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# Human Odor Detection of Homologous Carboxylic Acids and Their Binary Mixtures

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## Abstract

Does structural similarity of odorants influence detectability of their mixtures? To address this question, psychometric (probability of correct detection vs. concentration) functions were measured for aliphatic carboxylic acids and selected binary mixtures thereof. Unmixed stimuli included acetic ($C_2$), butyric ($C_4$), hexanoic ($C_6$), and octanoic ($C_8$) acids. Mixtures included $C_2 + C_4$, $C_2 + C_6$, and $C_2 + C_8$. Vapor-phase concentrations of individual compounds, as measured by a combination of solid-phase micro extraction and gas chromatography/mass spectrometry, were always the same, whether presented singly or in a binary mixture. Additivity of detectability was assessed with respect to response addition (independent processing of mixture components). For $C_2 + C_6$, for which the mixture components differed by 4 methylene units, and $C_2 + C_8$, which differed by 6 methylene units, response addition provided a reasonably good description of detection at all levels of performance. In contrast, for $C_2 + C_4$, which differed by only 2 methylene units, detection showed a tendency to exceed additivity at low concentrations but fell below additivity at higher concentrations. These results suggest that interaction among odors in binary mixtures does depend on structural similarity, at least for detection of carboxylic acids. Future studies can determine if this result is particular to carboxylic acids.

**Key words:** interaction, olfaction, psychophysics, sensitivity, structure–activity

## Introduction

The majority of work on odor detection has focused on single compounds. Starting with a simple model stimulus is logical, but the odors we encounter in natural environments are generally composed of many compounds. Thus, we must not only understand detection of single compounds but also understand how molecules interact in mixtures. In a number of studies, researchers have measured odor thresholds, that is, the concentration needed for a criterion level of detection, for both single compounds and mixtures (e.g., Rosen et al. 1962; Baker 1963; Guadagni et al. 1963; Laska and Hudson 1991; Patterson et al. 1993). In general, molecules cooperate to some extent. Concentrations of individual components in a threshold-level mixture usually fall below thresholds for individual components. These studies are informative, but most did not include vapor-phase calibration of stimuli. The relationship between concentration presented singly and concentrations presented in mixtures were estimated, not known. Further, the studies only estimated a single point in the detection function, that is, threshold, so they did not allow detailed analyses of mixture interactions across a range of concentrations.

Cometto-Muñiz et al. (1997, 1999, 2003, 2005) overcame some limitations of former studies by measuring vapor-phase concentrations of stimuli. In 3 studies, they not only measured vapor-phase concentrations of stimuli but also collected complete psychometric functions, that is, proportion correct detection versus concentration, for single compounds (Cometto-Muñiz et al. 1999, 2003, 2005). The psychometric functions for single compounds were used to formulate binary mixtures with different levels of predicted performance. As in past studies, the authors found cooperation among mixture components. In addition, they found level dependence. At low levels of detection, that is, above chance but below the level typically defined as threshold, odors showed a greater degree of cooperation than at higher levels of detection, that is, above threshold level but less than 100% correct.

Interactions in human odor detection depend on concentration, but what role does structural similarity play? Cometto-Muñiz and colleagues found a similar pattern of level dependence for both a pair of molecules within the same aliphatic series (ethyl propanoate and ethyl hexanoate) and for a pair with less structural similarity (butyl acetate and toluene) (Cometto-Muñiz et al. 2003, 2005). They concluded that some general rules of interaction might apply for a variety compounds. In contrast, an earlier study on butanol and heptanone found dose addition over a wide range of levels of detection (Cometto-Muñiz 1999). Analysis of the data from this earlier study followed different procedures, and thus, the results are not directly comparable to those of the later studies. Nevertheless, the results provide a hint that compound-specific differences may also occur. However, the experiments cited above are not ideal to examine differences among compounds for at least 3 reasons. First, the different odor combinations were tested at different times, presumably with different subject samples. Second, methods of stimulus presentation and analysis differed somewhat among the experiments. Finally, structural differences among compounds were not manipulated gradually and systematically.

The current study extends previous results by examining odor interactions within a fixed family of homologous compounds, namely, aliphatic carboxylic acids. Mixtures whose constituent compounds differed by 2, 4, and 6 methylene units were studied. The same subjects were tested in all conditions, using the same methods. An air dilution olfactometer tightly controlled stimulus concentration, and experimenters measured vapor-phase concentration at the output of the olfactometer for all stimuli.

## Experiment 1

### Materials and methods

#### *Subjects*

In total, 20 healthy adults (12 female) participated. Ages ranged from 22 to 47 years (average = 28.6 years). All subjects completed 2 threshold runs with butyric acid to determine if sensitivity was unusually low or high. One male, who could easily detect the lowest concentration, was disqualified. We could not easily change the range of stimuli to accommodate individuals. Most subjects were employees of the Monell Chemical Senses Center. Other subjects were recruited from the local community. Both employees and outside subjects were paid. All subjects provided written informed consent on forms approved by the Institutional Review Board of the University of Pennsylvania.

#### *Materials*

Subjects received 4 aliphatic, carboxylic acids: acetic acid (CAS# 64-19-7; Nagase ChemteX Corporation, Osaka, Japan, 99.7% pure), butyric acid (CAS# 107-92-6; Daicel Chemical Industries, Ltd, Tokyo, Japan, 99.6% pure), hexanoic acid (CAS# 142-62-1; Chisso Corporation, Tokyo, Japan, 98.5% pure), and octanoic acid (CAS# 124-07-2; Inoue Perfumery Co., Ltd, Tokyo, Japan, 97.3% pure). Subjects received a 6-step dilution series of each unmixed compound. Successive concentration steps differed by a factor of about 2.2. In addition, subjects received a 6-step dilution series of each of 3 binary mixtures: acetic acid mixed with butyric acid ($C_2 + C_4$), acetic acid mixed with hexanoic acid ($C_2 + C_6$), and acetic acid mixed with octanoic acid ($C_2 + C_8$). Concentrations of individual compounds were the same in binary mixtures as they were when presented alone (see Olfactometer and calibration). For example, the dilution series for $C_2 + C_4$ consisted of the lowest concentration step of $C_2$ added to the lowest step of $C_4$, the second lowest step of $C_2$ added to the second lowest step of $C_4$, and so forth up to the highest step of each component. Extensive pilot work suggested that the range of concentrations would span a wide range of detection performance for most subjects, with comparable levels of detection at a given step across compounds.

#### *Olfactometer and calibration*

Nitrogen that had flowed through odor vessels containing pure chemicals was mixed with filtered air to create each 6-step dilution series (see above). Chemical mixtures were formed by combining nitrogen streams from 2 separate odor vessels, that is, in vapor phase, before subsequent air dilution. Electronic valves could gate any of the 6 concentrations, or a clean air blank, to a glass cone. Subjects sampled by placing their noses in the cone. The olfactometer provided a total flow of 30 l/min to allow subjects to sniff without inhaling room air.

Samples at the output of the olfactometer (collected in Teldar bags) were quantified using gas chromatography/mass spectrometry (GC/MS). To enhance analytical sensitivity, solid-phase micro extraction (SPME) fibers were extended into sample bags for 45 min, and subsequently, the compounds were desorbed in the injection port of the GC/MS system. A liquid dilution series of each acid (in chloroform) provided standards to convert GC area to parts per million (ppm) (by mass). Standards were sampled in 2 ways. First, liquid standards were injected into Tedlar bags filled with nitrogen and sampled using SPME fibers. Second, liquid standards were injected directly into the GC/MS system. To a first approximation, the 2 methods yielded comparable values (Table 1). Values in the figures come from bag standards.

A detailed description of the design and calibration of the olfactometer can be obtained from the corresponding author. In brief, calibration yielded 3 results important to the interpretation of the psychophysical data. First, 2.2-fold air dilutions in the olfactometer actually produced 2.2-fold drops in ppm. Second, concentrations were stable, both within and between days. Third, concentrations for a given

single compound precisely matched concentrations of that compound when presented in a binary mixture.

### *Procedure*

Subjects received 5 samples on each trial: 2 odors of the same concentration interspersed in random order with 3 blanks (subjects knew there would be exactly 2 odors per trial). Trials began with a prompt for subjects to get in position and click a mouse. Two seconds after the mouse click, the subject received a series of five 2.5-s pulses separated by pauses of 3 s. Subjects exhaled through the nose between pulses to help clear odorant and rehumidify the nose. A window on a computer monitor identified each odor sample according to position in the sequence (1–5). A set of 5 virtual buttons were continuously available. A mouse click changed the label on a button from "blank" to "odor." At this point, subjects could mark as many samples as "odor" as they wished. After the initial sequence of 5 samples, responses carried over to another screen. The second screen included 5 additional buttons that allowed subjects to resample odors if they wished and change responses. After subjects were satisfied with their response, they ended the trial by clicking another virtual button. The program made sure that exactly 2 samples had been marked as "odor." If this condition was not met, a prompt asked the subject to modify the response. If exactly 2 samples had been marked as "odor," the computer wrote the response to the hard drive and ended the trial. Fifteen seconds separated successive trials.

Each experimental session (approximately 40 min) included 6 presentations of each concentration (see Olfactometer and calibration) of a single stimulus, that is, one pure compound or a particular binary mixture. The stimulus that subjects received varied across sessions. To lessen the effects of adaptation, concentrations were presented in blocked, ascending order. The computer presented the lowest concentration on the first 3 trials, the next lowest in the next 3 trials, and so forth. After 3 presentations of the highest concentration, the computer prompted a 5-min break. After the break, the sequence started again with the lowest concentration. Thus, in each experimental session, the subject contributed 6 trials per concentration. In addition, each single compound and each binary mixture were tested in 2 sessions, for a total of 12 trials per condition. The design started with an ideal of blocked random order across sessions. However, due to schedule changes caused by subjects and the fact that cleaning the olfactometer required more time for octanoic and hexanoic acids, the order of presentation was more haphazard than random. In particular, a disproportionate number of runs for acetic and butyric acid, including the runs for the acetic–butyric mixture, occurred early.

**Table 1** Odorant concentrations in log ppm

| Acetic[a] | Butyric[a] | Hexanoic | Octanoic |
|---|---|---|---|
| −3.55 (−4.34) | −4.87 (−4.73) | −3.83 (−4.04) | −3.58 (−3.80) |
| −3.20 (−3.98) | −4.51 (−4.40) | −3.48 (−3.68) | −3.24 (−3.47) |
| −2.85 (−3.63) | −4.16 (−4.08) | −3.13 (−3.33) | −2.90 (−3.13) |
| −2.50 (−3.27) | −3.80 (−3.76) | −2.78 (−2.97) | −2.56 (−2.80) |
| −2.15 (−2.91) | −3.44 (−3.43) | −2.44 (−2.62) | −2.22 (−2.46) |
| −1.80 (−2.55) | −3.09 (−3.11) | −2.09 (−2.26) | −1.89 (−2.13) |

Values outside parentheses based on prepared bag standards. Values in parentheses based on liquid injection.
[a]Lowest concentration estimated based on calibration curves.

### *Data analysis*

Basic data consisted of psychometric functions, that is, a transformation of proportion correct detection versus log stimulus concentration (Figure 1). Experimenters computed proportion correct for each subject. Next, a correction for chance was applied so that performance ranged from 0, that is, no ability to identify the odors among the blanks beyond that predicted by chance alone, to 1, that is, perfect ability to identify the odors among the blanks (Macmillan and Creelman 1991). This correction was applied to make additivity calculations (see below) easier. Next, a log odds ratio transform was applied: log odds = ln[p/(1 − p)], where p represents chance-corrected proportion correct and ln indicates natural log. This particular transform was chosen because pilot work showed that cumulative logistic functions fit detection data better than other sigmoidal forms, including

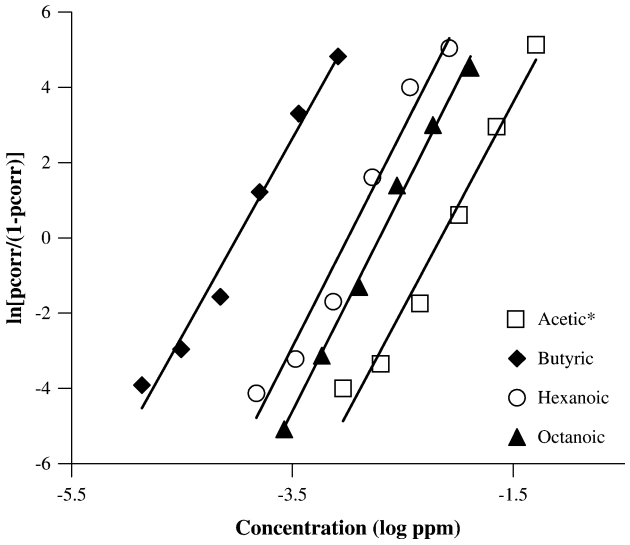


**Figure 1** In the *y* axis, log odds ratio of chance-corrected proportion correct is shown. The *x* axis shows vapor concentration in log ppm. Functions represent least squares linear fits. For acetic, log odds (pcorr) = 5.45log(ppm) + 11.79, $r^2$ = 0.97; for butyric, log odds (pcorr) = 5.24log(ppm) + 20.99, $r^2$ = 0.98; for hexanoic, log odds (pcorr) = 5.82log(ppm) + 17.53, $r^2$ = 0.97; for octanoic, log odds (pcorr) = 5.82 + 15.81, $r^2$ = 0.99. The asterisk indicates that the function for acetic acid has been shifted 0.5 log units to the right for clarity. The psychometric functions for octanoic and acetic acids largely overlap.

cumulative Gaussian and Wiebul functions. The transform made psychometric functions approximately linear, which allowed experimenters to fit data with simple linear functions using least squares regression. Finally, the log odds ratio of chance-corrected proportion was averaged across subjects for each compound and concentration.

Psychometric functions fit to detection data for pure compounds (for each individual subject) were used to generate individual predictions of response addition for the mixtures (see below). Differences in trends across compounds were assessed using repeated measures analysis of variance (ANOVA), with appropriate correction of degrees of freedom to compensate for violations of sphericity (Greenhouse and Geisser 1959).

In addition to analysis of basic data via ANOVA, patterns of mixture interaction were compared with a simple model, namely, response addition. Response addition assumes statistical independence of detection, that is, that the probability of detecting a binary mixture equals the probability of detecting one or both of the components. This is computed as $p(AB) = p(A) + p(B) - p(A)p(B)$, where p(AB) represents the probability of detecting the mixture, p(A) represents the probability of detecting component A, and p(B) represents the probability of detecting component B. One would use this equation to calculate the probability of rolling at least one "3" with a pair of fair dice (Feller 1968). Within the framework of the model, if detection performance for the mixture matches response addition, then little or no mixture interaction has occurred. If performance falls below response addition, some degree of suppression has occurred relative to statistical independence. If performance falls above response addition, then some form of mutual enhancement, or synergy, has occurred. The relationship between mixture detection and the predictions of response additivity can be compared across odor pairs as part of an assessment of structure–activity relationships.

Other models of additivity exist, including the dose addition model described by Cometto-Muñiz et al. (2003, 2005). Data in the current study were also analyzed with respect to dose addition, but these results do not appear below because they support the same conclusions. For the compounds and concentrations in the current study, response addition and dose addition made very similar predictions.

## Results

### *Psychometric functions for individual components*

Functions of proportion correct versus concentration (Figure 1) demonstrated an orderly dose–response relationship, with good linear fits in the coordinate space used. Further, the slopes of the functions seem quite consistent across stimuli. A 4 (odorant) × 6 (concentration step) ANOVA confirmed these impressions. Concentration step reached significance, $F(1.94, 34.96) = 221.60$, $P \ll 0.001$, but the effect of odorant and the odorant × concentration interaction failed to reach significance ($P > 0.50$). Thus, slopes of the functions for the 4 single compounds were quite similar and spanned a similar range of performance across the 6 concentration steps. Thresholds, that is, the concentrations that would lead to detection performance half way between chance level and 100% correct, did differ among compounds: −2.66 log ppm for $C_2$, −4.00 log ppm for $C_4$, −3.00 log ppm for $C_6$, and −2.71 log ppm for $C_8$.

### *Psychometric functions for mixtures*

As observed in single-compound functions, psychometric functions for binary mixtures demonstrated an orderly dose–response relationship (in all analyses reported from here on, the effect of concentration reached significance, $P \ll 0.001$). Unlike single-compound functions, functions for the binary mixtures were not parallel across compounds (Figure 2). Analyses omit the highest concentration step for each mixture. Because most subjects achieved maximum performance at these levels, the highest concentrations are of little use in evaluating mixture interactions. A 3 (odor mixture) × 5 (concentration step) ANOVA revealed a significant odor mixture × odorant interaction, $F(4.69, 84.35) = 3.94$, $P < 0.005$. The main effect of odor mixture failed to reach significance ($P > 0.50$). Follow-up analyses (pair wise, 2-way ANOVAs) found significant odor pair × concentration step interactions for $C_2 + C_4$ versus $C_2 + C_6$, $F(3.02, 54.40) = 6.3034$, $P < 0.0001$, and for $C_2 + C_4$ versus $C_2 + C_8$, $F(3.20, 57.52) = 5.17$, $P < 0.003$. There was no significant interaction for $C_2 + C_6$ versus $C_2 + C_8$ ($P > 0.76$). All in all, these analyses suggest that the pattern of mixture interaction with acetic acid was different for butyric acid than it was for hexanoic and octanoic acids.

### *Mixture interaction with respect to response addition*

Mixture data appear with additivity predictions (filled squares) in Figure 2. One test of model fits ($\chi^2$) indicates that predictions do not differ significantly from data for any mixture ($P > 0.20$). To a first approximation, data resemble response additivity. However, the $\chi^2$ analysis omits a great deal of information. Predictions for individual subjects allow a more powerful analysis by ANOVA. Data were submitted to a 3-way ANOVA: 3 (mixtures) × 2 (data vs predictions) × 5 (concentration). The main effect of data versus predictions reached significance, $F(1, 18) = 5.96$, $P < 0.03$. Overall, mixture detection fell below response addition. Further, a significant interaction between data versus prediction and concentration, $F(2.47, 44.37) = 4.10$, $P < 0.02$, revealed concentration dependence: mixture interactions fell further below response addition for higher concentrations than for lower concentrations. Furthermore, a significant 3-way interaction, $F(4.77, 85.88) = 3.60$, $P < 0.01$, revealed that the observed concentration dependence differed among mixtures. Subsequent 2-way ANOVAs (data vs prediction

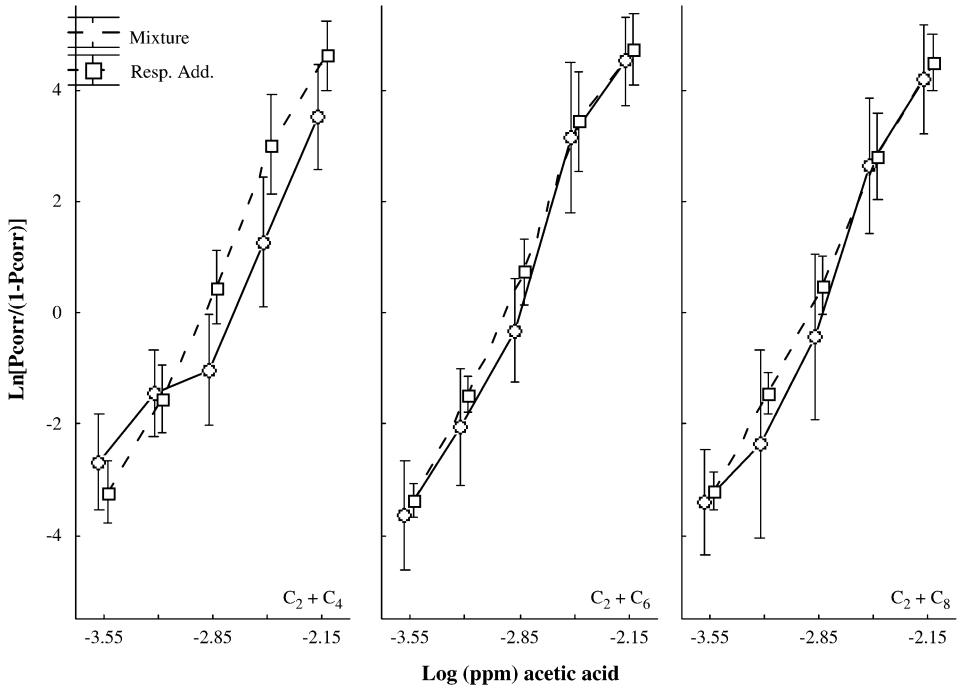


**Figure 2** Psychometric functions for binary mixtures. Filled squares with dashed lines represent predictions for response addition. Open circles with solid lines represent mixture data. Error bars represent 95% confidence intervals.

by concentration) conducted separately for the 3 mixtures revealed a main effect of data versus prediction for the $C_2 + C_4$ mixture, $F(1, 18) = 7.55$, $P < 0.02$, as well as a significant interaction, $F(3.51, 63.12) = 10.78$, $P \ll 0.001$. In contrast, neither the main effect of data versus prediction nor the interaction reached significance for the other 2 mixtures ($P$ ranged from 0.11 to 0.40). Thus, detection for mixtures did not differ significantly from response addition for $C_2 + C_6$ (4 methylene unit difference) or $C_2 + C_8$ (6 methylene unit difference), even using a relatively powerful statistical test. However, for $C_2 + C_4$ (2 methylene unit difference), cooperation in the mixture was better at low concentrations than at higher concentrations.

## Experiment 2

As noted in the procedure for Experiment 1, most sessions for acetic and butyric acid occurred early in testing. The results for the acetic–butyric mixture may have differed from those for the other mixtures for this reason. To help test whether practice or other order effects had a substantial influence on results, we replicated tests with acetic acid, butyric acid, and the acetic–butyric mixture.

### Materials and methods

The methods exactly matched those of Experiment 1. The same subjects who participated in Experiment 1 also served in Experiment 2. The only difference was a restricted stimulus set. Acetic acid, butyric acid, and an acetic–butyric mixture were tested in blocked, random order, that is, one session for each of the 3 stimuli in random order, then another session for each of the 3 stimuli in random order.

### Results

The results of Experiment 2 and the psychometric functions for single compounds agreed well with those of Experiment 1 (Figure 3). Slopes of best-fit functions were similar between the experiments (see caption for Figures 1 and 3). Further, in a 3-way ANOVA (experiment × concentration × compound), the effect of experiment, that is, Experiment 1 versus Experiment 2, and all interactions involving experiment failed to reach significance ($P$ values ranging from 0.36 to 0.96). Furthermore, the function for the acetic–butyric mixture looks strikingly similar to the corresponding function from Experiment 1 (compare the leftmost panel of Figure 2 with Figure 4). Again, the interaction between data versus response addition and concentration reached significance, $F(2.58, 46.46) = 8.36$, $P < 0.001$. Accordingly, psychometric functions for pure $C_2$ and $C_4$ collected at different times were almost identical. Further, the pattern of mixture interaction, that is, better cooperation at lower concentration levels than at higher levels, replicated well.

## Discussion

To our knowledge, this work constitutes the first systematic study of structure–activity relationships in mixture

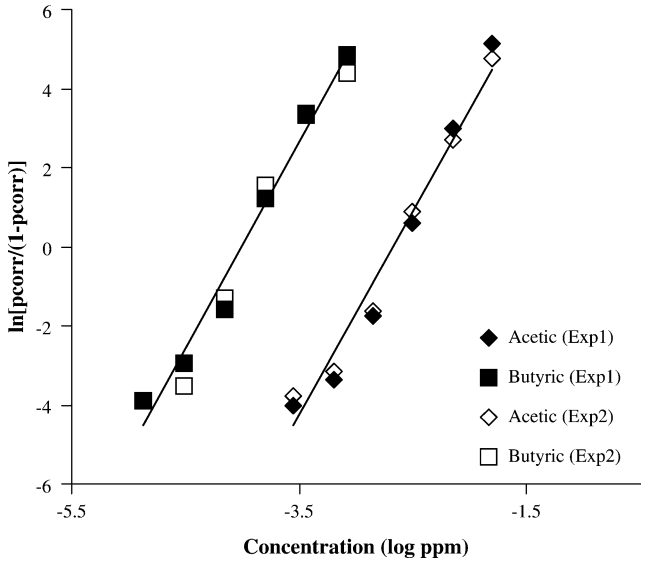


**Figure 3** Psychometric functions for acetic (diamonds) and butyric (squares) acid. Filled symbols represent data from Experiment 2 (retest). Open symbols represent data from Experiment 1, replotted from Figure 3. Lines represent least squares linear fits to data from Experiment 2. For acetic acid, log odds (pcorr) = 5.14log(ppm) + 10.23, $r^2$ = 0.98; for butyric acid, log odds (pcorr) = 5.26log(ppm) + 18.28, $r^2$ = 0.97.

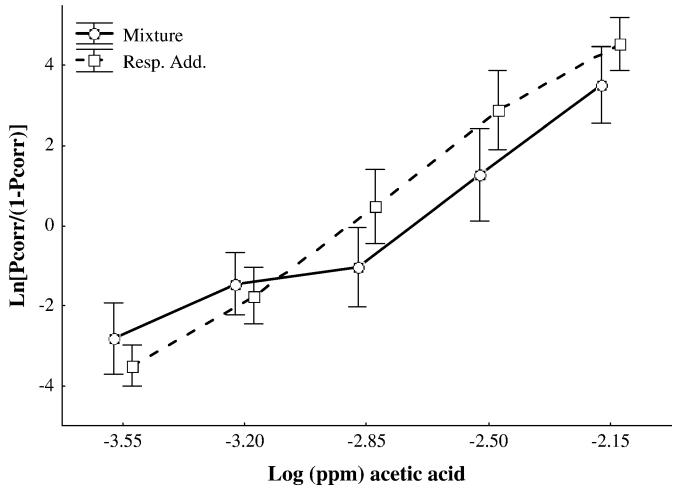


**Figure 4** Psychometric function for the acetic–butyric mixture in Experiment 2. Filled squares (dashed lines) represent predictions for response addition. Open circles (solid lines) represent mixture data. Error bars represent 95% confidence intervals.

interactions at the perithreshold level in humans. Despite the fact that the slopes of psychometric functions for unmixed compounds differed very little, the slope of the psychometric function for $C_2$ + $C_4$ (separated by 2 methylene units) differed from those of $C_2$ + $C_6$ and $C_2$ + $C_8$ (separated by 4 and 6 methylene units, respectively). Further, only $C_2$ + $C_4$ deviated significantly from response addition, that is, statistical independence in detection. Relative to the other mixtures, $C_2$ and $C_4$ demonstrated a tendency toward better cooperation at low perithreshold concentrations (synergy with respect to response addition) and worse cooperation at higher perithreshold concentrations (suppression with respect to response addition). In short, the pair that differed by 2 methylene units showed stronger mixture interactions than the pairs that differed by 4 and 6 methylene units. These data suggest that structural similarity does play a role in mixture interactions near threshold.

### Current findings in the context of psychophysical literature

In broadest terms, the current findings agree with many past studies of mixture interactions in odor detection, which have also shown some degree of cooperation (e.g., Rosen et al. 1962; Baker 1963; Guadagni et al. 1963; Laska and Hudson 1991; Patterson et al. 1993; Cometto-Muñiz et al. 1997). Because most of these studies did not include vapor-phase calibration of stimuli and did not involve collection of full psychometric functions, more detailed comparisons seem inappropriate.

Cometto-Muñiz and colleagues, who did calibrate their stimuli and collect full detection functions, found concentration dependence (Cometto-Muñiz et al. 2003; 2005). Low perithreshold concentrations showed better cooperation than did higher perithreshold concentrations. This pattern held both for a homologous pair of odors that differed by 4 methylene units and a pair that showed even greater structural dissimilarity. In broad terms, a similar pattern of concentration dependence held for the $C_2$ + $C_4$ mixture in the current study. However, as noted above, $C_2$ + $C_6$ and $C_2$ + $C_8$, which differed by 4 and 6 methylene units, respectively, showed little if any concentration dependence. Future studies can determine if the discrepancy in results comes from stimulus differences or methodological differences.

Above threshold, the range in which most work on mixture interactions has been done, a mixture of 2 odorants will generally smell less intense than the sum of the intensities of the unmixed components but stronger than the weaker of the 2 components (Berglund et al. 1973; Cain 1975; Laing et al. 1984; Cain et al. 1995; Laing 1995; Lawless 1997). However, analogous to the findings of concentration dependence in the perithreshold work of Cometto-Muñiz and colleagues, studies have found more complete suprathreshold summation when the unmixed components are of relatively low intensity (Laing et al. 1984; Cain et al. 1995). Current results for $C_2$ + $C_4$, together with those of Cometto-Muñiz et al. (2003, 2005), suggest that loss of additivity with increasing concentration may begin at high perithreshold concentrations, at least in some cases.

If one assumes that stronger mixture interactions indicate greater overlap in the sensory mechanisms (either receptors or more central mechanisms) that mixture components stimulate, the finding of a stronger mixture interaction for $C_2$ + $C_4$ than for the more structurally dissimilar pairs

suggests that the mechanisms involved are "tuned" to carbon chain length to some extent. This conclusion is consistent with other psychophysical work, including structure–activity studies of suprathreshold cross-adaptation (Pierce et al. 1995) and magnitude of "anosmic defect" in specific anosmia (Amoore 1970). This conclusion is also consistent with work on suprathreshold quality discrimination, which finds that pure compounds become progressively easier to discriminate from one another as difference in carbon chain length increases (Laska and Hubener 2001; Laska and Tuebner 1998, 1999 ). For several aliphatic series, including carboxylic acids, discrimination reached an asymptote at a difference of about 3 or 4 methylene units. The finding of asymptotic discrimination suggests minimal overlap in the sensory mechanisms that process molecules that differ by 4 methylene units. At a difference of 4 methylene units and greater, current results are also consistent with minimal overlap of sensory mechanisms.

There is a small literature on the relationship between threshold and carbon chain length in both human and nonhuman primates (e.g., Cometto-Muñiz et al. 1998; Laska and Seibt 2002a, 2002b; Laska et al. 2003, 2004). Current data more closely resemble detection of carboxylic acids by pigtail macaques (Laska et al. 2004), which follow a U-shaped trend (higher thresholds for shorter and longer chain-lengths), but are inconsistent with the negative correlation found in squirrel monkeys and humans (Cometto-Muñiz et al. 1998; Laska et al. 2000). Why current results differ from previous data on humans is unclear. Three facts seem worth considering: 1) in most past studies, correlations tended to be modest, 2) no previous study combined both rigorous olfactometry and vapor-phase calibration of stimuli, and 3) the number of acids studied in the current experiments was too small for meaningful analysis. Regardless, the matter may deserve further investigation.

### Current results in the context of physiology

At least some olfactory receptor proteins, which determine the molecular specificity of individual receptor neurons, are tuned to carbon chain length within an aliphatic series (Kajiya et al. 2001; Touhara 2002). This tuning is true of many cells in the olfactory bulbs as well, though tuning is narrower (Yokoi et al. 1995; Leon and Johnson 2003; Mori et al. 1999, 2006). Some work suggests that the median molecular receptive range for bulbar output neurons spans about 3 or 4 methylene units (Fletcher and Wilson 2003). If some odor–odor interactions occur at this level, then one would expect stronger interactions at a difference of 2 methylene units within an aliphatic series, consistent with current results. In addition, physiological studies suggest that molecular tuning of bulbar output neurons is refined by lateral, inhibitory connections, with stronger inhibitory interactions for carbon chain lengths closer to the receptive range (Mori and Shepherd 1994; Yokoi et al. 1995; Mori et al. 1999). This result is also consistent with stronger mixture interactions between more similar molecules.

The concentration dependence observed for $C_2 + C_4$, and for other compounds in the studies of Cometto-Muñiz et al. (2003, 2005) might also arise at various levels of the olfactory system. Given the low concentrations involved in odor detection, substantial chemical interaction in the nose and nasal mucosa seems unlikely. However, if such interactions do occur, one would expect stronger interactions at higher concentrations. In addition, a study of mixture interactions in individual olfactory receptor neurons from mice demonstrated concentration dependence: when suppression occurred, it was stronger at higher concentrations (Duchamp-Viret et al. 2003). The mechanism of suppressive interactions at the level of the olfactory receptor neuron is unclear, but there is some evidence of competition for receptor sites between structurally similar molecules for some olfactory receptors (Oka et al. 2004). One would expect such antagonism, either competitive or noncompetitive, to grow stronger as the number of molecules increased with respect to the number of free receptors. More central inhibitory interactions, that is, at the level of the olfactory bulbs, might also grow stronger with concentration.

Regardless of the particular physiological mechanisms involved, better summation at low concentration levels might allow organisms to detect weak mixtures, for example, the odor of a distant predator, at lower levels than they otherwise could. The odor could serve as a general alert, even if too weak to be properly identified. At higher concentrations, where absolute detection poses no problem, the benefits of inhibitory interactions in refining odor signals for superior discrimination might outweigh the drawback of reduced sensitivity.

### Limitations

Our study employed stimuli of high purity (the highest obtainable within practical limits). Further, our combination of SPME and GC/MS failed to find measurable cross-contamination among the carboxylic acids used. Nevertheless, some trace compounds, present in concentrations below instrument sensitivity, might have influenced the results. In addition, to learn whether the observed relationship between structural similarity and degree of mixture interaction will appear in other families of compounds will require further experiments. Finally, the mixtures were always comprised of components that were approximately matched in detection probability at each concentration step. The study provides no information on how imbalances between compounds will affect the observed structure–activity relationships. This work constitutes a successful first step toward understanding structure–activity relationships in detection of perithreshold odor mixtures, but more work is needed.

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