Odor Detection of Mixtures of Homologous Carboxylic Acids and Coffee Aroma Compounds by Humans

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Mixture summation among homologous carboxylic acids, that is, the relationship between detection probabilities for mixtures and detection probabilities for their unmixed components, varies with similarity in carbon-chain length. The current study examined detection of acetic, butyric, hexanoic, and octanoic acids mixed with three other model odorants that differ greatly from the acids in both structure and odor character, namely, 2-hydroxy-3-methylcyclopent-2-en-1-one, furan-2-ylmethanethiol, and (3-methyl-3-sulfanylbutyl) acetate. Psychometric functions were measured for both single compounds and binary mixtures (2 of 5, forced-choice method). An air dilution olfactometer delivered stimuli, with vapor-phase calibration using gas chromatography−mass spectrometry. Across the three odorants that differed from the acids, acetic and butyric acid showed approximately additive (or perhaps even supra-additive) summation at low perithreshold concentrations, but subadditive interactions at high perithreshold concentrations. In contrast, the medium-chain acids showed subadditive interactions across a wide range of concentrations. Thus, carbon-chain length appears to influence not only summation with other carboxylic acids but also summation with at least some unrelated compounds.

KEYWORDS: Odor threshold; mixture interaction; sensory; psychophysics

INTRODUCTION

The physical stimuli associated with the aromas of many foods and beverages are complex chemical mixtures. For example, about 25 compounds make important contributions to the aroma of coffee (see ref *1*). Analytical techniques such as gas chromatography−mass spectrometry (GC-MS) can be used to identify these chemical constituents. Furthermore, hybrid sensory/analytical techniques, including aroma extraction dilution analysis (AEDA) and gas chromatography−olfactometry (GC-O), can be used to evaluate the intensity or character (quality) of individual constituents (*2−5*). However, attempts to mimic natural aromas by combining important components identified through hybrid techniques often fail (see ref *6* for some discussion). Thus, minor components may interact with other compounds to shape aroma perception.

One key question is whether chemicals below the sensory detection threshold can combine to form a detectable mixture. Some studies have determined olfactory detection thresholds, that is, the concentration needed for a criterion level of detection, for both single compounds and mixtures (see, e.g., refs *7−11*). In general, concentrations of individual components in a threshold-level mixture fall below threshold concentrations for unmixed components. In other words, summation occurs, such that subjects may reliably detect a mixture even when unable to reliably detect the individual mixture components. However, a detailed examination of the results reveals that patterns of summation vary greatly. Approximately additive interactions are most common, but subadditivity and synergy have also been observed.

Is variance in degree of summation meaningful? Because most studies failed to include vapor-phase calibration of stimuli, the relationship between the concentration of each odorant presented singly and the corresponding concentrations in mixtures was not known. The rigor of stimulus control has seldom been clear. Furthermore, the studies only estimated thresholds and did not measure complete proportion correctly versus concentration (psychometric) functions. Thus, detailed analyses of mixture interactions across a range of concentrations have seldom been possible.

Cometto-Muñiz et al. (*12−15*) overcame some of these limitations. They measured vapor-phase concentrations of stimuli and measured complete psychometric functions for single (unmixed) compounds in three studies (*13−15*). The psychometric functions for unmixed compounds, together with models of additivity, allowed the authors to formulate binary mixtures with different levels of predicted detection performance. As in previous studies, substantial summation occurred. However, mixtures below the level typically defined as threshold (but detected at greater than chance level) showed a greater degree of summation than mixtures that were above threshold (but detected less than 100% of the time). Thus, Cometto-Muñiz and colleagues demonstrated concentration dependence (weaker odorants summed more completely than stronger odorants).

Interestingly, Cometto-Muñiz and colleagues found a similar pattern of concentration dependence for ethyl propanoate mixed with ethyl hexanoate (a structurally similar pair of molecules within the same aliphatic series) and for butyl acetate mixed with

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toluene (a pair with less structural similarity) (*14*, *15*). Thus, some general rules of interaction might apply for diverse compounds. However, structural differences among compounds were not manipulated gradually and systematically, so the studies were not ideal to draw conclusions regarding differences among compounds.

A subsequent study, which also included vapor-phase calibration, examined interactions among four homologous carboxylic acids (*16*). Full psychometric functions were measured for acetic (C_2), butyric (C_4), hexanoic (C_6), and octanoic acids (C_8). Full psychometric functions were also measured for C_2 mixed with C_4 (difference of two methylene units), for C_2 mixed with C_6 (difference of four units), and for C_2 mixed with C_8 (difference of six units). An additivity model (see Materials and Methods) was applied to psychometric functions for the unmixed acids to predict psychometric functions for the binary mixtures. Level dependence only occurred for C_2 mixed with C_4. For this pair that differed by only two methylene units, summation was subadditive at higher concentrations and additive (or perhaps even supraadditive) at lower concentrations. For the mixtures that differed by four and six methylene units, summation was essentially additive across the full range of measured concentrations. Thus, close structural similarity within an aliphatic series may be required to observe nonadditive interactions.

The current study tests the hypothesis that carbon chain length is an important factor in determining interactions among compounds that differ more in structure, that is, between members of an aliphatic series and compounds outside that series. Model stimuli will again include aliphatic carboxylic acids (C_2, C_4, C_6, and C_8). Stimuli will also include three common flavor compounds that differ greatly from the acids in both molecular structure and suprathreshold odor character, namely, 2-hydroxy-3-methylcyclopent-2-en-1-one, furan-2-ylmethanethiol, and (3-methyl-3-sulfanylbutyl) acetate. If very close structural similarity is required to observe nonadditive interactions, then mixtures of acids and other compounds should all show additive interactions. On the other hand, if carbon-chain length is important in general, then there should be systematic differences among the carboxylic acids in how they sum with various flavor compounds.

MATERIALS AND METHODS

Subjects. Twelve healthy, nonsmoking adults (seven female, five male) participated. Ages ranged from 22 to 47 years. All provided written informed consent on forms approved by an Institutional Review Board at the University of Pennsylvania. All experiments were conducted in compliance with the appropriate laws and institutional guidelines. 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 had extensive experience in previous experiments using the same procedures and apparatus used in the current study.

Materials. Stimuli included four aliphatic, carboxylic acids: acetic acid (CAS Registry No. 64-19-7, Nagase ChemteX Corp., Osaka, Japan, 99.7% pure), butyric acid (CAS Registry No. 107-92-6, Daicel Chemical Industries, Ltd., Tokyo, Japan, 99.6% pure), hexanoic acid (CAS Registry No. 142-62-1, Chisso Corp., Tokyo, Japan, 98.5% pure), and octanoic acid (CAS Registry No. 124-07-2, Inoue Perfumery Co., Ltd., Tokyo, Japan, 97.3% pure). Stimuli also included three common flavor compounds: 2-hydroxy-3-methylcyclopent-2-en-1-one (commonly called maple lactone, abbreviated here as ML, CAS Registry No. 80-71-7, Toyotama International Inc., Tokyo, Japan, 98.3% pure), furan-2-ylmethanethiol (commonly called furfuryl mercaptan, abbreviated here as FM, CAS Registry No. 98-02-2, Oxford Chemicals Limited, 95.1% pure), and (3-methyl-3-sulfanylbutyl) acetate (MSA, CAS Registry No. 50746-09-3, Ogawa & Co., Ltd., 99.2% pure). GC-MS (see Olfactometer and Calibration) was used to verify that all stimuli met or exceeded manufacturer claims regarding purity.
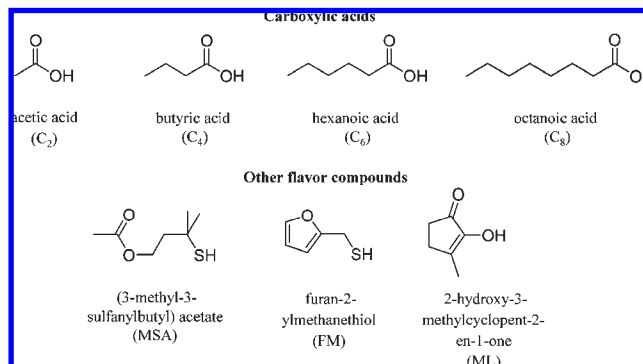
Figure 1. Molecular structures of the odor materials used in the experiment.

Table 1. Vapor-Phase Concentrations of Stimulus Materials (log ppm by Mass)

dilution	compound[a]						
	C_2	C_4	C_6	C_8	ML	MSA	FM
5	−2.86	−4.34	−3.46	−3.27	−3.00	−5.57	−6.74
4	−2.51	−3.99	−3.11	−2.93	−2.67	−5.23	−6.40
3	−2.17	−3.63	−2.76	−2.60	−2.35	−4.89	−6.06
2	−1.82	−3.28	−2.41	−2.27	−2.02	−4.55	−5.71
1	−1.47	−2.93	−2.06	−1.94	−1.70	−4.20	−5.37
0	−1.12	−2.58	−1.71	−1.60	−1.37	−3.86	−5.03

[a] C_2, acetic acid; C_4, butyric acid; C_6, hexanoic acid; C_8, octanoic acid; MSA, (3-methyl-3-sulfanylbutyl) acetate; FM, furan-2-ylmethanethiol; ML, 2-hydroxy-3-methylcyclopent-2-en-1-one.

The acids are homologous compounds that share the same functional group, but differ in number of methylene units in the base chain (**Figure 1**). The other three compounds are diverse in structure. ML has a sweet, maple syrup aroma. FM has a roasted, coffee aroma. MSA has a fruity odor. Both FM and MSA are thiols and share a slight sulfurous note. All compounds are found in foods and beverages, either naturally or as added flavors.

Subjects received a six-step dilution series of each unmixed compound (**Table 1**). Successive concentration steps differed by a factor of about 2.2. In addition, subjects received a six-step dilution series of each acid paired with each of the other three compounds. For example, acetic acid was mixed with ML (C_2–ML), with MSA (C_2–MSA), and with FM (C_2–FM). Accordingly, subjects received 12 mixtures in total. Concentrations of individual compounds were the same in binary mixtures as they were when presented alone. For example, the dilution series for C_2–ML consisted of the lowest concentration step of C_2 added to the lowest step of ML, the second lowest step of C_2 added to the second lowest step of ML, etc., up to the highest step of each mixture component. Extensive pilot work suggested 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. All stimuli were presented using an automated, air dilution olfactometer constructed in our laboratory. A more detailed description of the olfactometer is available at http://chemse.oxfordjournals.org/cgi/content/full/bjp062/DC1. Briefly, nitrogen flowed through odor vessels containing pure chemicals (powdered ML was diluted with Milli-Q filtered water at 0.01 g/mL before being placed in an odor vessel). Odorized nitrogen was mixed with filtered air to create a six-step dilution series of each stimulus. Chemical mixtures were formed by combining nitrogen streams from two separate odor vessels, that is, in vapor phase, before subsequent air dilution. All concentrations were generated continuously and vented out of the room when not presented to subjects. Electronic valves could gate any of the six concentrations, or a clean air blank, to a glass cone. Subjects sampled by placing their noses in the cones. The olfactometer provided a total flow of 30 L/min at output to allow subjects to sniff without inhaling room air.

Flow from the olfactometer was collected in 4.7 L Tedlar gas sampling bags. Analytical sensitivity was enhanced by collecting odor molecules

using solid-phase microextraction (SPME). SPME fibers used for collection were 2 cm, 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/carboxen/PDMS) "Stableflex" fibers (Supelco Corp., Bellefonte, PA). Odorants in the Tedlar bags were then sampled by inserting the tapered end of the SPME fiber holder through a septum on the sampling bags and extending the SPME fiber into the sample for 45 min.

To desorb and analyze the collected sample, the SPME fiber containing the adsorbed odorant was inserted into the hot injection port of a Voyager GC-MS system (Thermoquest/Finnigan, San Jose, CA). The GC-MS system was equipped with Xcalibur software (version 1.2, ThermoElectron Corp., San Jose, CA). A Stabilwax column, 30 M × 0.32 mm with 1.0 μm coating (Restek Corp., Bellefonte, PA), was used for separation and analysis of the odorants collected from all samples. The following chromatographic protocol separated components: 60 °C hold for 4 min, followed by a temperature ramp of 6 °C/min to 230 °C with an 8 min hold at this final temperature. Accordingly, each run lasted 42.3 min before the instrument recycled and readied itself for the next run. The injection port was set at 230 °C. Helium carrier gas was used at a constant column flow rate of 2.5 mL/min throughout the analysis. Data acquisition and operating parameters for the mass spectrometer were set as follows: scan rate, 2 scans/s; scan range, m/z 40−200; ion source temperature, 200 °C; and an ionizing energy, 70 eV.

An initial calibration for a particular condition (i.e., a particular single compound or mixture) occurred before any subjects were tested under that condition. Samples of all concentrations were collected in Tedlar bags. SPME samples were taken within 24 h of filling the bags. After absorption, SMPE samples were submitted to GC-MS analysis as soon as possible (within minutes). "Spot check" samples were collected during psychophysical testing to verify stability over time (three or four samples each day, randomly selected concentrations). These spot check samples, collected between subjects or during breaks were also analyzed within 24 h of initial collection.

To convert GC peak areas to parts per million (ppm; by mass), a liquid dilution series of each stimulus compound (in chloroform) was used to make gas-phase standards. Liquid standards were injected into Tedlar bags filled with nitrogen and allowed to evaporate in the bags overnight. The resulting standards were sampled using SPME fibers, with the same procedure used to quantify samples from the olfactometer, namely, 45 min of sampling before desorption in the injection port of the GC-MS system.

Calibration yielded three important results with respect to interpretation of the psychophysical data. First, 2.2-fold air dilutions in the olfactometer 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. A previous paper describes the general procedure in greater detail (*16*). In brief, we used a 2 of 5, forced-choice procedure. Subjects received two identical odors and three clean-air blanks (in random order) each trial. We used a 2 of 5 procedure because it has a lower percent correct by chance (10%) than the more commonly used 2-alternative (50%) and 3-alternative (33%) forced-choice procedures. Lower chance performance allows more stable estimates of proportion correct for mixtures in the low perithreshold range with fewer trials collected per subject. Odor pulses lasted 2.5 s, with 3 s pauses between pulses. Subjects knew that exactly two stimuli in each trial were odors and were required to identify the two odor samples, guessing if uncertain. After the initial presentation of the five stimuli each trial, subjects were allowed to resample the stimuli if they wished (preliminary work suggested that resampling helped optimize detection performance). At least 15 s elapsed between trials.

During an experimental session (about 40 min) subjects received six presentations of each of six concentrations (see **Table 1**) of a fixed stimulus. The stimulus could be either a pure compound or a binary mixture (see Stimulus Materials for more detail). Subjects received the stimuli in blocked, ascending order of concentration. Subjects received the lowest concentration on the first three trials, the next lowest in the next three trials, and so forth up to the highest concentration. After a break of at least 5 min, the sequence was repeated, again starting with the lowest concentration. Subjects received each binary mixture and each single compound in two sessions, for a total of 12 trials per condition. The 19 stimuli, 7 unmixed compounds and 12 binary mixtures, were tested in blocked,
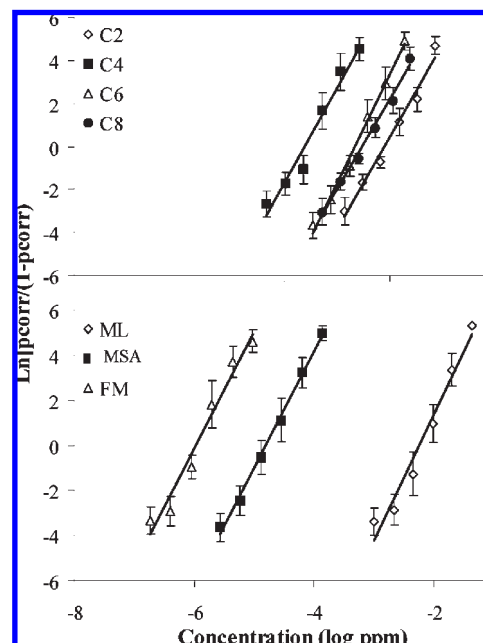
Figure 2. Psychometric functions for single (unmixed) compounds: *Y*-axis, log-odds ratio of chance-corrected proportion correct (averaged across subjects); *X*-axis, stimulus concentration in log ppm (by mass). The lines represent best fit linear functions (least-squares regression). Error bars represent ± SEM.

random order (although subjects needed to reschedule sessions on occasion and thereby deviated from a strictly random order).

Data Analysis. Data analysis has been described in more detail in a previous paper (*16*). In brief, experimenters computed proportion correct for each subject, then applied a correction for chance so that performance ranged from 0 (chance-level ability to identify the two odors among the three blanks) to 1 (perfect ability to detect the two odors): chance-corrected proportion correct = (raw proportion correct − 0.10)/(1 − 0.10). 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. We chose this transform because pilot work showed that cumulative logistic functions fit detection data better than other sigmoidal forms. Finally, the log-odds ratio of chance-corrected proportion was averaged across subjects for each compound and concentration and plotted against log concentration to form psychometric functions. Transformation made psychometric functions approximately linear, so functions could be fit using least-squares linear regression.

Patterns of mixture interaction were compared with probability summation, that is, the assumption that detecting a binary mixture equals the probability of detecting one or both of the components: $p(AB) = p(A) + p(B) − p(A)p(B)$, 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 (*17*). Within the framework of the model, if detection performance for the mixture falls below probability summation, some degree of suppression has occurred relative to statistical independence. If performance falls above probability summation, then some form of mutual enhancement, or synergy, has occurred.

Linear fits to psychometric functions for individual subjects were used to generate individual predictions of probability summation for each binary mixture. Probability summation predictions were compared to observed psychometric functions for binary mixtures using repeated measures analysis of variance (ANOVA) models, with appropriate correction degrees of freedom to compensate for violations of sphericity.

RESULTS

Psychometric Functions for Unmixed Compounds. Proportion correct increased smoothly with concentration, with good linear fits in the coordinate space used (R^2 ranged from 0.97 to 0.99, see **Figure 2**). Fits to data from individual subjects ranged from
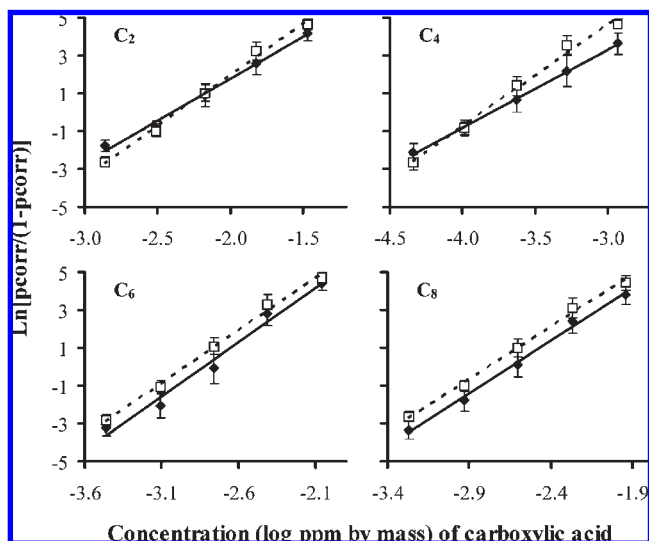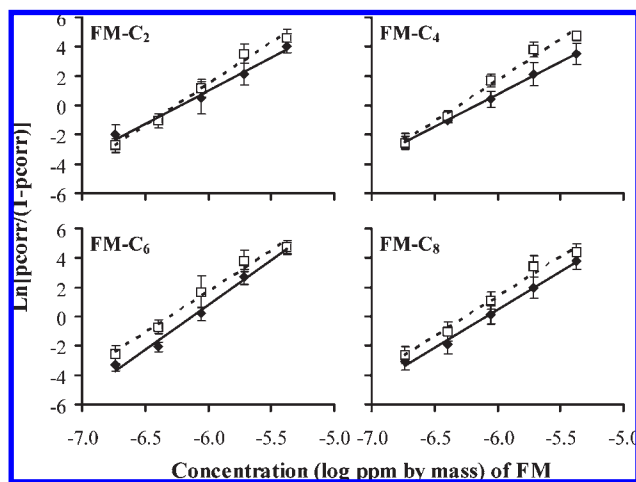
Figure 3. Psychometric functions for mixtures that included four aliphatic carboxylic acids: *Y*-axis, log-odds ratio of chance-correct proportion correct; *X*-axis, concentration of carboxylic acid in mixtures in log ppm by mass. Each acid was mixed with three other unrelated odor compounds. For each carboxylic acid, data from three series of mixtures were averaged to depict general trends in mixture interactions with other compounds. Solid symbols (solid lines) represent observed detection data. Open symbols (dashed lines) represent additivity predictions based on detection functions for individual components. Error bars represent ± SEM.

0.55 to 0.99 (mean = 0.83, SD = 0.11). In short, cumulative logistic functions fit the data reasonably well. For average data, detection of all compounds rose from near chance to essentially perfect with an increase in concentration of 1.7 log units (50-fold increase). Slopes of psychometric functions were relatively similar across compounds: In a concentration step × compound ANOVA, the interaction failed to reach significance ($p > 0.20$). Thresholds, that is, the concentrations that would lead to detection performance halfway between chance-level and 100% correct, are not a central focus of this study, but may have archival value: -2.09 log ppm for C_2, -3.62 log ppm for C_4, -2.66 log ppm for C_6, -2.51 log ppm for C_8, -5.98 log ppm for FM, -4.81 log ppm for MSA, and -2.25 log ppm for ML.

General Analysis of Detection Data for Binary Mixtures Compared to Additivity Predictions Based on Detection of Unmixed Compounds. As an initial step, detection data for all binary mixtures, together with corresponding predictions based on probability summation of detection data for the unmixed components, were submitted to a repeated-measures ANOVA. Note that, because predicted detection of binary mixtures was close to perfect for the second-highest concentrations, data for the highest concentrations were not analyzed. ANOVA factors follow: concentration step (1−5) × carboxylic acid (C_2, C_4, C_6, and C_8) × aroma compound (FM, MSA, and ML) × data type (observed detection for binary mixtures vs predicted values).

Most main effects failed to reach significance, with the notable exception of concentration step, $F(4, 44) = 146.74$, $p \ll 0.0001$, demonstrating an expected dose−response relationship. Most interactions also failed to reach significance, including all interactions involving aroma compound. Thus, the analysis provided no evidence that ML, MSA, and FM differed greatly in their mixture interactions with the carboxylic acids. In contrast, the three-way interaction between carboxylic acid, concentration, and data type reached significance, $F(9.8, 108.1) = 2.22$, $p < 0.03$, suggesting that mixture interactions differed among the carboxylic acids (**Figure 3**).
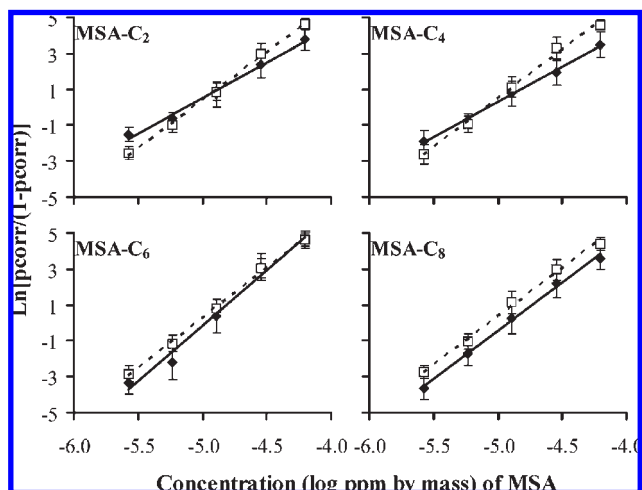

Figure 4. Psychometric functions for four aliphatic carboxylic acids mixed with furan-2-ylmethanethiol (FM): *Y*-axis, log-odds ratio of chance-correct proportion correct; *X*-axis, concentration of FM in mixtures in log ppm by mass). Solid symbols (solid lines) represent observed detection data. Open symbols (dashed lines) represent additivity predictions based on detection functions for individual components. Error bars represent ± SEM.

Simple, concentration step × data type ANOVAs on data for each carboxylic acid (averaged across aroma compounds) revealed significant main effects of data type for C_6, $F(1, 11) = 5.67$, $p < 0.001$, and for C_8, $F(1, 11) = 6.53$, $p < 0.01$, but no significant main effects of data type for C_2 or C_4 ($p > 0.14$). The analysis also revealed significant concentration step × data type interactions for C_2, $F(3.4, 38.9) = 6.25$, $p < 0.001$, and for C_4, $F(3.1, 34.0) = 4.39$, $p < 0.01$, but no significant interaction for C_6 or C_8 ($p > 0.30$). Thus, short-chain carboxylic acids (C_2 and C_4) demonstrated additive, or perhaps even supra-additive, mixture summation with the aroma compounds at low concentration steps, but subadditive mixture summation at higher concentration steps. In contrast, medium-chain (C_6 and C_8) carboxylic acids demonstrated subadditive mixture summation at all concentration levels tested.

Analysis of Data for Each Binary Mixture. Although the overall ANOVA suggested that mixture interactions were similar across the three model aroma compounds (FM, MSA, and ML), it is possible that averaging across the compounds might produce patterns of interaction that are not present in data from individual aroma compounds. Thus, data for each binary mixture were submitted to concentration step × data type ANOVAs (**Figures 4−6**). Inspection of **Figures 4−6** suggests patterns of mixture interaction generally consistent with those seen for data averaged across aroma compounds (**Figure 3**).

For mixtures including FM (**Figure 4**), the interaction between concentration and data type reached significance for FM mixed with C_2, $F(3.7, 41.2) = 6.89$, $p < 0.001$, and for FM mixed with C_4, $F(3.4, 37.9) = 3.22$, $p < 0.03$, but failed to reach significance for FM mixed with C_6 or C_8 ($p > 0.25$). Main effects of data type failed to reach significance, but there were trends toward subadditive interactions for FM mixed with C_6, $F(1, 11) = 4.26$, $p = 0.06$, and for FM mixed with C_8, $F(1, 11) = 4.19$, $p = 0.07$. Thus, analysis yielded significant interactions for mixtures with short-chain acids and trends toward main effects for the medium-chain acids, in good general agreement with analysis of data averaged across the three aroma compounds.

For mixtures including MSA (**Figure 5**), the interaction between concentration and data type reached significance for MSA mixed with C_2, $F(4, 44) = 4.28$, $p < 0.01$, and for MSA mixed with C_4, $F(2.8, 31.2) = 3.80$, $p < 0.03$, but failed to reach

Figure 5. Psychometric functions for four aliphatic carboxylic acids mixed with (3-methyl-3-sulfanylbutyl) acetate (MSA): *Y*-axis, log-odds ratio of chance-correct proportion correct; *X*-axis, concentration of MSA in mixtures in log ppm by mass). Solid symbols (solid lines) represent observed detection data. Open symbols (dashed lines) represent additivity predictions based on detection functions for individual components. Error bars represent ± SEM.
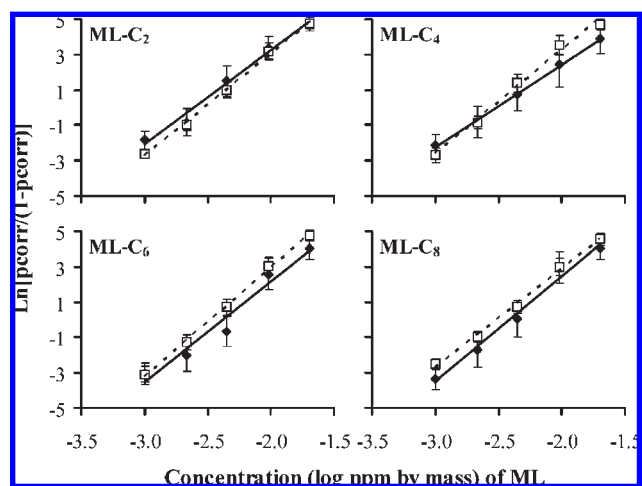
Figure 6. Psychometric functions for four aliphatic carboxylic acids mixed with 2-hydroxy-3-methylcyclopent-2-en-1-one (ML): *Y*-axis, log-odds ratio of chance-correct proportion correct; *X*-axis, concentration of ML in mixtures in log ppm by mass). Solid symbols (solid lines) represent observed detection data. Open symbols (dashed lines) represent additivity predictions based on detection functions for individual components. Error bars represent ± SEM.

significance for MSA mixed with C_6 or C_8 ($p > 0.65$). The main effect of data type reached significance for MSA mixed with C_8, $F(1, 11) = 6.31$, $p < 0.03$, but failed to approach significance for the mixtures with the other three carboxylic acids ($p > 0.35$). Again, the pattern of significant interactions for mixtures with short-chain acids, and general subadditivity for one of the medium-chain acids, is roughly consistent with analysis of data averaged across the three aroma compounds.

For mixtures including ML (**Figure 6**), the main effect of data type reached significance for ML mixed with C_6, $F(1, 11) = 5.16$, $p < 0.05$. For all other mixtures that included ML, both the main effect of data type and the interaction failed to reach significance ($p > 0.30$). Although statistical analysis suggested weaker deviations from additivity for mixtures of ML and carboxylic acids,

inspection of **Figure 6** does not reveal any gross qualitative differences in patterns of interaction with respect to those seen for mixtures including FM or MSA (but see Limitations).

DISCUSSION

Interaction among Mixture Components. Overall, mixture interactions did not deviate drastically from additivity. In broad terms, this finding agrees with a growing body of literature showing summation among perithreshold odorants: Concentrations of individual chemicals in a threshold level mixture tend to fall below individual threshold concentrations (see, e.g., refs *7−16* and *18*).

Mixture summation also occurs in detection of perithreshold tastes (*19*). Because perithreshold additivity occurs among bitter, sweet, sour, and salty stimuli that are known to stimulate diverse taste receptors (*20*), some more central mechanisms are able to function as general detectors of sapid stimuli, independent of kind. It is becoming increasingly clear that olfaction functions in a similar fashion. Unlike taste, we cannot yet predict whether a given pair of odorants will stimulate nonoverlapping groups of receptors because the molecular receptive ranges of most vertebrate olfactory receptor proteins have not been characterized. Furthermore, physiological studies have found both olfactory receptor neurons (peripheral mechanisms) and neurons in the brain (central mechanisms) that respond to diverse odorants and, therefore, constitute possible substrates for detection mechanisms that integrate across diverse odorants (*21−25*).

Regardless of specific mechanism, a general tendency toward mixture summation has practical significance. For example, it could prove to be very difficult to identify the source of an odor in a food, beverage, or personal product when the target is a mixture of components having little or no perceptual impact on their own (discussed in ref *6*). Mixture summation might also have implications for odor complaints in indoor air.

Effect of Concentration and Compound on Degree of Summation. In the suprathreshold (clearly perceptible) range, binary mixtures often smell less intense than the sum of the intensities of their unmixed components, even when one accounts for the compressive nature of psychophysical functions (*26−28*). Furthermore, suprathreshold summation is more complete when the unmixed components are of relatively low intensity (*26, 29*). The first rigorous studies that examined detection of perithreshold mixtures at different concentrations suggested that summation is more complete in the low perithreshold range than in the high perithreshold range (*14, 15*). Accordingly, suprathreshold subadditivity may begin at high perithreshold concentrations in some cases (*15, 16*).

Subsequent work at the perithreshold level showed that patterns of summation also depended on the compounds that comprise the mixture (*16*). Specifically, Wise and colleagues found that aliphatic carboxylic acids that differed by two methylene units showed more complete summation at weaker concentrations than at higher concentrations but that pairs of acid that differed by four or six methylene units showed essentially additive interactions across a wide range of concentrations (*16*). This work suggested that structural similarity, at least within an aliphatic series, influences how odors sum in mixture. Previous work at the suprathreshold level also found substantial differences in summation among odor pairs, but did not involve systematic variation of molecular parameters (reviewed in ref *26*).

In the current study, both acetic and butyric acid showed more complete summation at lower concentrations than at higher concentrations when paired with structurally dissimilar molecules. For hexanoic and octanoic acids, subadditive interactions were seen across a wide range of concentrations. To the best of

our knowledge, the data on mixtures that include C_6 and C_8 constitute the first evidence of a reduction from (simple) additivity across a wide range of concentrations generated using vapor-phase calibration and rigorous psychophysical methods. More generally, these results suggest that carbon chain length may be an important factor in mixture interactions between carboxylic acids and at least some compounds outside this aliphatic series.

One might conclude that close structural similarity is not important in mixture interactions. However, because both olfactory receptor neurons and cells in the brain may respond to molecules from more than one chemical class, carbon chain length cannot be the only factor that determines functional similarity (*30*, *31*). In other words, what appears to be structurally dissimilar to the chemist may not be structurally dissimilar to the olfactory brain. Further work, continuing to use rigorous psychophysical methods and vapor-phase calibration of stimuli, is needed to generate and refine hypotheses regarding structure–activity relationships in mixture interactions.

Regardless, both the current results and the previous work on mixtures of carboxylic acids (*16*) suggest that carbon chain length might be an important factor in mixture interactions at the perithreshold level. This hypothesis is consistent with other psychophysical work that has found systematic effects of carbon chain length, including work on suprathreshold cross-adaptation and discrimination (*32*, *33*). Furthermore, work in physiology suggests that at least some olfactory receptor proteins (and, thus, the olfactory receptor neurons that express these proteins) are tuned to carbon chain length to some extent (*34*). Many cells in the olfactory bulbs are also tuned to carbon chain length, although tuning is narrower than in olfactory receptor neurons (*35*). In short, future structure–activity studies of mixture interactions should continue to consider carbon chain length as a potentially important factor.

Limitations. We used stimuli of the highest obtainable purity within practical limits. We verified purity using GC-MS. Still, it is possible (although unlikely) that some trace compounds, present in concentrations below instrument sensitivity, might have influenced the results.

In addition, the sample of test compounds is limited. The finding that short-chain carboxylic acids seemed to interact differently with other compounds than did medium-chain acids might not generalize to other aliphatic series. Even for carboxylic acids, the finding might not generalize to interactions with other aroma compounds. Furthermore, we cannot rule out differences among the three aroma compounds we tested. The overall ANOVA described under General Analysis of Detection Data for Binary Mixtures Compared to Additivity Predictions Based on Detection of Unmixed Compounds suggested that differences among carboxylic acids were more reliable than differences among the three aroma compounds. Thus, an examination of the data averaged across the three aroma compounds (**Figure 3**) suggests a testable hypothesis: Carbon chain length is important for mixture interactions between carboxylic acids and other compounds. However, with a sample of 12 subjects, our data may not provide enough power to distinguish more subtle differences among the three aroma compounds we tested. In general, firm conclusions regarding structure–activity relationships in mixture interactions must await additional data.

Furthermore, stimuli included only balanced mixtures, that is, mixtures of components that roughly matched with respect to detection probability. Other research, using some of the same stimuli that this study employed, suggests that relative proportion of components in mixtures can strongly affect degree of additivity (*18*). Finally, work on higher order mixtures (mixtures of three or more components) will eventually be required to understand processing of natural mixtures. In short, more work will be required to fully elucidate the rules that govern mixture interactions in the detection of odors.

ABBREVIATIONS USED

C_2, acetic acid; C_4, butyric acid; C_6, hexanoic acid; C_8, octanoic acid; FM, furan-2-ylmethanethiol; ML, 2-hydroxy-3-methylcyclopent-2-en-1-one; MSA, (3-methyl-3-sulfanylbutyl) acetate.

SAFETY

No unusual risks were involved that required special precautions beyond standard laboratory safety procedures.

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