) procedure with presentation of ascending concentrations. This method required participants to select

which of 3 vessels on a trial smelled different from the other two, guessing if necessary. Subjects were not aware that, on a trial, 2 vessels contained blanks (mineral oil) and one contained a target chemical stimulus at some concentration. Position of blanks and stimulus were randomized. In a *test series*, each dilution step of a chemical was presented to the participant twice, in ascending order of concentration. Since we prepared duplicate vessels per dilution step, each vessel was presented only once in a series, thus minimizing depletion of headspace vapor. The inter-trial interval was at least 45 sec. After selecting the vessel smelling different, participants had to rate their level of confidence with that choice. Confidence was rated on a scale raging from "1" (not confident) to "5" (extremely confident).

Subjects participated in 3 to 6 sessions of 1 to 3 hours to complete a total of 15 *test series* for each chemical in irregular order (i.e., 30 presentations per concentration, although 3 subjects completed all 30 presentations for EH but only between 10 and 20 presentations for EP before becoming unavailable). The data from all series for each chemical were averaged, first, within individuals and, then, across individuals to obtain group data.

Experiment 2. Odor detectability of selected mixtures and single chemicals. We used the same procedure and instructions as in Experiment 1. In a session, stimuli from the first or second set were presented in random order. The same vessel was not sampled again until the remaining four replicas for that stimulus had been sampled once.

Subjects participated in 4 to 6 sessions of 1 to 3.5 hours to complete a total of 30 trials per stimulus in both sets. Individual and group data were calculated as before.

Data Analysis

Plots of odor detection probability (i.e., odor detectability) as a function of vapor concentration or stimulus composition summarized the outcome. Detection probability (P) was corrected for chance [45] and adjusted to a scale ranging from 0.0 for chance detection to 1.0 for perfect detection. A repeated measures analysis of variance (ANOVA) (SuperANOVA v. 1.11, Abacus Concepts, Inc.) served to test for significance. The ANOVA included the main factor "target detectability" (with two levels: P≈0.4 and P≈0.8), the main factor "stimulus" (with five levels: two being single chemicals and three being mixtures), and their interaction. To calculate the theoretical values of detectability under an assumption of independence of detection, i.e., response-additivity, between components of a mixture we used the following formula [24]:

$$P_{det.EP,EH} = 1 - [(1-P_{det.EP})(1-P_{det.EH})] \quad (3)$$

In this formula, $P_{det.EP,EH}$ = Probability of detection of the binary mixture of ethyl propanoate and ethyl heptanoate, $P_{det.EP}$ = Probability of detection of the ethyl propanoate component alone, and $P_{det.EH}$ = Probability of detection of the ethyl heptanoate component alone. The formula uses the experimentally-obtained probability of detection for the individual components of any mixture to calculate the expected detectability for the mixture assuming that the detection of one component is independent of the detection of the other. In such case, the components would show what we call complete (or perfect)

response-addition, and the experimentally-obtained probability of detection for the mixture will not be significantly different from that calculated in equation (3).

Results

Experiment 1. Psychometric functions for the odor of single chemicals. Figure 1 shows the concentration-detectability function for the odor of ethyl propanoate and heptanoate and the corresponding confidence levels. As expected, confidence of detection increased as actual detectability increased. Conversion of detection probabilities into Z-scores produced linear functions. The slopes of the odor function for propanoate and heptanoate were 1.17 and 0.95, respectively, according to the following equations:

For ethyl propanoate: $y = 1.172x + 0.676$ with $r = 0.992$ (4)

For ethyl heptanoate: $y = 0.953x + 1.359$ with $r = 0.998$ (5)

where "y" represents detectability expressed as Z-score and "x" represents vapor concentration expressed as log ppm.

Insert Figure 1 about here

Experiment 2. Odor detectability of selected mixtures and single chemicals. Figure 2 shows the results in terms of detectability and confidence for the seven stimuli in the first set (left panel) and for the seven stimuli in the second set (right panel). (First and second sets as described under "Stimuli and equipment. Experiment 2".) The outcome indicates that single stimuli targeted at $P \approx 0.4$ and corresponding mixtures were all equally

detectable at P≈0.4. The two single stimuli targeted at P≈0.2 were detectable at P=0.37 (EP) and P=0.30 (EH), above expectation but close to one another (Figure 2, left panel). Single stimuli targeted at P≈0.8 were detectable at P=0.64 (EP) and P=0.76 (EP), slightly below expectation but also close to one another. In contrast, the detectability of the mixtures in this set was clearly lower than that of the corresponding single stimuli (Figure 2, right panel). Confidence ratings tended to follow the trend for detectability performance (Figure 2).

Insert Figure 2 about here

Response-addition in mixtures. The contrast between the two sets (i.e., targeted at P≈0.4 and at P≈0.8) in terms of detectability of mixtures referenced to detectability of the single chemicals is clearly observed in Figure 3, where we compare experimentally obtained detectability with calculated detectability assuming independence of detection, i.e., response-addition, according to equation (3). The mixtures in the set targeted at P≈0.4 are detectable at levels close to calculated values, whereas the mixtures in the set targeted at P≈0.8 fall well below calculated values. These trends found statistical support in the outcome of a repeated measures ANOVA that included the factors "target detectability" (two levels: P≈0.4 and P≈0.8) and 'stimulus" (five levels: two single chemicals and three mixtures). The results showed that: 1) detectability of stimuli at target P≈0.8 was significantly higher than at target P≈0.4 ($F(1,19) = 8.727$, $p = 0.0081$; 2) detectability among the five stimuli was significantly different ($F(4,76) = 10.282$, $p = 0.0001$; and 3) there was a significant interaction between the two factors ($F(4,76) =$

15.538, p = 0.0001, indicating that the trend observed among stimuli at P≈0.4, i.e., a flat function, differed significantly from that observed at P≈0.8, i.e., a "U" shaped function. Furthermore, a means comparison contrast between the detectability of the two single stimuli, on the one hand, and that of the three mixtures, on the other, failed to reach significance for the P≈0.4 series, but did reach significance for the P≈0.8 series (p = 0.0001).

Insert Figure 3 about here

Dose-addition in mixtures. A complementary way of looking at the results from mixtures is to probe into dose-addition, in contrast to the response-addition analysis performed above. Complete (or perfect) dose-addition would occur when the detectability of a mixture, expressed in concentrations units of only one component (as described below), can be predicted by the psychometric function for that component. In the context of testing the mixtures from both the P≈0.4 and P≈0.8 series (Experiment 2), we also tested the single stimuli EP and EH at the target detectabilities P=0.2, P=0.4, P=0.6, and P=0.8. We can then use the results from these stimuli to build a psychometric function for EP and another for EH, in much the way we did in Experiment 1 (Figure 1), but, in this case, these functions would have been obtained in the same context as testing the mixtures, including identical procedure and same subjects. Using this psychometric function for EP and EH we can convert any vapor concentration of one chemical, e.g., EP, into the "odor–equivalent" concentration of the other chemical, i.e., EH. By odor-equivalent we simply mean finding the concentration of the second chemical that

produces the same odor detectability as the selected concentration of the first chemical. Through this method, we can express the mixtures in concentration units of only EP or only EH, and then use the psychometric function for that compound to calculate an estimated detectability under an assumption of dose-addition. This estimated detectability can also be compared with the experimentally obtained detectability for that mixture (Figure 3). The results show, first, that the values estimated expressing the mixtures as all-EP concentrations agree closely with those estimated expressing them as all-EH concentrations; and, second, that the mixtures in the set targeted at P≈0.4 are more detectable than estimated via dose-addition (Figure 3, left), whereas those in the set targeted at P≈0.8 are detectable at levels close to those estimated via dose-addition (Figure 3, right).

In summary, the outcome reveals that at relatively low levels of detectability of the reference single components the mixtures follow a model of response-addition (i.e., independence of detection as formalized in equation (3)), whereas at relatively high levels of detectability of the reference single components they follow more closely a model of dose-addition (Figure 3).

Discussion

Considerable progress has been made on the molecular biology of olfaction [47] particularly since the seminal discovery of a multigene family encoding odorant receptors [10]. It is estimated that there are about 1,000 genes encoding odorant receptors in

rodents [57], but in humans many of these are believed to be pseudogenes, leaving only about 350 functional odorant receptor genes [58]. The number of qualitatively different odors perceived by humans can be estimated in, at least, the tens of thousands (almost all volatile organic compounds have odor), an observation that fits well with the prevalent notion that even single-chemical odorants produce a neural message consisting of the combined output (pattern) of many odorant receptors with overlapping odorant specificity [27, 44, 46]. These coding characteristics further highlight the relevance of approaches that, like ours, address the integrated functional response of the human olfactory system.

The study of the odor detection of mixtures vis-à-vis that of the individual constituents has the potential to reveal: 1) general rules through which olfaction integrates the input, and 2) the structural and chemical boundaries that underlie a larger or smaller degree of cooperation between constituents to elicit detection. In turn, data of this sort can help to define the characteristics of chemical tuning displayed by the intact olfactory sense. So far we have completed the study of three different chemically contrasting odorant pairs: 1-butanol/2-heptanone [18], butyl acetate/toluene [15], and ethyl propanoate/ethyl heptanoate (this study). All cases included measurement of the psychometric function for the odor detection of the individual components of the binary mixture as a basis for, first, preparing the mixtures and, second, assessing their odor detectability compared to that of the individual constituents. Furthermore, for the two most recently studied mixtures, the approach employed allowed a direct comparison of results obtained when the single components used as references in the set reflected relatively low levels of detectability (i.e., $P \approx 0.4$) versus when they reflected relatively

high levels of detectability (i.e., P≈0.8). Despite the considerable dissimilarity in chemical functionality and structure between the ester/alkylbenzene pair and the ester/ester pair, the outcome in both cases revealed that a model of response-addition only holds at low levels of detectability.

Considering that we are probing olfaction at the near-threshold level it is reasonable to assume that, to a large extent, only the most sensitive odorant receptors for each component of the mixture are being activated. At relatively low levels of detectability, i.e., of stimulation, it is unlikely that there will be significant competition between molecules (of the same or different kind) for free receptors. Under these conditions, whether molecules are expected to share few, if any, common receptors (butyl acetate/toluene case) or a larger share of common receptors (ethyl propanoate/ethyl heptanoate case) the net effect observed across the integrated system is response-addition or, in other words, independence of detection (Figures 2 and 3, left panels). As the concentration of stimulating molecules increases, the likelihood for various types of interactions also increases and, in fact, our data indicate that interactions begin to emerge such that detection of mixtures now approaches a model of dose-addition (Figures 2 and 3, right panels). At the peripheral level, this change could result, most commonly, from higher competition for common receptors, i.e., competitive agonism, from blocking of each other's receptors, i.e., antagonism (see below), or from a combination of both. At a more central level, in the olfactory bulb, the change could result from activation of inhibitory circuits in the bulb (see below). The seemingly larger departure from response-addition observed at relatively high detectability for the dissimilar mixture (butyl

acetate/toluene) might not be due to increased bulbar inhibition since it is believed that such inhibition [39] would be stronger for a pair that stimulates similar receptors (e.g., ethyl propanoate/ethyl heptanoate), and, thus, projects to neighboring glomeruli than for a structurally much more dissimilar pair, likely to stimulate receptors projecting to distant glomeruli. Nevertheless, a recent study has suggested that the extent of the bulbar inhibitory circuit might involve not only neighboring but also much distant glomeruli than hitherto suspected [7].

The issue of odor mixture additivity from a psychophysical perspective has received considerably more attention in the suprathreshold range. At such levels, it is the rule to observe hypoadditivity whereby the perceived odor intensity of a mixture falls below the sum of the perceived odor intensities of the single components (e.g., [9]). This outcome holds even in models considering dose-addition and not only response-addition [13]. In some cases, a level-dependent effect has been found such that a low level of odor stimulation shows greater additivity than a higher level [13, 35]. The present results trace the beginning of the loss of response-additivity to the near-threshold level, specifically as detectability approaches the suprathreshold range. The finding seems consistent with neurophysiological observations that, at near-threshold concentrations, only a few, neighboring glomeruli within the olfactory bulb are activated but, as concentration increases, more widely distributed glomeruli become activated [31, 55], probably potentiating inhibitory circuits in the bulb (e.g., [7, 21]).

The decrease in odor detection (i.e., response) additivity observed here psychophysically at high detectability has a further neurophysiological correlate, measured as spike frequency of single olfactory neurons [23]. The responses of receptor cells to binary mixtures shifted towards decreasing additivity as the concentration of the mixed chemicals increased. Selection of odorants in the mixtures was based on whether they were reported to activate the inositol triphosphate (IP_3) or the cyclic adenosine monophosphate (cAMP) olfactory transduction pathway, not on contrasting chemical functionality/structural features. Nevertheless, the analysis of spike activities failed to reveal differences between IP_3- and cAMP-increasing odorants.

A mechanism that could account for the observed decrease of odor detection additivity in mixtures is molecular antagonism. In other words, one odorant might bind to one or more of the same receptors as a second odorant but fail to activate them, thus blocking them. For example, the response of the mouse mOR-EG olfactory receptor, encoded by the MOR174-9 gene [57], to eugenol (EG) is antagonized by the related odorant methyl isoeugenol (MIEG) [48]. In our case, since we are probing olfaction at the integrated-system level, each odorant is likely to activate more than one type of olfactory receptor, but, still, activation is probably restricted to the most sensitive ones given that we stimulate at the absolute detection level. The study with the mOR-EG receptor showed a varied picture even when the approach included only peripheral events: There were neurons that responded only to EG, only to MIEG, to both EG and MIEG, to EG but not to the mixture of EG and MIEG (revealing antagonism of EG responses by MIEG), and to MIEG but not to the mixture of EG and MIEG (revealing antagonism of MIEG

responses by EG). This findings of antagonism at the molecular and cellular level fall into register with psychophysical observations at the suprathreshold level that since long have pointed out to odor counteraction and masking effects (e.g., [12]).

In conclusion, we have now investigated the relationship between the olfactory detection of binary chemical mixtures and that of their single components across three pairs of odorants varying in their degree of structural and physicochemial similarity. The combined outcome of these studies indicates the following: At relatively low levels of detectability, the components approximate response-addition, that is, independence of detection, where the probability of detection of the mixtures results from the combined probability of detection of each individual odorant. In contrast, at relatively high levels of detectability, the components approximate dose-addition, where the probability of detection of the mixtures is governed by the psychometric function of the individual odorants. We have discussed the most likely physiological and molecular mechanisms, both at the periphery and at central locations, that might underlie the psychophysical results observed.

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Figure legends

Figure 1. Odor detectability (left *y*-axis, filled symbols) and confidence rating (right *y*-axis, empty symbols) as a function of vapor concentration of ethyl propanoate (squares) and heptanoate (circles). Each point represents the average of 610 trials (propanoate) or 658 trials (heptanoate) from 22 normosmics. Bars indicate standard errors (SE).

Figure 2. Left panel. Detectability (left *y*-axis, filled symbols) and confidence rating (right *y*-axis, empty symbols) for the seven stimuli in the first set (as defined under "Stimuli and Equipment. Experiment 2"), including single chemicals and mixtures. The horizontal line marks the exact target level P=0.4. Right panel. Analogous to the left panel but for the seven stimuli in the second set. The horizontal line marks the exact target level P=0.8. In both panels, bars represent standard errors (SE).

Figure 3. Comparison between experimentally obtained detectability of single stimuli and mixtures (filled squares, continuous lines) and calculated values assuming response-addition (empty squares, dashed lines) (see equation (3)), or assuming dose-addition expressing all concentrations in terms of either EP (circles, dashed lines) or EH (triangles, dashed lines) (see text). The comparison is made for the series targeting P≈0.4 (left graph) and the series targeting P≈0.8 (right graph). Bars represent standard errors (SE) of experimental data.

FIGURE 1

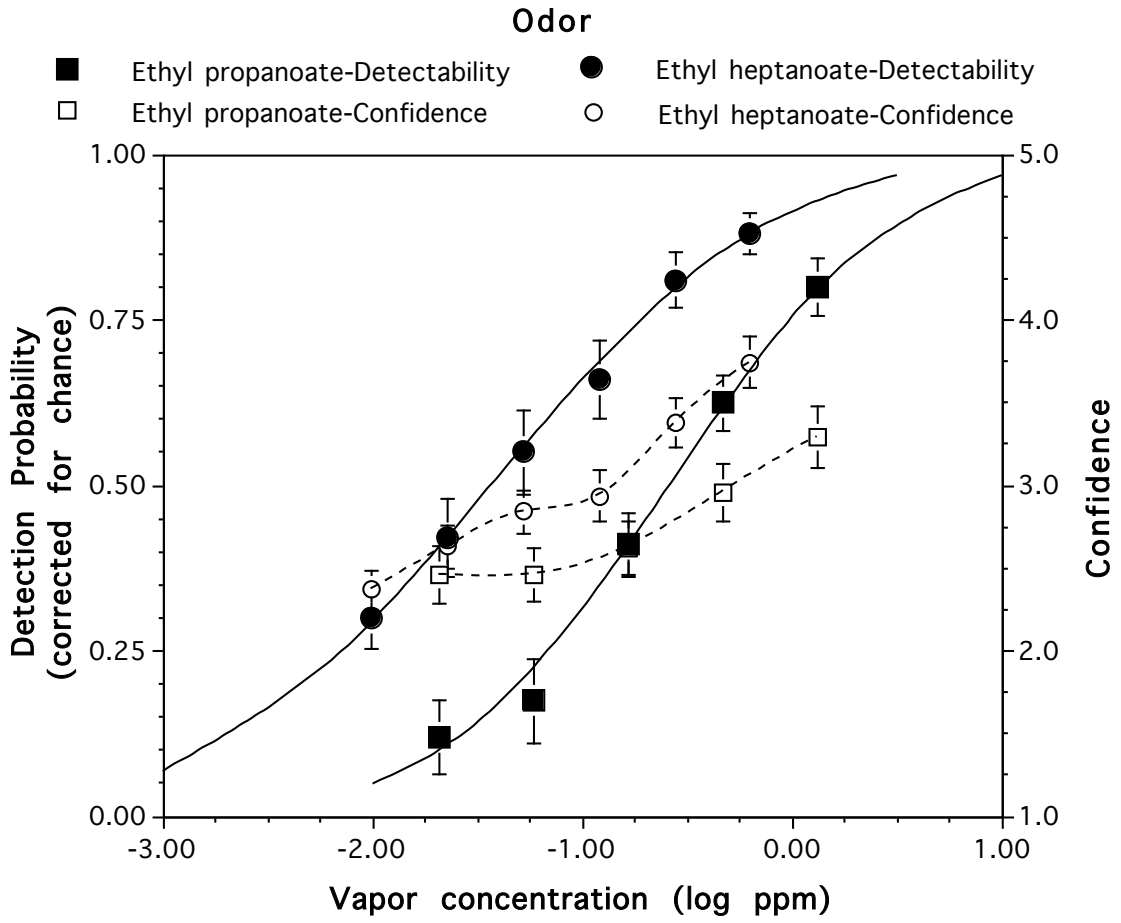

Odor
Ethyl propanoate-Detectability
Ethyl heptanoate-Detectability
Ethyl propanoate-Confidence
Ethyl heptanoate-Confidence
Detection Probability (corrected for chance)
Confidence
Vapor concentration (log ppm)
1.00
0.75
0.50
0.25
0.00
5.0
4.0
3.0
2.0
1.0
-3.00
-2.00
-1.00
0.00
1.00

FIGURE 2

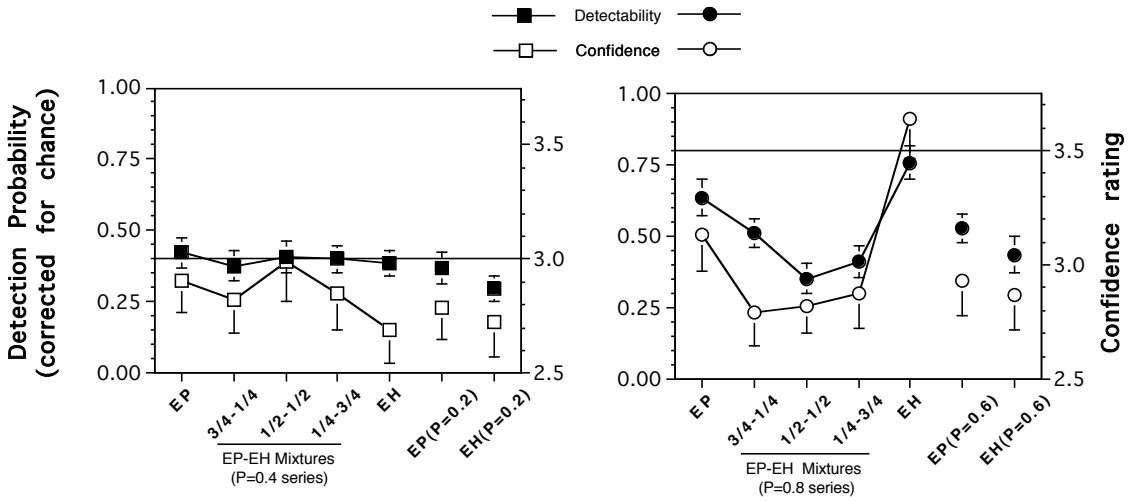
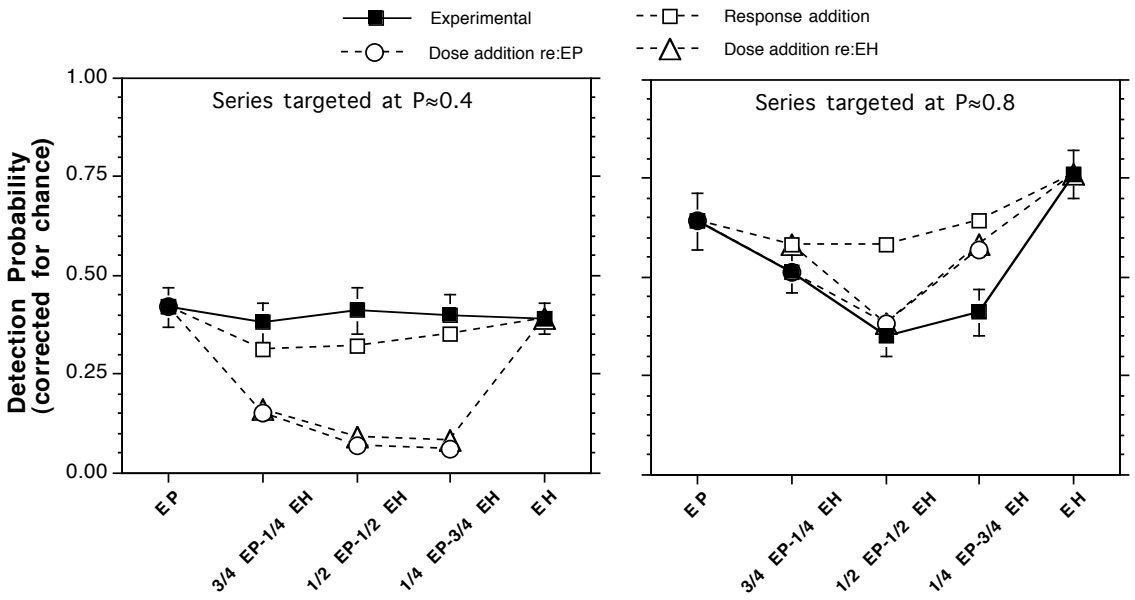

Detectability
Confidence
Detection Probability (corrected for chance)
Confidence rating
1.00
0.75
0.50
0.25
0.00
3.5
3.0
2.5
EP
3/4-1/4
1/2-1/2
1/4-3/4
EH
EP(P=0.2)
EH(P=0.2)
EP-EH Mixtures (P=0.4 series)
EP(P=0.6)
EH(P=0.6)
EP-EH Mixtures (P=0.8 series)

FIGURE 3


Experimental
Dose addition re:EP
Response addition
Dose addition re:EH
Series targeted at P≈0.4
Series targeted at P≈0.8
Detection Probability (corrected for chance)
1.00
0.75
0.50
0.25
0.00
E P
3/4 EP-1/4 EH
1/2 EP-1/2 EH
1/4 EP-3/4 EH
E H

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