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Dose-Addition of Individual Odorants in the Odor Detection of Binary Mixtures

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Running head: Olfactory Detection of Mixtures

Abstract

In a series of experiments, we have explored the rules of olfactory detection agonism between the odorants butyl acetate and toluene. First, we obtained the concentration–detection function for the odor of the individual compounds. Second, we selected the concentrations of the two substances producing three levels of detectability (low, medium, and high) and, for each level, tested the comparative detectability of the two single chemicals and three mixtures of varying proportions. In each case, the mixtures were prepared in such way that, if a rule of complete dose addition were to hold, all five stimuli (two single, three mixtures) should be equally detected. The outcome revealed complete dose addition at relatively low detectability levels but fell short of dose addition at medium and high levels. A recent analogous study on trigeminal chemosensory detection via nasal pungency and eye irritation of these same stimuli have shown a similar trend but showed a less dramatic loss of dose additivity with increased detectability. These results on detection of mixtures suggest a more selective window of chemical tuning (i.e., less dose-addition) in olfaction than in trigeminal chemoreception.

Keywords: olfaction; odor detection; chemical mixtures; psychometric odor functions; dose-additivity; butyl acetate; toluene

Introduction

Despite the considerable progress made over the last decade in our understanding of the cellular and molecular events triggered in olfactory receptor neurons upon their interaction with an odorant molecule (particularly regarding the second messenger systems involved) [22, 23], much less is known about the primary odorant–receptor interaction. We are far from understanding, for example, to which crucial structural and physicochemical properties of the incoming odorants the array of receptor neurons is tuned. Each receptor neuron might express as little as one receptor type [35] although the outcome of some studies favors multiple receptors [29, 39]. Despite this, there is overall agreement that each receptor is broadly tuned to a range of possible ligands and that the olfactory neural message consists of the combined output (pattern) of many receptors with overlapping odorant specificity [29, 33, 35]. In view of this, investigations that address these issues via the integrated response of the olfactory system, be it physiologically, psychophysically, or via imaging techniques, become particularly revealing.

A strategy based on a systematic testing of homologous chemical series (or, at least, structurally closely–related substances), rather than using blends of compounds varying irregularly in structure and properties, has proved useful to begin to unveil the physicochemical basis for olfactory activity at all neural levels, and in both cellular/subcellular and integrative approaches. This has been a fruitful approach both in studies focused on the properties of the first [28, 41, 42, 49] and second order [25, 27, 30, 32, 36, 48] neurons within the olfactory pathway [37], and in studies focused on the relevant features of the chemicals acting as odorant stimuli by probing the response of the integrated olfactory system [1, 2]. Another strategy that can contribute to our understanding of the odorant – olfactory receptor interaction is the study of mixtures. This is the focus of the present study.

From a perceptual point of view, many studies of odorant mixtures have dealt with the suprathreshold range (e.g., [6]). Fewer studies have addressed the topic at the threshold level. Since odors in the environment rarely originate from a single compound, understanding the rules that govern the odor detection of mixtures has pressing theoretical and practical significance. From a basic perspective, information on how the olfactory system processes the detection of mixtures hold clues, for example, to the degree of generality with which the integrated olfactory sense interact with odorants. From an applied perspective, the topic has relevance in such diverse areas as food aromas, perfumery, odor masking, and odor pollution.

An early report testing organic compounds associated with food aromas (principally saturated, unsaturated, and branched aldehydes but also a limited number of other compounds: dimethyl sulfide, octanol, butyric acid, and butylamine) found that a simple rule of sub-threshold additivity fitted the results quite accurately [24]. Since then, other studies have found various degrees of sub-threshold additivity in olfactory detection, including some possible cases of synergism or hyperadditivity [4, 31, 38, 40]. Such stimulus-additivity for chemosensory detection has also been recently reported for the gustatory modality, irrespective of whether the mixture contained like-quality, unlike-quality, or both types of tastants [43, 44]. As argued in a recent study [16], a better knowledge of the actual extent of these effects requires the measurement of a complete psychometric (i.e., detectability) function for each individual chemical (e.g., [14]), a time-demanding undertaking that none of the above-mentioned studies pursued. Nevertheless, in the case of binary mixtures, it is still feasible to obtain the psychometric functions for the olfactory detection of each chemical and use the information to create and test mixtures in search of their rules of additivity. The outcome of such an approach with binary mixtures of 1-butanol and 2-heptanone lent

support, as a first approximation, to the notion of dose additivity in olfactory detection [12].

The present study continues this line of research by selecting a new pair of odorants, butyl acetate and toluene, and an experimental strategy that probes with increased detail into the degree of dose additivity at various levels of detectability within the psychometric function. At the present state of knowledge on the structural and physicochemical basis governing degree of dose additivity in chemosensory detection of mixtures (both olfactory and trigeminal) the choice of which new pairs to select is quite broad and, perhaps, arbitrary. Our choice of butyl acetate and toluene followed a pattern of structural and physicochemical contrasts described in a recent study of the trigeminal detectability of mixtures of these two compounds [13].

Materials and Methods

Subjects

The study protocol was approved by the Human Subjects Committee of the University of California, San Diego. All subjects gave written inform consent on forms approved by the Committee.

All subjects employed performed in the normosmic range, i.e., had a normal sense of smell, on a standardized clinical olfactory test [7]. All participants were nonsmokers.

Experiment 1. Odor detectability of the single chemicals. For butyl acetate, we tested a group of 12 subjects (6 females, 6 males) with an average age (±SD) of 27 (±12) years. For toluene, we tested a group of 10 subjects (5 females, 5 males), all of whom were also tested for butyl acetate, with an average age (±SD) of 29 (±12) years.

Experiment 2. Odor detectability of binary mixtures at target p=0.80. We tested a group of 20 subjects (12 females, 8 males) with an average age (±SD) of 27 (±10) years. Two of them (a female and a male) participated in Experiment 1.

Experiment 3. Odor detectability of binary mixtures at target p=0.95. We tested a group of 21 subjects (14 females, 7 males) with an average age (±SD) of 22 (±5) years. Four of them (3 females and a male) participated in Experiment 1.

Experiment 4. Odor detectability of binary mixtures at target p=0.60. We tested a group of 17 subjects (9 females, 8 males) with an average age (±SD) of 22 (±3) years. Two of them (2 females and a male) participated in Experiment 1.

Stimuli and Equipment

Experiment 1. Odor detectability of the single chemicals. Stimuli included butyl acetate (99+%) and toluene (99.8%). Mineral oil (light, Food Chemical Codex quality) served as solvent and blank. Duplicate dilution series for each chemical were prepared in 3-fold dilution steps. Pilot odor testing suggested the following starting concentration of the series: 0.03% v/v for butyl acetate and 0.10% v/v for toluene (both labeled dilution step 1). Ensuing 3-fold dilution steps were labeled 2, 3, 4, and so forth. Stimuli were stored and delivered from glass vessels (1,900 ml capacity) containing 200 ml of solution. The vessels have been recently described in a study of nasal pungency thresholds [15].

Vapor concentration in the headspace of the vessels was measured off-line by gas chromatography (GC) (flame ionization detector, FID) via direct sampling with a gas-tight syringe, up to the limit of sensitivity of the instrument. The measurements revealed a linear relationship between the response of the detector and stimulus

concentration. GC readings were taken shortly after preparation of stimuli and after all subjects were tested to confirm stability. The coefficient of variation of GC measurements across dilution steps averaged (±SD) 11% (±4.4) for butyl acetate and 12.8% (±7.5) for toluene. A calibration curve based on repetitive liquid injections of known masses of the respective compounds into the GC allowed conversion of GC readings into concentration units (in ppm by volume).

Experiment 2. Odor detectability of binary mixtures at target p=0.80. Butyl acetate, toluene, and the solvent mineral oil were the same as those used in Experiment 1. There were a total of five stimuli here: three binary mixtures (in the concentrations and proportions described below), and two single chemicals (one concentration of butyl acetate and one of toluene). The concentration of each single chemical was taken from the results of Experiment 1 and represented the concentration producing an odor detectability of p=0.80 (Figure 4) on a scale ranging from p=0.00 (i.e., chance detection) to p=1.00 (i.e., perfect detection). The first binary mixture had butyl acetate at a concentration producing odor detectability (always according to Experiment 1) at a level equal to 3/4 of 0.80 (i.e., p=0.60) and toluene at a concentration producing odor detectability at 1/4 of 0.80 (i.e., p=0.20). The second mixture had each compound at a concentration producing odor detectability at a level equal to 1/2 of 0.80 (i.e., p=0.40). Finally, the third mixture had butyl acetate at a concentration producing odor detectability at a level equal to 1/4 of 0.80 (i.e., p=0.20) and toluene at a concentration producing odor detectability at 3/4 of 0.80 (i.e., p=0.60). Thus, in each of the three binary mixtures, the simple addition of the expected detectabilities of the two components always equaled 0.80, precisely the same level of detectability as that expected from the two single stimuli.

To avoid depletion of the headspace from the bottles while testing, we prepared each of the five stimuli described above in quintuplicate. As in Experiment 1, gas

chromatography served to quantify the vapor-phase concentration of each stimulus via headspace vapor-sampling and creation of a calibration curve. To enhance analytical sensitivity in quantifying these stimuli, headspace samples were concentrated in adsorption tubes (Sorbent tube, 4-1/2"L x 4mm ID, packed with 20:35 Tenax-TA/ Carboxen1000/CarbosieveSIII) and thermally desorbed via a thermal desorption unit connected to the GC. Figure 1 summarizes the GC measurements obtained (up to the limit of sensitivity of our system) in this and all following experiments with mixtures. Overall, all stimuli followed the expected trends except for some deviation toward higher values in the level of toluene measured at target p=0.80 for the 1/2 and 1/2 mixture.

Insert Figure 1 about here

Experiment 3. Odor detectability of binary mixtures at target p=0.95. All considerations are analogous to those in Experiment 2, only that, here, the concentrations of the five stimuli referred to p=0.95 (instead of 0.80) always according to the results in Experiment 1 (Figure 4). In other words, the concentration of butyl acetate and toluene, respectively, in the two single stimuli equaled that producing p=0.95 (Figure 4) and the concentrations of butyl acetate and toluene in the three binary mixtures were made in the same proportions than before: 3/4 and 1/4, 1/2 and 1/2, and 1/4 and 3/4, but using 0.95 (instead of 0.80) to derive the calculations. In Experiment 3, the simple addition of the expected detectabilities of the two substances in each mixture always equaled 0.95, precisely the same level of detectability as that expected from the two single stimuli.

Experiment 4. Odor detectability of binary mixtures at target p=0.60. All considerations are analogous to those in Experiment 2, only that, here, the concentrations of the five stimuli referred to p=0.60 (instead of 0.80) always according to the results in Experiment 1 (Figure 4). In Experiment 4, the simple addition of the

expected detectabilities of the two substances in each mixture always equaled 0.60, precisely the same level of detectability as that expected from the two single stimuli.

Procedure

Experiment 1. Odor detectability of the single chemicals. To obtain the concentration-response (i.e., psychometric) function for each chemical, we employed a three-alternative, forced-choice procedure (3AFC) with presentation of ascending concentrations. Starting from the lowest concentration, each trial entailed the presentation of a dilution step and two blanks in random sequence. The participant had to decide which stimulus was "different". The same dilution step was tested twice (using the duplicate vessels for each step) before continuing to the next higher concentration. At least 45 sec separated trials. In addition to selecting the "different" stimulus from the triad, subjects had to rate the confidence with which they made their decision on the following scale: 1 = not confident, 2 = a little confident, 3 = confident, 4 = very confident, 5 = extremely confident. Testing continued, each step given for two trials, until reaching the highest concentration, that is, dilution step 1 defined above. At this time a 2-min rest period began. Then, testing resumed at the lowest concentration and proceeded upward again. This regimen was repeated between 3 and 6 times per session. Typically, subjects participated in 2-4 such sessions.

Experiment 2. Odor detectability of binary mixtures at target p=0.80. As in Experiment 1, we used a 3AFC procedure and the confidence rating scale described above. Order of presentation of the stimuli and position of blanks and stimulus in each 3AFC triad were randomly assigned. In a typical 3- to 4-hour session each subject made 20 3AFC judgements for each of the five stimuli. At least 45 sec elapsed between one triad and the next one. There was a 10-15 min break in the middle of the session.

Experiment 3. Odor detectability of binary mixtures at target p=0.95. Same considerations as in Experiment 2.

Experiment 4. Odor detectability of binary mixtures at target p=0.60. Same considerations as in Experiment 2.

Data Analysis.

Plots of detection probability and/or confidence ratings as a function of stimulus concentration (in ppm by volume) and/or composition summarized the outcome. Detection probability was corrected for chance [34] and ranged from 0.0, that is, chance detection, to 1.0, that is, perfect detection. Significance of trends was tested by analysis of variance (ANOVA). To calculate the theoretical values of detectability under an assumption of complete dose addition, the following formula was used:

$$P_{det.\ BAc,Tol} = 1 - [(1 - P_{det.\ BAc}) \cdot (1 - P_{det.\ Tol})]$$

Where $P_{det.\ BAc,Tol}$ = Probability of detection of the binary mixture of butyl acetate and toluene, $P_{det.\ BAc}$ = Probability of detection of butyl acetate alone, and $P_{det.\ Tol}$ = Probability of detection of toluene alone. The formula reflects the formally strict expression [21] of the probability of detection of a binary mixture under the assumption of complete dose addition, although the simple approximation $P_{det.\ BAc,Tol} = P_{det.\ BAc} + P_{det.\ Tol}$ could also have been used.

Results

Experiment 1. Odor detectability of the single chemicals. Figures 2 and 3 show the psychometric functions for the odor detectability of butyl acetate and toluene, respectively. In addition, they show for each chemical, respectively, the average

confidence rating given by the group of subjects at each concentration, considering: 1) all trials, 2) trials where detection occurred, and 3) trials where detection did not occur.

Insert Figures 2 and 3 about here

In turn, Figure 4 stresses the linear section of the psychometric function for each chemical and depicts the corresponding equation. These figures reveal that: 1) In absolute terms, butyl acetate is a more powerful odorant than toluene since its detectability function is displaced to the left of the concentration range. 2) Both chemicals present relatively similar slopes. 3) For both chemicals, confidence ratings averaged over all trials and over trials showing detection fall closely into register and systematically increase with concentration; in contrast, confidence ratings averaged over trials showing no detection remain low and relatively constant across all the concentration range.

Insert Figure 4 about here

Experiment 2. Odor detectability of binary mixtures at target p=0.80. Figure 5 shows the results for all five stimuli. The detectability of the two single chemicals (which were the internal standards against which to compare the detectability of the mixtures) is virtually identical. It averages slightly below 0.60, somewhat lower than the target 0.80. Even when the detectability of the two single compounds came out lower than expected in absolute terms, it is virtually the same for both (confirming the validity of their iso-detectability, which was precisely the basis for selecting those concentrations), and it is much higher than the detectability of the three mixtures. In turn, the detectability among the mixtures is also similar and barely reaches 0.10. Results of an ANOVA gave statistical support to these trends: the odor detectability of the five stimuli was significantly different [$F(4,76)=31.41$, $p<0.0001$], and specific statistical

comparisons within the stimuli showed that the average detectability of the single substances was significantly higher than that of the mixtures ($p<0.0001$). No significant differences in detectability were found between the two single chemicals or among the three mixtures.

Insert Figure 5 here

Experiment 3. Odor detectability of binary mixtures at target p=0.95. Figure 6 illustrates the results obtained. Again, the detectability of the single chemicals is virtually identical (around 0.90, very close to the target 0.95) and is much higher than that of the three mixtures. An ANOVA revealed a significant difference in the detectability of the five stimuli [$F(4,80)=81.14$, $p<0.0001$], with the average detectability of the single compounds significantly higher than that of the mixtures ($p<0.0001$). No significant differences were found between the two single chemicals but the three mixtures did show significant differences among them ($p<0.0001$).

Insert Figure 6 about here

Experiment 4. Odor detectability of binary mixtures at target p=0.60. Figure 7 presents the outcome, showing that, once again, the detectability of the two single chemicals came out very close to one another (around p=0.30) and uniformly lower than the target p=0.60. Nevertheless, in contrast with the results from Experiments 2 and 3, the three mixtures were as detectable as the single compounds. An ANOVA gave statistical support to this trend, showing no significant differences in detectability among the five stimuli.

Insert Figure 7 about here

Discussion

The issue of the specific molecular characteristics defining the receptive range of a chemoreceptor has been explored in animal models both at the peripheral (i.e., olfactory epithelium) (e.g., [20]) and central (i.e., olfactory bulb) [25–27, 30, 36, 48] levels. It has also been addressed by physiological methods [19, 33, 41, 42], which might reflect a relatively closer approach to "in vivo" conditions, and by functional expression of cloned receptors [3, 28, 45–47, 49]. The present study probes into the topic by: a) using human psychophysics, thus providing an overall physiological perspective that integrates the response across all olfactory levels, b) focusing on a detection/no detection paradigm (i.e., threshold range), thus covering function at a level minimizing odor quality issues, and c) employing binary mixtures to explore degrees of dose additivity as an indicator of the extent of sensory agonism (in turn reflective of the receptive range of the complete system) across a broad peri-threshold span.

Systematic studies of odor detection thresholds (ODT) for up to five dozen single volatile organic compounds (VOCs) belonging to homologous chemical series and other structurally-related chemical families (e.g., terpenes) (see review in [9]) have provided the basis for a recent model for odor thresholds based on general physicochemical and structural properties [2]. A study of mixtures of up to nine VOCs from the same and different homologous series indicated that physicochemical properties, shown to play a large role in the determination of chemosensitivity to individual VOCs, seemed to have relevance for mixtures as well: Increased lipophilicity of the components of the mixtures (as well as their number) facilitated their degree of sensory agonism [16]. Due to the relatively high number of VOCs mixed, this investigation did not include measurement of the psychometric function for each compound as would be necessary for a more formal and quantitative look into the degree of dose addition responsible for the sensory agonism observed. A study of a

simple binary mixture did provide the opportunity to explore dose additivity by means of complete psychometric functions (for olfactory and trigeminal detection) of the alcohol 1-butanol and the ketone 2-heptanone [12]. As a first approximation, the overall outcome for both chemosensory modalities lent support to the notion of dose additivity at perithreshold levels, at least as gauged by expressing the mixtures as concentration units of one (or the other) chemical through the use of concentration-equivalent transformations based on the psychometric functions for each compound.

In the present investigation we have again resorted to measure the psychometric function for each substance and use the results to test the odor detection of binary mixtures of the ester butyl acetate and the alkylbenzene toluene, a pair characterized by different structural and physicochemical contrasts than the alcohol and ketone previously studied. As discussed recently, the new pair presents more drastic structural differences than the former pair, although from certain physicochemical criteria based on interaction between the two components, for example hydrogen bonding between the hydrogen bond acid (1-butanol) and hydrogen bond base (2-heptanone), the pair 1-butanol/2-heptanone presents a sharper contrast (see [13]).

Our present findings for olfactory detection reveal strong dose additivity at relatively low detectability levels of the single chemicals, i.e., levels clearly above chance detection but below half-way between chance and perfect detection (around $p \approx 0.30$) (see Figure 7). At somewhat higher detectability levels, i.e., levels close to half-way between chance and perfect detection (around $p \approx 0.55$), dose addition breaks down dramatically (see Figure 5). Finally, dose addition still remains quite incomplete at relatively high detectability levels, i.e., levels only slightly short of perfect detection (around $p \approx 0.90$) (see Figure 6). In this latter case, detectability is significantly higher for those mixtures dominated by one or the other component, compared to the 1/2-1/2 mixture (see Figure 6). Thus, the outcome for the present mixture indicates that the

degree of dose additivity for olfactory detection varies inversely with detectability level, being significantly higher at relatively low than at high detectability levels across the psychometric function of the mixed odorants. The possibility that the lower degree of dose addition seen at high detectability levels could derive from (subthreshold) odor adaptation through the 3-4 hour sessions cannot be completely ruled out. Nevertheless, it seems less likely in view that we strictly enforced the "at least 45 sec" waiting time between triads, and allowed a 10-15 min break within a session. Also, and perhaps more compelling, we did not see a trend of lower detectability for mixtures heavily laden towards one (i.e., 3/4 and 1/4) or the other (i.e., 1/4 and 3/4) component, as would have been expected from the observation that odor self-adaptation is stronger than cross-adaptation (e.g., [10]). In fact, the mixtures with highest expected detectability (Experiment 3, Figure 6) showed the opposite effect: the uneven mixtures were significantly more detectable than the even (i.e., 1/2 and 1/2) one.

Since the same two substances employed here were also tested in an analogous study of nasal and ocular trigeminal chemosensory detection of mixtures [13], it might be illustrative to compare those results with the present ones. Figure 8 depicts such comparison. The outcome shows that, at an approximately medium level of detectability (i.e., $0.55<p<0.65$), nasal trigeminal detection closely follows a rule of complete dose addition whereas, as we just discussed, olfactory detection falls quite short of that. Detection of eye irritation, the other trigeminal outcome, follows a trend somewhere between the other two sensory endpoints.

Insert Figure 8 about here

Taken together, the present olfactory data and the previous trigeminal data on detectability of mixtures paint a picture of strong dose addition at low detectability levels (i.e., $0.00<p<0.35$). As detectability increases, dose addition degrades more

dramatically for olfaction than for trigeminal chemesthesis. In the absence of purely chemical interactions between the stimuli at either the vapor-phase or the receptor biophase (an almost certain scenario at the very low olfactory concentrations, and a likely scenario at even the higher trigeminal concentrations as discussed in [13]), the loss of dose additivity has to originate in the biological sensory system. Since our psychophysical approach probes the outcome of the integrated output, it might be too speculative to ascribe the effect to a particular neural level: periphery, olfactory bulb/Gasserian ganglion, or more central regions. Nevertheless, the fact that we are working at perithreshold levels and tapping on a detection task provides added leverage to sensory aspects compared to higher cognitive processes. In any case, our results are compatible with a finer (i.e., more selective) degree of molecular tuning in olfaction than in trigeminal chemesthesis. Such finer tuning would be compatible with the strong loss of dose addition seen for odor detection (as detectability increases). This interpretation agrees with studies of quantitative structure-activity relationships that show trigeminal impact to be almost exclusively governed by transport-driven processes that carry the irritant from the vapor phase to the receptor biophase. Interestingly, this holds only up to a certain molecular size above which trigeminal impact is lost completely in a sharp chemical "cut off" effect [11]. In contrast, olfactory impact, although also transport-driven, depends considerably on specific molecular features [2].

How do the results on perception of mixtures at perithreshold levels compare with those at suprathreshold levels for chemesthesis and olfaction? One general observation is that, irrespective of level, the trigeminal chemosensory system tends to integrate the impact of mixed stimuli in a more complete way than olfaction [16-18]. This leads to a higher degree of dose additivity in the irritation modality across the complete perceptual range. Olfaction, on the other hand, shows a trend for complete dose addition at low detectability levels that breaks down substantially as the stimuli grow in intensity. In turn, this leads to the common finding of odor hypoadditivity at

suprathreshold levels whereby the odor intensity of mixtures falls below the sum of the perceived odor intensities of the individual components (e.g., [5, 6]), an outcome that holds even in models considering addition of mass of the stimuli rather than addition of sensation [8].

Future research needs to explore two important and interrelated aspects in mixture research at threshold levels: a structural/physicochemical aspect and a complexity aspect. The first aspect addresses the issue of whether a larger or smaller dissimilarity in chemical structure and/or physicochemical properties of the individual chemicals mixed can alter significantly the outcome obtained. Taken at face value, the results from the binary mixture 1-butanol/2-heptanone failed to detect substantial departures from dose addition across the detectability range and to find indications of less dose-addition for olfaction [12]. In fact, one of the reasons for selecting the new pair butyl acetate/toluene was to stretch the structural contrast between the members of the pair in an attempt to break the dose additivity observed. As the study of butyl acetate/toluene progressed, we re-focused the experimental strategy for quantifying dose addition and changed it to the one described here. This approach provided a more detailed and direct analysis of mixture effects but, at the same time, made it less comparable with the broader strategy employed with 1-butanol/2-heptanone. In terms of structural and physicochemical properties, the components of the chemically 'non reactive' pair 1-butanol/2-heptanone will interact with each other through hydrogen bonding to a great extent. However, if we include one member of the pair that is regarded as chemically ‘reactive’, then an example of an interactive pair can be butyl acetate/butanoic acid, and a most ‘reactive’ pair can be pentanal/butanoic acid, where both compounds are classed as ‘reactive’. If we wished to include shape effects, then menthol/2-heptanone would be an interesting comparison with 1-butanol/2-heptanone, and butyl acetate/pinene (alpha or beta) with butyl acetate/toluene. On the other hand, we can also choose binary combinations, such as propyl acetate/butyl acetate or

toluene/ethyl benzene, where, on chemical or 'shape' grounds, we would expect minimal interaction and maximum dose addition. Should deviations from dose additivity be observed in these cases, then effects due to the biological sensory system would have to be invoked.

The second aspect that also needs to be further explored relates to whether the presence of more components in the mixtures (i.e., added complexity) also can make a difference in the results obtained. Both aspects are likely to interact with each other since the particular kind of compounds making up complex mixtures (whether similar or dissimilar according to some relevant criteria) could modulate to some extent the outcome. We plan to address these issues in future investigations. Again, we can choose compounds from different chemical classes such as alcohols, ketones, esters, aldehydes, carboxylic acids, and aromatic hydrocarbons, among other series.

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Figure Legends

Figure 1. Vapor-phase concentrations, up to the limit of sensitivity of our gas chromatography system, for butyl acetate (left y-axis, empty symbols) and toluene (right y-axis, filled symbols) measured in the experiments testing mixtures: Experiment 2 (circles), Experiment 3 (squares), and Experiment 4 (triangles). Bars, sometimes hidden by the symbol, indicate standard deviation.

Figure 2. The left y-axis shows odor detection probability for butyl acetate (filled squares) as a function of vapor-phase concentration (in log ppm). Each point represents the average of 222 judgments made by 12 subjects. Bars indicate standard error. The right y-axis shows average confidence ratings for butyl acetate, also as a function of vapor concentration. Confidence ratings were averaged over all trials (squares with crosses), over trials were the stimulus was detected (empty squares), and over trials were the stimulus was not detected (triangles).

Figure 3. The left y-axis shows odor detection probability for toluene (filled circles) as a function of vapor-phase concentration (in log ppm). Each point represents the average of 224 judgments made by 10 subjects. Bars indicate standard error. The right y-axis shows average confidence ratings for toluene, also as a function of vapor concentration. Confidence ratings were averaged over all trials (circles with crosses), over trials were the stimulus was detected (empty circles), and over trials were the stimulus was not detected (triangles).

Figure 4. Same odor detectability data as in Figures 2 (butyl acetate) and 3 (toluene) but stressing the linear range of the functions (filled symbols) for butyl acetate (squares) and toluene (circles). The equation for each chemical is also shown. Bars indicate standard error.

Figure 5. The left y-axis shows the detection probability measured for each of the five stimuli tested in Experiment 2 (empty squares, continuous line) where the target detectability for the single chemicals was 0.80. Also shown are the detectability values expected from the simple combined detectability (i.e., complete dose-addition) of the individual components of the binary mixtures (filled squares, dashed line). (See Data Analysis.) Bars indicate standard error. The right y-axis shows the confidence ratings (averaged across all trials) (circles, dashed line) given for each of the five stimuli. Note how confidence ratings closely follow the trend for actual detectability.

Figure 6. Same as in Figure 5 but for the five stimuli tested in Experiment 3 where the target detectability for the single chemicals was 0.95.

Figure 7. Same as in Figure 5 but for the five stimuli tested in Experiment 4 where the target detectability for the single chemicals was 0.60.

Figure 8. a. Comparison of results obtained on odor detectability of the two single chemicals and mixtures (this study) (empty squares) with those obtained on nasal pungency detectability of the same two chemicals and proportional mixtures (triangles) [13]. The theoretical odor detectability expected assuming complete dose addition is also shown for comparison (filled squares, dashed line). Bars indicate standard error. b. Analogous to "a." but comparing odor (this study) with eye irritation detectability (circles) [13].

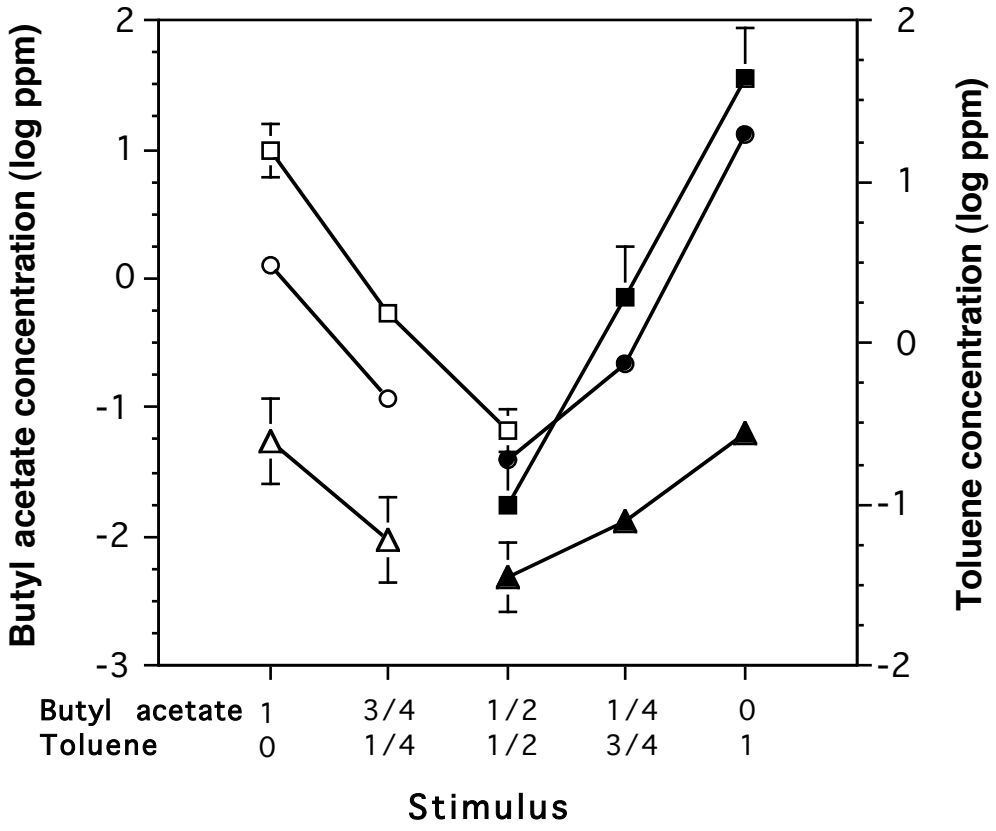
Butyl acetate concentration (log ppm)
Toluene concentration (log ppm)
2
1
0
-1
-2
-3
Butyl acetate 1 3/4 1/2 1/4 0
Toluene 0 1/4 1/2 3/4 1
Stimulus

FIGURE 1

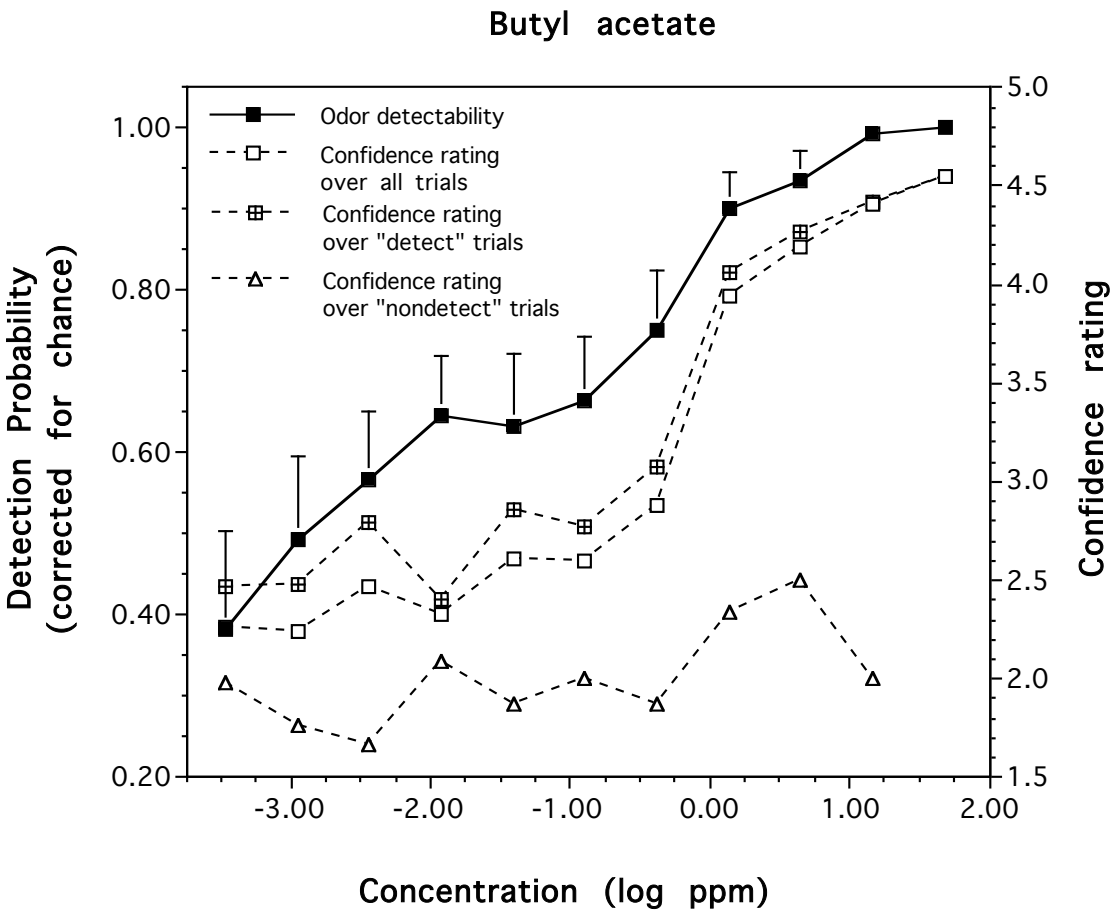
Butyl acetate
Odor detectability
Confidence rating over all trials
Confidence rating over "detect" trials
Confidence rating over "nondetect" trials
Detection Probability (corrected for chance)
Confidence rating
Concentration (log ppm)
1.00
0.80
0.60
0.40
0.20
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
-3.00
-2.00
-1.00
0.00
1.00
2.00

FIGURE 2

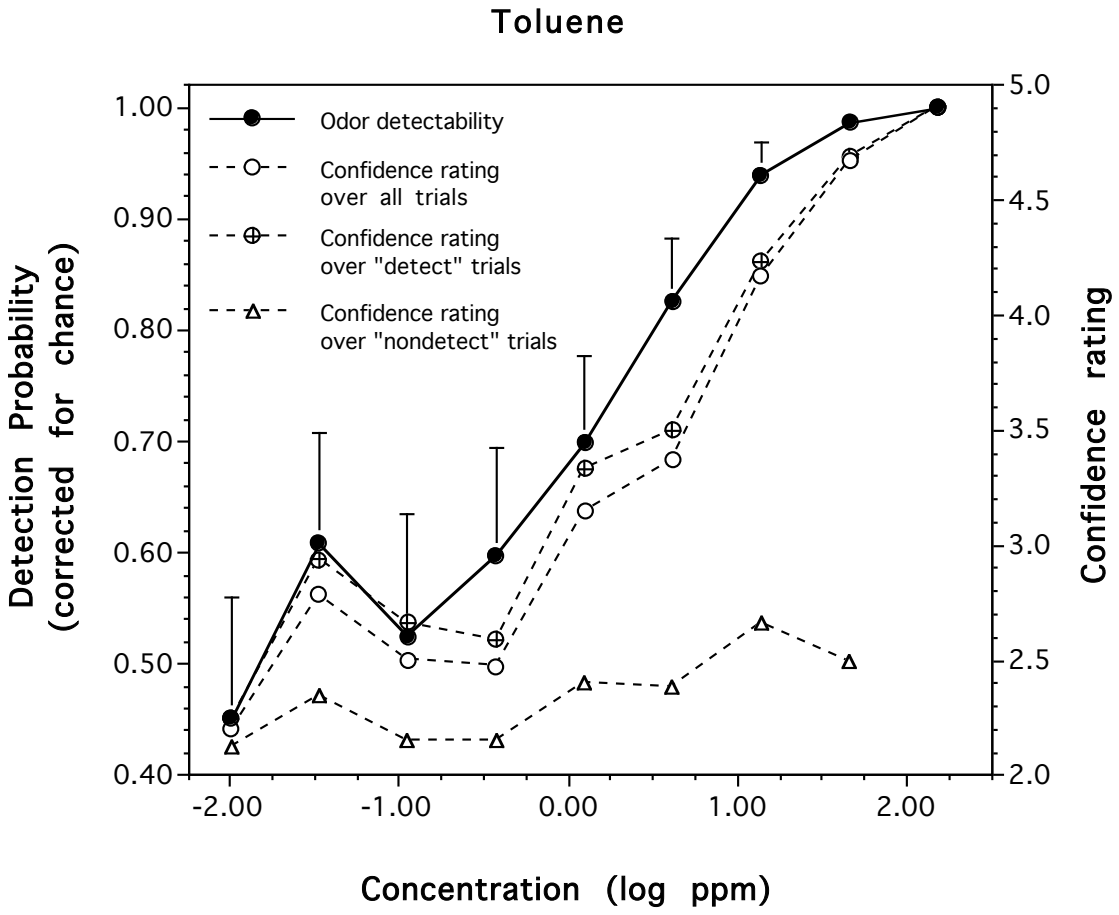
Toluene
Odor detectability
Confidence rating over all trials
Confidence rating over "detect" trials
Confidence rating over "nondetect" trials
Detection Probability (corrected for chance)
Confidence rating
Concentration (log ppm)
1.00
0.90
0.80
0.70
0.60
0.50
0.40
5.0
4.5
4.0
3.5
3.0
2.5
2.0
-2.00
-1.00
0.00
1.00
2.00

FIGURE 3

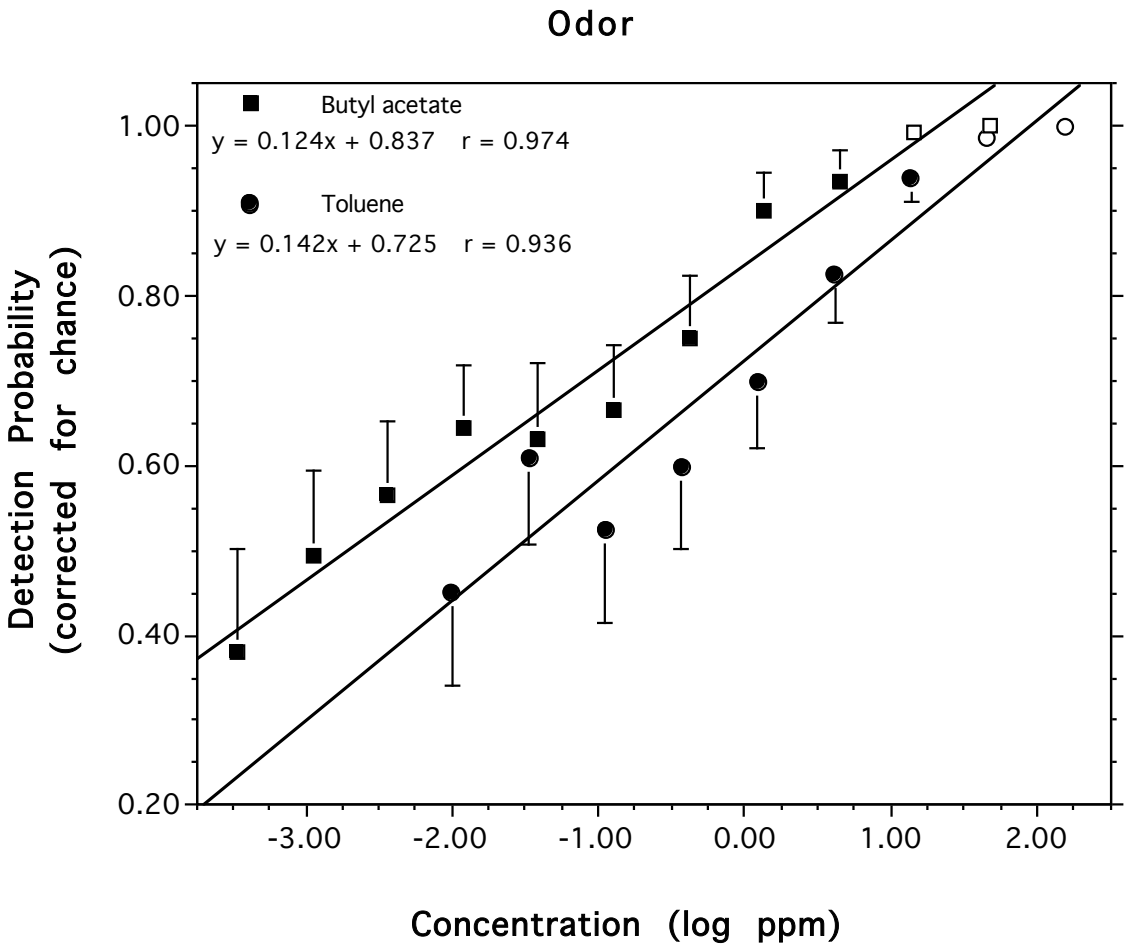
Odor
Butyl acetate
y = 0.124x + 0.837 r = 0.974
Toluene
y = 0.142x + 0.725 r = 0.936
Detection Probability
(corrected for chance)
1.00
0.80
0.60
0.40
0.20
-3.00
-2.00
-1.00
0.00
1.00
2.00
Concentration (log ppm)

FIGURE 4

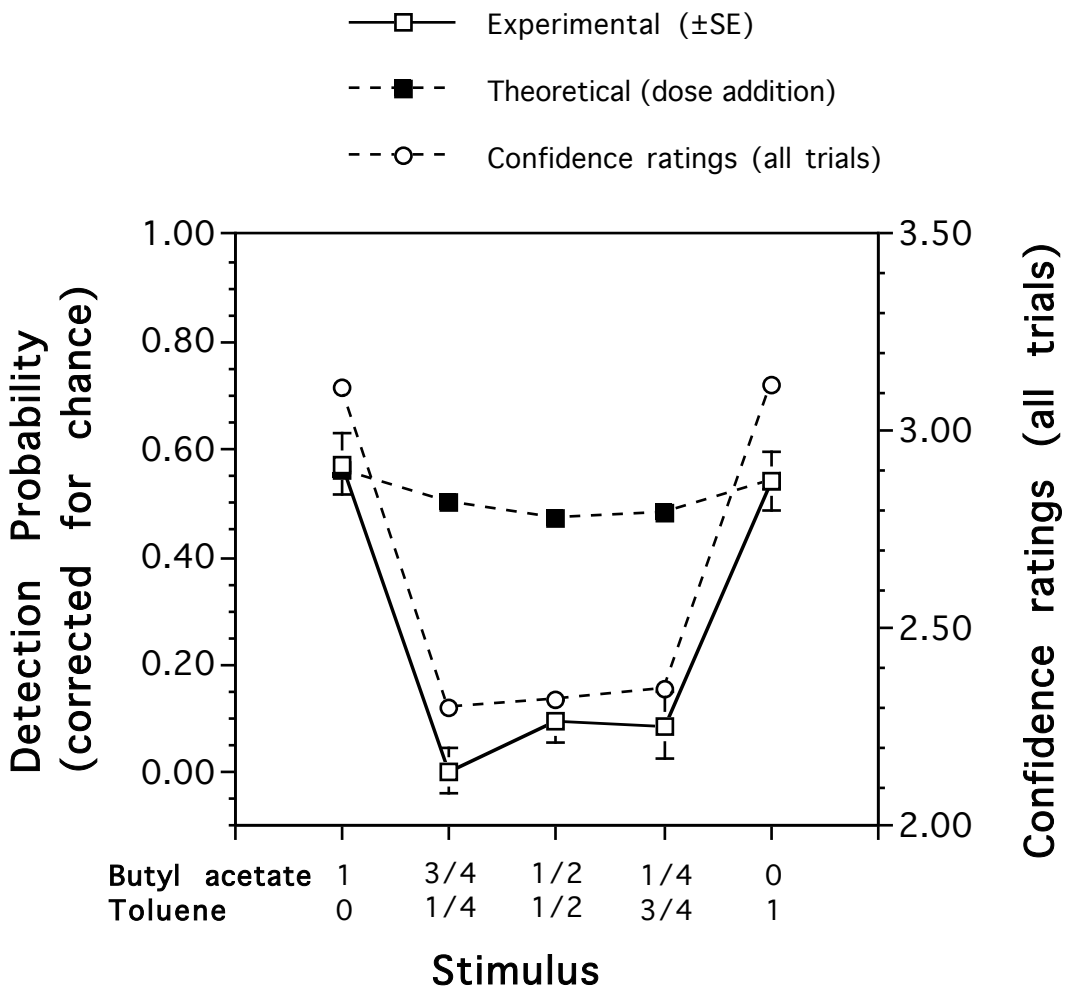
Experimental (±SE)
Theoretical (dose addition)
Confidence ratings (all trials)
Detection Probability (corrected for chance)
1.00
0.80
0.60
0.40
0.20
0.00
3.50
3.00
2.50
2.00
Confidence ratings (all trials)
Butyl acetate 1 3/4 1/2 1/4 0
Toluene 0 1/4 1/2 3/4 1
Stimulus

FIGURE 5

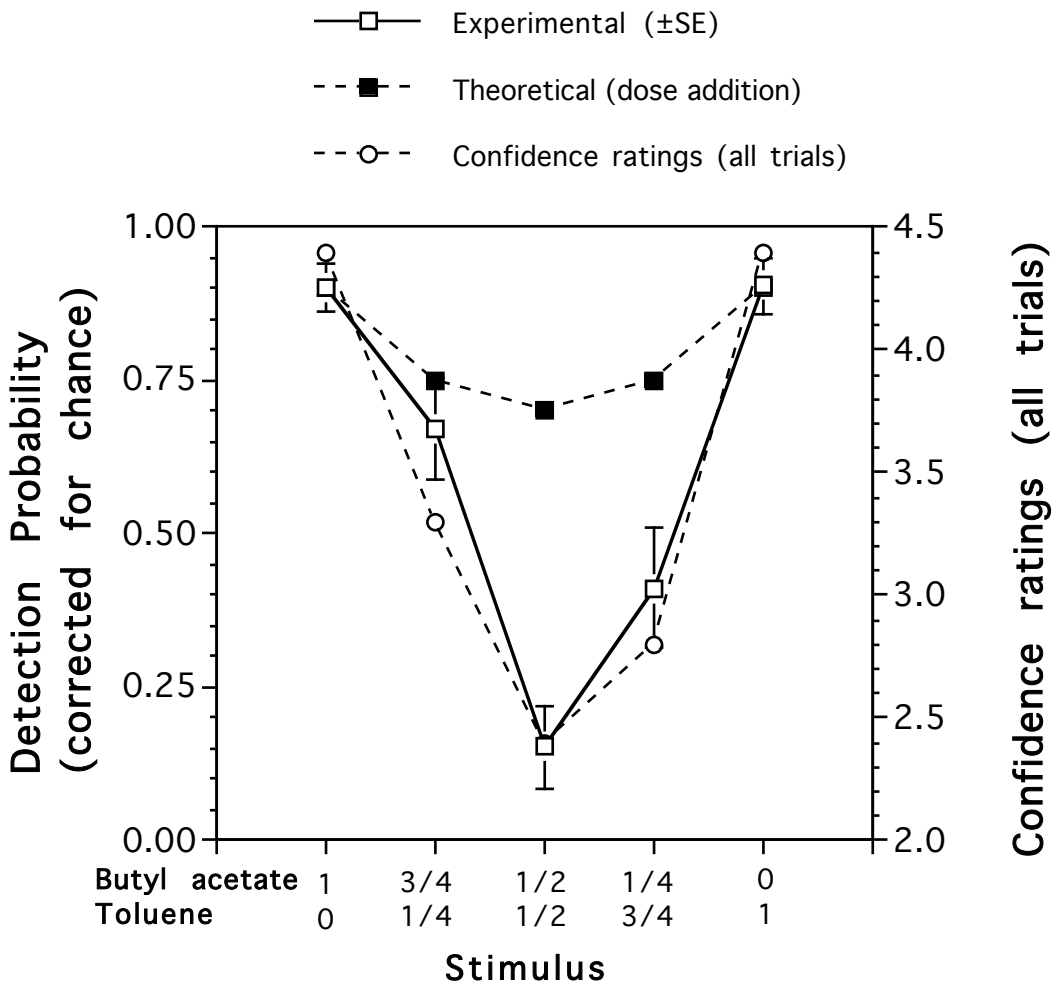
Experimental (±SE)
Theoretical (dose addition)
Confidence ratings (all trials)
Detection Probability (corrected for chance)
Confidence ratings (all trials)
1.00
0.75
0.50
0.25
0.00
4.5
4.0
3.5
3.0
2.5
2.0
Butyl acetate
Toluene
1
0
3/4
1/4
1/2
1/2
1/4
3/4
0
1
Stimulus

FIGURE 6

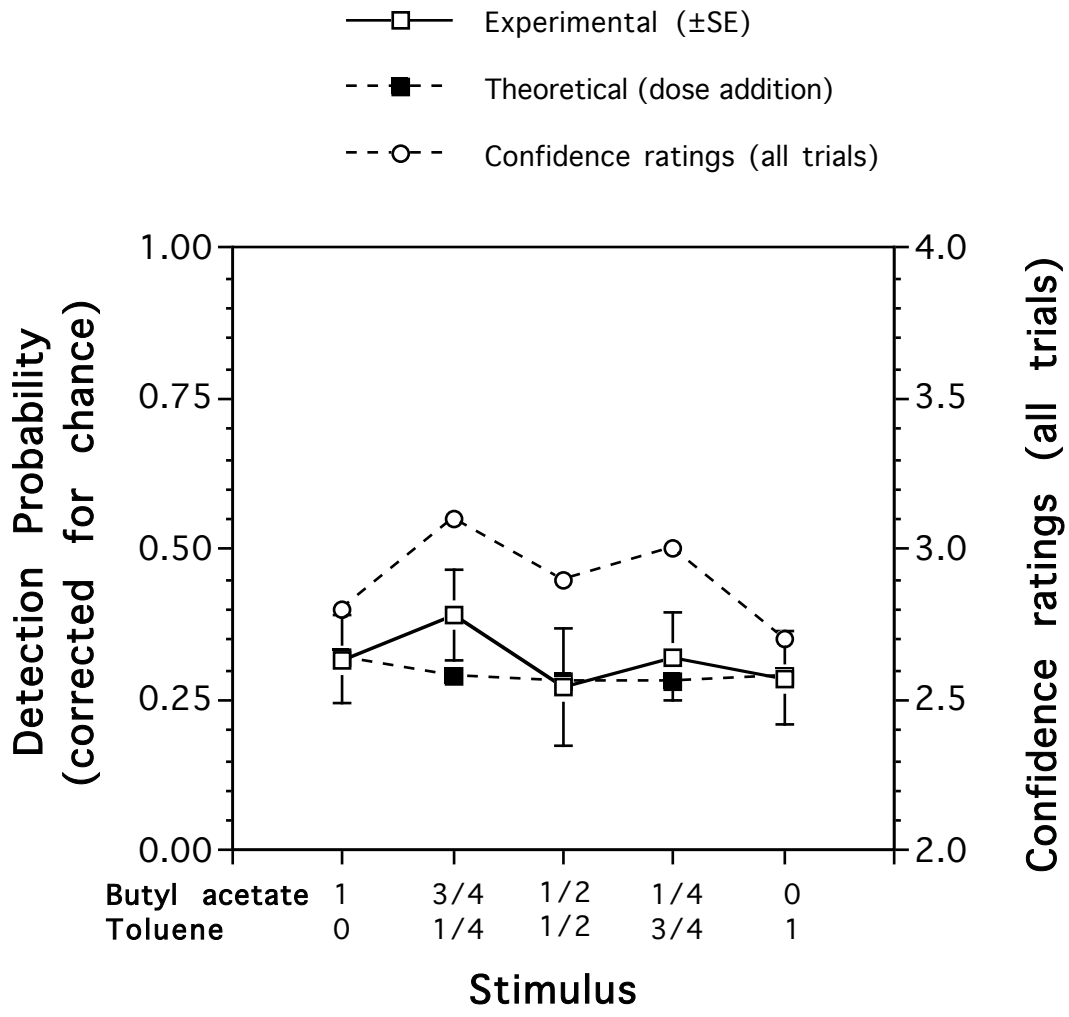
Experimental (±SE)
Theoretical (dose addition)
Confidence ratings (all trials)
Detection Probability (corrected for chance)
Confidence ratings (all trials)
1.00
0.75
0.50
0.25
0.00
4.0
3.5
3.0
2.5
2.0
Butyl acetate 1 3/4 1/2 1/4 0
Toluene 0 1/4 1/2 3/4 1
Stimulus

FIGURE 7

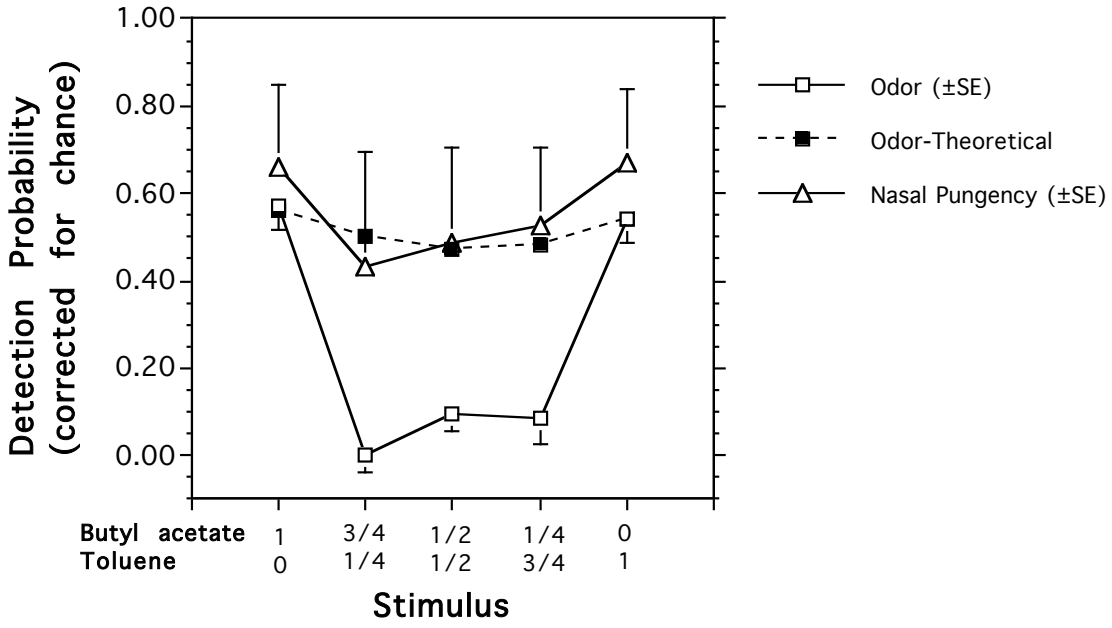

FIGURE 8 a

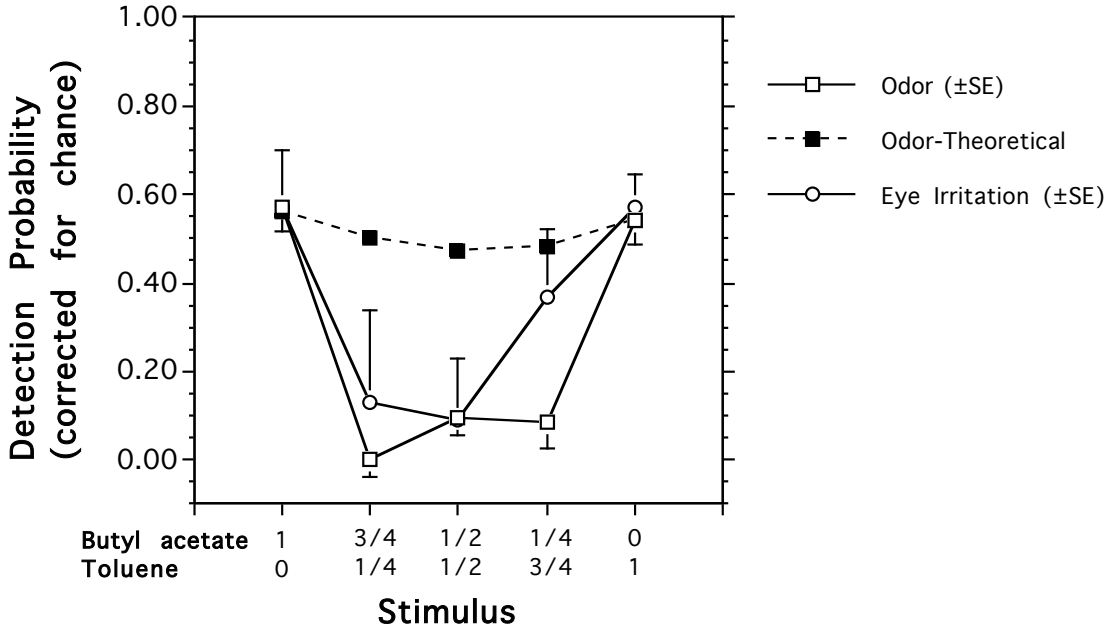

FIGURE 8 b

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