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BRIEF COMMUNICATIONS

A Critical Test of the Overlap Hypothesis for Odor Mixture Perception

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The overlap hypothesis of mixture perception is based on the observation that mixtures of perceptually similar odorants tend to smell different from their components (configural), whereas mixtures of dissimilar odorants smell like their components (elemental). Because input patterns of perceptually similar odorants tend to overlap more than dissimilar ones, it has been hypothesized that component pattern overlap can predict a mixture's perceptual quality, with high overlap predicting a configural response and low overlap an elemental response. The authors used 7 pairs of odorants chosen for different degrees of overlap in their monomolecular 2-deoxyglucose activation patterns to test the theory in a go/no-go behavioral assay that measured generalization from binary mixtures to components. The authors show that individual component odorant input patterns are not sufficient to predict mixture quality, falsifying the overlap hypothesis. An important finding is that different odorant pairs with similar glomerular overlap showed opposite behavioral–perceptual responses, suggesting nonlinear effects at the receptor or glomerular level or the critical involvement of higher order areas. Thus, the authors posit that imaging the mixtures themselves may provide additional information needed to reliably predict mixture quality.

Keywords: configural perception, elemental perception, synthetic perception, odor mixtures, 2-deoxyglucose imaging

Binary odorant mixtures can smell different from (configural) or like (elemental) their components. At the first stage of olfactory processing, odorants bind to receptors located on olfactory receptor neurons, which appear to express a single receptor type (Mombaerts, 1999, 2004); are broadly distributed within the olfactory epithelium (Schoenfeld & Cleland, 2005); and converge with other like receptor neurons onto the same glomeruli, which are spherical neuropil structures located in the superficial layer of the main olfactory bulb (Mombaerts et al., 1996). This convergence creates an activation map with the signal-to-noise ratio being further increased by a juxtaglomerular network (Aungst et al., 2003; Chen & Shepherd, 2005).

The topography created by olfactory receptor convergence poses the following question: Are olfactory bulb spatial input patterns sufficient to predict perceptual qualities of odors or their mixtures? Patterns of 2-deoxyglucose (2DG) have been used to predict perceptual similarities among monomolecular odorants (Johnson & Leon, 2007; Linster et al., 2001). However, when it comes to mixture quality, some recent studies have suggested that higher level interactions may be necessary and input patterns may not be sufficient to predict perceptual qualities (Grossman, Mallik, Ross, Kay, & Issa, 2008; Kay, Crk, & Thorngate, 2005; Linster & Cleland, 2004). In this study, we tested whether complete component input maps can predict a behavioral–perceptual response to binary mixtures.

Perception and discrimination of binary mixtures of monomolecular odorants represent a more complex problem than monomolecular odorants. One hypothesis of odor mixture perception was originally derived from observations of perceptual similarities among odorants. When similar odorants are combined, subjects often do not recognize the components, but when exposed to mixtures of different odorants, they often recognize the components (Linster & Smith, 1999; Wiltrout, Dogra, & Linster, 2003). Mixtures of chemically similar odorants can also show configural effects, and mixtures of chemically different odorants can show elemental effects (Kay et al., 2005; Kay, Lowry, & Jacobs, 2003; Linster & Smith, 1999; Wiltrout et al., 2003; Wise, Miyazawa, Gallagher, & Preti, 2007). Because odorants that smell alike often have similar glomerular activation patterns, the theory has been extended to hypothesize that similarities between input maps can predict configural perceptual quality in mixtures, with dissimilarity predicting elemental quality (Grossman et al., 2008; Linster & Cleland, 2004; Mandairon, Stack, & Linster, 2006). The hypothesis predicts that when combined in a binary mixture, odorants with highly similar activation patterns should produce a percept in which neither component smells like the mixture, termed *configural* or *synthetic*. At the opposite end of the spectrum, a binary mixture of two components with highly dissimilar activation pat-

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terns should smell like both individual components, termed *elemental*.

Building on past results in our laboratory, we explicitly tested the hypothesis in rats, using complete 2DG olfactory bulb activation maps of the individual components. Each of seven pairs of odorants tested had approximately equal theoretical vapor pressures and a degree of overlap at the glomerular layer from small (~0.0) to large (>0.7, complete overlap = 1.0; see Figure 1). We trained rats to respond (press a lever) to a binary mixture for a reward and then tested generalization of that response to the mixture components. Generalization to and recognition of a component is inferred from the number of trials in which a rat responds to that component significantly more than to an unrelated control odorant. We show that the input patterns of odorant mixture components cannot accurately predict mixture quality (i.e., elemental vs. configural behavioral–perceptual response). Therefore, we conclude that odor mixture quality is not the result of the linear sum of component input patterns.

Method

Subjects

Eight adult male Sprague–Dawley rats (300– 400 g; purchased from Harlan HSD, Madison, WI) were used for all behavioral tests, except for (+)/(–)-limonene (n = 7) and (+)-limonene/(+)-limonene (n = 4). Rats were dieted to 85% of their ad libitum weights prior to initial training and all test sessions. While not being tested, the rats were returned to ad libitum diets and were redieted prior to additional tests. Rats were housed in a 14:10-hr light–dark cycle (lights on at 0900 CST), with testing conducted during the light cycle in a dimly lit room. All experimental methods were approved and done under veterinary supervision and oversight by the University of Chicago IACUC in accordance with AAALAC standards.

Odor Sets

Odor sets were selected to produce a spectrum from high (Odor Sets 1, 2; (+)limonene/(–)limonene and propyl propionate/ethyl butyrate, respectively) to slight overlap (Odor Sets 6, 7; isoamyl butyrate/butyric acid and hexanal/ethyl benzene) and were drawn from odorants previously published (Johnson, Farahbod, & Leon, 2005; Johnson, Farahbod, Saber, & Leon, 2005; Johnson, Farahbod, Xu, Saber, & Leon, 2004; Johnson et al., 2002; see also http://leonserver.bio.uci.edu/; see Figure 1). Calculated overlap values were determined by a matrix correlation of individual component patterns (odorant A to odorant B), based on activation as seen in pixel intensity (Johnson et al., 2004). Control (CS–) odorants were not related to test odorants.

Odorants were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Atlanta, GA): acetone (99.5%), amyl acetate (98%), butanone (99+%), butyric acid (99+%), cumene (99.9%), cycloheptane (98%), cyclohexanone (99.8%), diacetyl (97%), ethyl butyrate (99%), ethyl 2-methylbutyrate (99%), ethylbenzene (99+%), hexanal (98%), isoamyl butyrate (99+%), (+)-limonene (97%), (–)-limonene (96%), methyl acetate (99.8%), methyl salicylate (99+%), nonanone (99+%), octanol (99.8%), propyl propionate (99%), and 1-proponol (99.8%). Ethyl 2-methylbutyrate, methyl acetate, diacetyl, and acetone were diluted to a 1% solution with mineral oil prior to being placed in their respective test tubes. All other odorants were undiluted in test tubes.

Behavioral Task

All behavioral training and test sessions were conducted in the same operant conditioning chamber. Behavioral session events and scoring were controlled by MedPC-IV software (Med Associates, St. Albans, VT) on a PC (Windows XP Professional SP2), interfaced by Med Associates hardware. The task was the same as that previously validated (Kay, Krysiak, Barlas, & Edgerton, 2006) and described below (see *Partial reinforcement paradigm*). Odorants were delivered to the odor port through a solenoid manifold with individual odorants kept in separate test tubes. Equal parts of saturated vapor from each component odorant were combined in a carrying tube, which ran for ~0.25 m, before being injected—just before the odor port—into a plain airstream, which ran continuously throughout each session, creating an approximately 1:4 dilution of binary mixture to clean air. Individual component odorants and the CS– (control) odorants were delivered at a concentration of approximately 1:8, saturated vapor:air.

Illumination of a house light marked the beginning of a trial within sessions. At this time, a nose poke in the odor port tripped a photobeam detector activating odor delivery. Rats were trained to lever press for the CS+ mixture (mixture AB; go) to receive a 45-mg sucrose pellet (Research Diets, Inc., New Brunswick, NJ), except for unrewarded trials in the partial reinforcement paradigm (see below), and to withhold lever pressing for the CS– (monomolecular odorant C; no go). For the CS–, if no response (lever press) was made during a 10-s window, the house light was extinguished and an intertrial interval of 15 s commenced. If the rat pressed the lever for the CS– during the 10-s window, the house light was immediately extinguished and a penalty of 10 s was added to the normal intertrial interval. Rats were neither rewarded nor punished for a lever press for a component odorant. A lever press for a component is the behavioral–perceptual response marker for generalization of the mixture to the pressed component.

A partial reinforcement paradigm was implemented in test sessions of 250 trials. The first 40 trials of a session were classified as training for the rat to learn the odor set for that session. The remaining trials were for testing generalization to the component odorants. We used a partial reinforcement reward schedule, which dispensed a sucrose pellet on only two thirds of the correct CS+ trials (pseudorandomly drawn without replacement in blocks of 24 trials), so that with 75% CS+ and 25% CS– trials, the rats could be rewarded on 50% of the total trials. This method allowed for component odorant substitution in the unrewarded CS+ trials after the initial 40 training trials without any change in the amount of reinforcement the rats could receive. After the first 40 trials, the 25% of total trials that previously were unrewarded CS+ trials were divided approximately in thirds, so that each corresponded to ~8.3% of the remaining 210 trials—one third each (17–18 trials) for the mixture, component odorant A, and component odorant B trials (with none of these trials reinforced).

Rats were trained to high accuracy (≥ 90%) on a training set of CS+ mixture (anisole, odorant A; nonanone, odorant B) and CS– (amyl acetate) prior to beginning testing. After the initial training set, rats were tested on a different odor set in each session, one

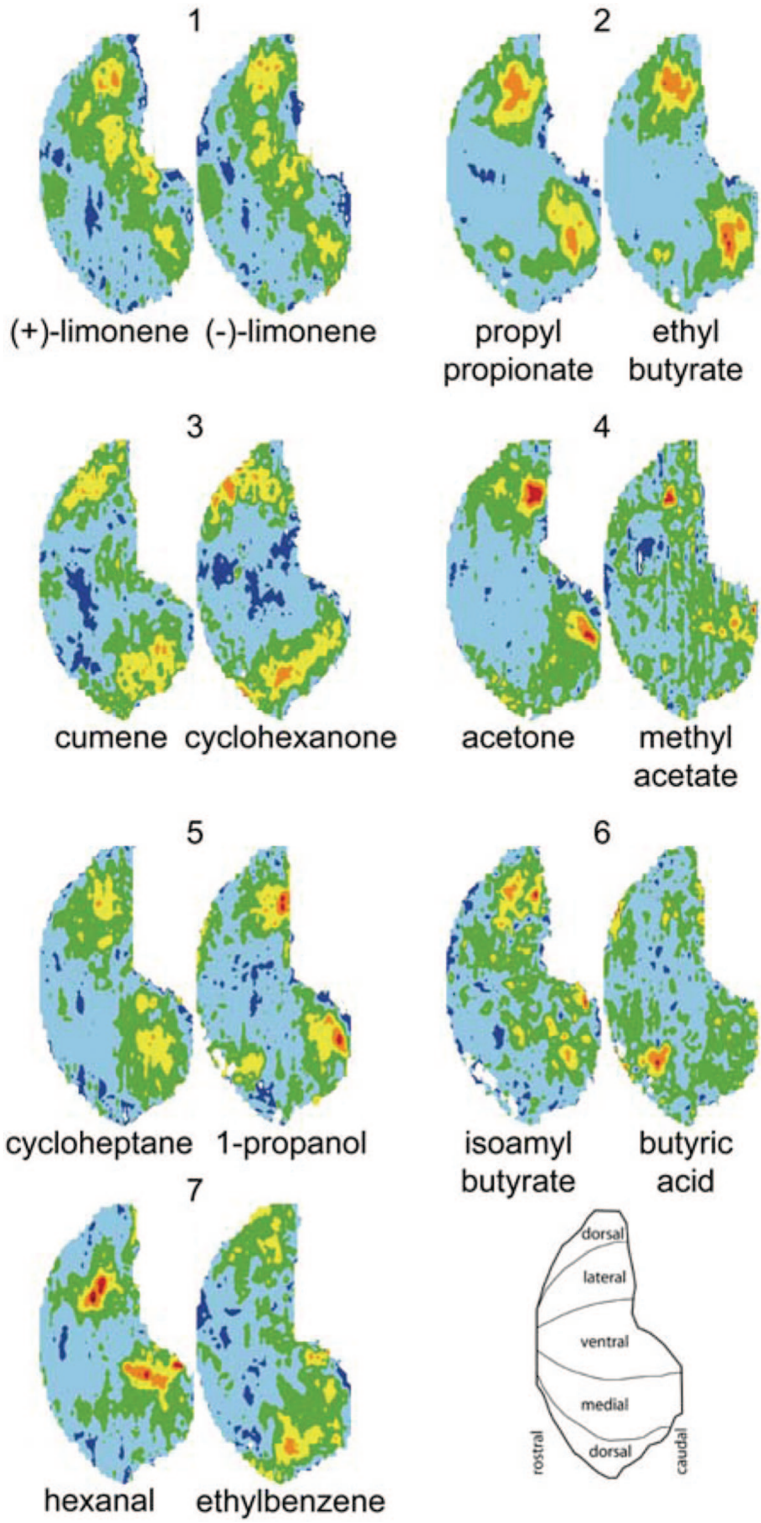


Figure 1. 2-deoxyglucose (2DG) activation maps of components (odorants A, left; odorants B, right). Notice descending correlation between components from Odor Set 1 to Odor Set 7. Odor Set 1: (+)-limonene (p-limonene), (–)-limonene (m-limonene), $r = .74$; Odor Set 2: propyl propionate, ethyl butyrate, $r = .79$; Odor Set 3: cumene, cyclohexanone, $r = .46$; Odor Set 4: acetone, methyl acetate, $r = .24$; Odor Set 5: cycloheptane, 1-propanol, $r = .23$; Odor Set 6: isoamyl butyrate, butyric acid, $r = -.05$; Odor Set 7: hexanal, ethylbenzene, $r = .05$. (Figures from Leon Lab and available at http://leonserver.bio.uci.edu/, used with permission.)

session on a given day, with the order of tests balanced across animals. Odorant solenoids were counterbalanced so that half the rats received odorants A and B through solenoids designated A and B. The other half received the odorants driven by the opposite solenoids (B and A) to control for possible differences in flow or auditory cues through the two solenoids. If a rat performed at less than 90% accuracy for the CS+/CS– responding in a given session, that odor set was repeated at a later date. All subjects reached criterion on all odor sets.

Data Analysis

A lever press was used as the metric for determining whether or not a subject perceived a presentation as like the mixture. By using the presence or absence of a lever press, we could construct two categories: "like mixture" and "not like mixture." The trained CS– was designed to be the standard for "not like mixture." Responding significantly more than to the CS– indicated that an odorant was in the "like mixture" category, whereas responding below this level indicated it was "not like mixture."

We used a criterion-based method for filtering data, validated previously (Kay et al., 2006). Because the lever is in the arena during the session and it is easily pressed (low resistance), subjects sometimes press the lever either unintentionally (due to grooming and other behaviors) or as an apparent afterthought, as has been seen in other go/no-go tasks with relatively mild negative reinforcement for wrong answers (e.g., Kay & Laurent, 1999). To count only those lever presses for which the subject mistook the odorant for the CS+ mixture, we used a simple filter based on latency to press. We calculated the mean and standard deviation of the latency to lever press for the unrewarded CS+ trials for each animal for each odor set and set a 95% confidence interval. All trials that fell within the confidence interval were regarded as those for which the rat mistook an odorant for the CS+ mixture and were used for filtered analysis.

Data analyses were conducted with StatView (SAS Institute, Cary, NC). An analysis of variance (ANOVA) omnibus null hypothesis was tested for each odor set to confirm that the variances were homogeneous. A Bonferroni–Dunn post hoc test was then performed for each odor set to determine statistically significant differences pairwise among means. The Bonferroni–Dunn test sets statistical significance at $p = .0083$ ($\alpha = .05$) for any given pairwise contrast.

Results

Generalization to Component Odorants

The overlap hypothesis of binary mixture perception predicts that mixtures of odorants that have highly overlapping input patterns should show a configural behavioral–perceptual response, whereas mixtures of odorants that overlap very little should show an elemental response, with those in between showing various levels of responding. We tested generalization patterns for seven binary mixtures chosen for component overlap ranging from near zero to greater than 70% overlap (see Figure 1). Within each pair, odorant volatility was approximately equal to avoid confounds due to differences in airborne concentration within an odor pair. Any threshold for the degree of overlap predicting elemental over configural responses is arbitrary. We assumed, however, that odorants at the extremes (highest and lowest overlap) should conform to the hypothesis. Furthermore, odorant pairs with similar overlap should show similar response patterns, and this was a primary assessment for evaluating whether the patterns themselves were sufficient to explain component recognition.

After training to associate each CS+ mixture with a reward, rats responded significantly to each CS+ over the CS– and over each of the two component odorants. We tested responses to component odorants over the CS– to assess configural versus elemental responding. To make sure that a lever press was intentional, we used a filter wherein we constructed a 95% confidence interval for latency to lever press, as we have done previously (Kay et al., 2006). Even so, only two odor sets showed a difference in outcome between filtered and unfiltered data (see Figure 2).

Odor Sets 1 and 2 have the highest overlap in 2DG activation patterns (0.74 and 0.79, respectively) and were predicted to produce configural behavioral–perceptual responses. For odor set 1—(+)-limonene/(–)-limonene—an elemental response was observed, with rats responding significantly to both components. For Odor Set 2, a configural response was observed, with rats responding to neither component, although the unfiltered data show a marginal response to ethyl butyrate (ethyl butyrate∗control: mean difference = 0.239, critical difference = 0.234, $p = .0073$, significant).

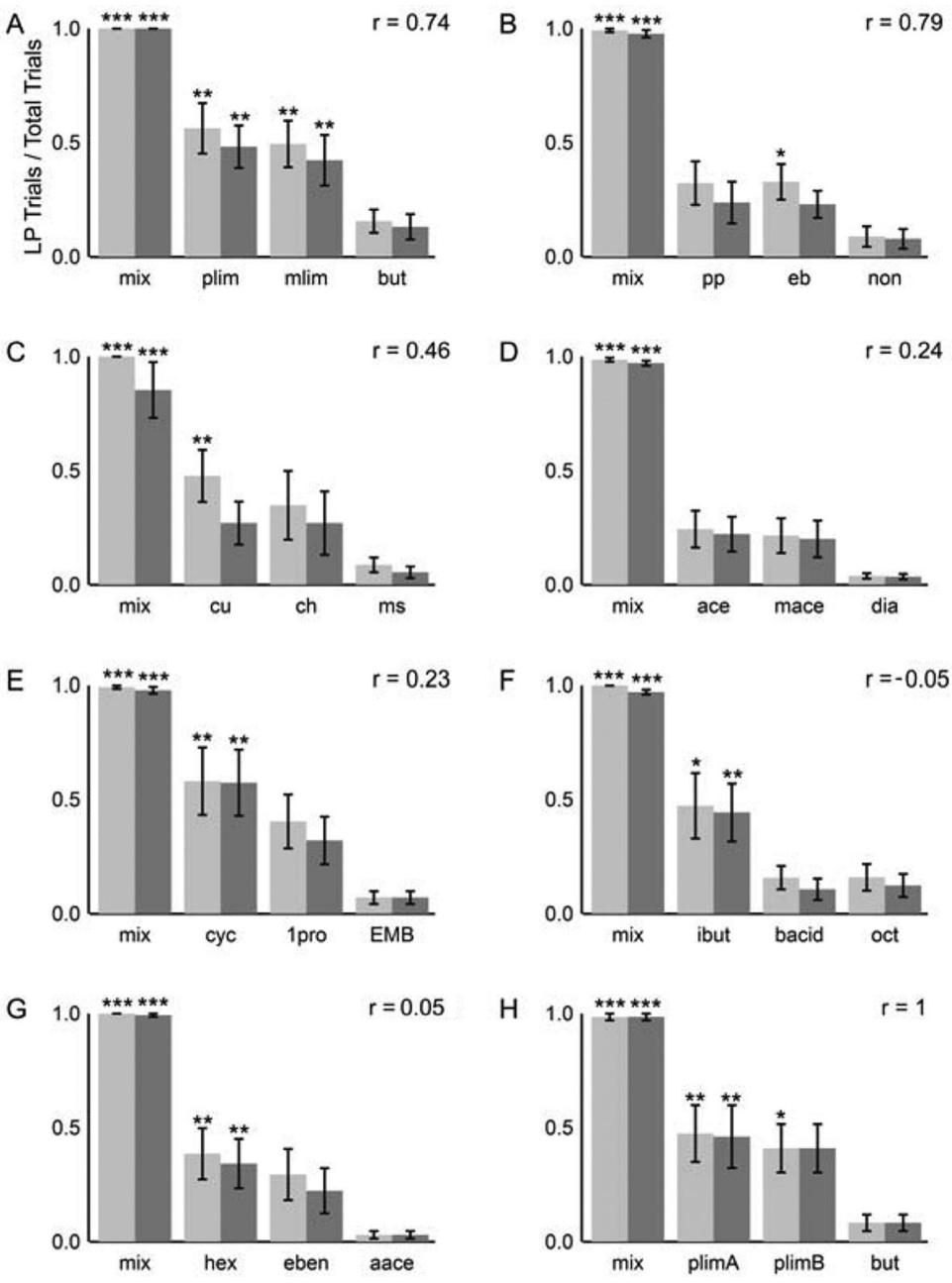
Odor Sets 3–5 had overlap values between the extrema, so the hypothesis predicted only that the responses should fall somewhere in between configural and elemental. For Odor Set 3 (cumene/cyclohexanone, overlap, $r = .46$), before filtering, a slight overshadowing was observed, with cyclohexanone overshadowed by cumene. A configural response was revealed when the data were filtered. For Odor Sets 4 and 5 (acetone/methyl acetate and cycloheptane/1-propanol, overlap, $r = .24$ and $r = .23$, respectively), we found overshadowing responses. For Odor Set 4, rats responded significantly to acetone and not to methyl acetate. For Odor Set 5, rats responded significantly to cycloheptane and not to 1-propanol.

Odor Sets 6 and 7 have close to zero overlap (isoamyl butyrate/butyric acid and hexanal/ethylbenzene, $r = -.05$ and .05, respectively), so the hypothesis predicted elemental responses to the mixture components. Both odor sets showed overshadowing responses. For Odor Set 6, the rats responded significantly to octanol, but not to butyric acid over CS–. For Odor Set 7, the rats responded significantly to hexanal but not to ethylbenzene.

In summary, the most overlapping pairs (Odor Sets 1 and 2) did not produce the same type of response patterns (elemental for Odor Set 1 and configural for Odor Set 2). Odor Sets 2 and 3 (with filtering) and Odor Set 4 (with and without filtering) were the only instances of true configural responses, and Odor Set 1 (high overlap +/– limonene) was the only odor set producing a true elemental response.

Overlap–Generalization Correlation

It is possible that the binary nature of our assessment (elemental vs. configural) influenced the results. We therefore computed a post hoc generalization metric (average response to components/average response to the mixture) to determine whether there might be a generalization magnitude continuum correlated with overlap



(see Figure 3). There was no correlation between the two measures overall (Figure 3A, solid line). When restricting the correlation estimate to only those trials in which the difference in responding to the two odorants was within 1 standard deviation of zero (Figure 3B), we found a weak positive correlation between generalization to components and pattern overlap (Figure 3A, dotted line). The hypothesis predicted a negative correlation. In addition, this significant correlation was driven by four rats' elevated responses to the (+)-limonene/(–)-limonene components. When this odor set is removed, the correlation is again insignificant (Figure 3A, dashed line). We therefore conclude that there is no correlation between input pattern overlap and generalization magnitude.

Concentration Effects

The logical extension of the tested overlap hypothesis predicts that at the most extreme level of overlap (100%), a mixture of an odorant with itself should result in an odor that smells different from itself. The predicted absurdity draws attention to the issue of concentration effects that might contribute to the perceptual similarities and differences between mixtures and their components (McNamara, Magidson, & Linster, 2007). Rats trained on a binary mixture of (+)-limonene/(+)-limonene ((+)-limonene in each of the two odor tubes) showed no difference in responding to either (+)-limonene (unfiltered: $p = .5048$; filtered: $p = .622$). Rats

responded significantly to plimA ((+)-limonene through solenoid A) over control for both filtered and unfiltered conditions but significantly to plimB ((+)-limonene through the B solenoid) only for the unfiltered data. (The filter reduced the number of lever presses by one for one rat; mean difference = 0.326; critical difference = 0.333, $p = .0094$, *ns*). For both conditions, there was no statistical difference between the two components (plimA∗plimB); we therefore conclude that response to plimB is like the response to plimA. Responding to each component was roughly half that of the CS+ mixture, which suggests a concentration effect (Figure 2H).

Discussion

Mixtures of large numbers of odorants tend to produce synthetic percepts (Jinks & Laing, 2001), and this gives rise to the notion that mixtures are synthetic odors. Several studies have now shown that laboratory rodents and humans do discern individual components within some, but not all, binary odor mixtures (Cometto-Muniz, Cain, & Abraham, 2005; Kay et al., 2003, 2005; Wiltrout et al., 2003). Theory is needed to be able to predict which odorants in mixture will give rise to elemental and which to configural effects and to allow for additional strong hypotheses that can then be explicitly tested.

Derived from configural and overshadowing perceptual responses to some mixtures of chemically or perceptually similar odorants and odorants that are known to activate overlapping receptor populations, one hypothesis posits that if two components have similar input patterns, then their mixture should smell different from the components (Jinks & Laing, 2001; Kay et al., 2003, 2005; Linster & Cleland, 2004; Linster & Smith, 1999; Livermore & Laing, 1998a, 1998b; Wiltrout et al., 2003). In contrast, if two components have dissimilar input patterns, the theory predicts that their mixture should smell like both components. This theory was based on a small number of odor sets and had not been previously tested using odorants for which complete input patterns were known. To test this hypothesis, we used odor sets for which we had complete 2DG glomerular layer response patterns to predict the qualitative properties of these odorants in mixture. We compared behavioral generalization to component odorants to responses predicted by their input patterns.

The monomolecular patterns did not accurately predict binary mixture quality in very high and very low overlap mixtures, where clear predictions could be formed. It is possible that the reinforcement nature of the task itself may contribute to larger differences between a mixture and its components, and this would favor a configural response (Linster, Johnson, Morse, Yue, & Leon, 2002). Thus, it is also important to examine responses among pairs of odorants with similar amounts of overlap. Odorant pairs with similar overlap could produce different, even opposite, behavioral–perceptual responses, as in the case of the high overlap in Odor Sets 1 and 2. We also examined whether a continuous generalization metric might correlate with input pattern overlap and found no evidence for a predicted negative correlation (see Figure 3).

If input pattern overlap cannot reliably predict mixture quality, then this suggests that odorants' input patterns do not necessarily sum linearly in binary mixtures, that inhibitory or higher order effects may exist at the receptor or glomerular level in mixtures, or that higher order areas are implicated. One case in which inhibitory action has been reported at the receptor level involves two chem-

Figure 2. (*opposite*) Proportion of trials for which rats lever pressed in response to presented odorants. From left to right, sets of two bars represent means ± *SEM* of unrewarded mixture, component A, component B, and control. Light gray bars are unfiltered data, and dark gray bars are filtered data (outside the 95% confidence interval in latency to lever press). Bonferroni–Dunn post hoc tests were applied to each odor set (unfiltered and filtered independently). For this test, contrasts between two treatments (e.g., mixture and control) are said to be significant at $\alpha = .05$ when the corresponding $p \leq .0083$ ($^{*}p \leq .0083$; $^{**}p \leq .005$; $^{***}p \leq .0001$). Correlations (*r* values) in each panel represent the amount of overlap between the corresponding pair of 2-deoxyglucose (2DG) patterns in Figure 1. Following are means and 95% confidence interval (constructed as mean ± 2∗*SEM*) presented as abbreviation (full name). A. Odor Set 1: Unfiltered: mix (+limonene/–limonene) = 1 (1,1), +lim (+limonene) = 0.563 (0.341, 0.785), –lim (–limonene) = 0.494 (0.29, 0.698), but (butanone) = 0.156 (0.054, 0.258). Filtered: mix (+limonene/–limonene) = 1 (1,1), +lim (+limonene) = 0.482 (0.389, 0.575), –lim (–limonene) = 0.423 (0.312, 0.534), but (butanone) = 0.132 (0.077, 0.187). B. Odor Set 2: Unfiltered: mix (propyl propionate/ethyl butyrate) = 0.992 (0.976, 1.008), pp (propyl propionate) = 0.323 (0.133, 0.513), eb (ethyl butyrate) = 0.328 (0.172, 0.484), non (nonanone) = 0.089 (−0.001, 0.179). Filtered: mix (propyl propionate/ethyl butyrate) = 0.977 (0.961, 0.993), pp (propyl propionate) = 0.238 (0.147, 0.329), eb (ethyl butyrate) = 0.23 (0.171, 0.289), non (nonanone) = 0.079 (0.036, 0.122). C. Odor Set 3: Unfiltered: mix (cumene/cyclohexanone) = 1 (1, 1); cu (cumene) = 0.478 (0.25, 0.706); ch (cyclohexanone) = 0.349 (0.049, 0.649); ms (methyl salicylate) = 0.088 (0.022, 0.154). Filtered: mix (cumene/cyclohexanone) = 0.853 (0.731, 0.975); cu (cumene) = 0.271 (0.177, 0.365); ch (cyclohexanone) = 0.271 (0.132, 0.41); ms (methyl salicylate) = 0.055 (0.029, 0.081). D. Odor Set 4: Unfiltered: mix (acetone 1%/methyl acetate 1%) = 0.986 (0.968, 1.004); ace (acetone 1%) = 0.245 (0.083, 0.407); mace (methyl acetate 1%) = 0.216 (0.064, 0.368); dia (diacetyl 1%) = 0.039 (0.015, 0.063). Filtered: mix (acetone 1%/methyl acetate 1%) = 0.971 (0.96, 0.982); ace (acetone 1%) = 0.223 (0.147, 0.299); mace (methyl acetate 1%) = 0.202 (0.122, 0.282); dia (diacetyl 1%) = 0.036 (0.024, 0.048). E. Odor Set 5: Unfiltered: mix (cycloheptane/1-propanol) = 0.993 (0.979, 1.007); cyc (cycloheptane) = 0.581 (0.285, 0.877); 1pro (1-propanol) = 0.404 (0.168, 0.64); EMB (ethyl 2-methylbutyrate 1%) = 0.071 (0.015, 0.127). Filtered: mix (cycloheptane/1-propanol) = 0.979 (0.964, 0.994); cyc (cycloheptane) = 0.574 (0.429, 0.719); 1pro (1-propanol) = 0.321 (0.217, 0.425); EMB (ethyl 2-methylbutyrate 1%) = 0.071 (0.043, 0.099). F. Odor Set 6: Unfiltered: mix (isoamyl butyrate/butyric acid) = 1 (1, 1); but (isoamyl butyrate) = 0.158 (0.056, 0.26); bacid (butyric acid) = 0.473 (0.185, 0.761); oct (octanol) = 0.16 (0.044, 0.276). Filtered: mix (isoamyl butyrate/butyric acid) = 0.971 (0.96, 0.982); ibut (isoamyl butyrate) = 0.444 (0.317, 0.571); bacid (butyric acid) = 0.107 (0.06, 0.154); oct (octanol) = 0.124 (0.074, 0.174). G. Odor Set 7: Unfiltered: mix (hexanal/ethylbenzene) = 1 (1, 1); hex (hexanal) = 0.482 (0.296, 0.668); eben (ethylbenzene) = 0.423 (0.201, 0.645); aace (amyl acetate) = 0.132 (0.022, 0.242). Filtered: mix (hexanal/ethylbenzene) = 0.993 (0.986, 1.000);hex (hexanal) = 0.343 (0.235, 0.451); eben (ethylbenzene) = 0.224 (0.125, 0.323); aace (amyl acetate) = 0.031 (0.014, 0.048). H. (+)-limonene/(+)-limonene mixture: Unfiltered: mix (+lim/+lim) = 0.98 (0.94, 1.02); plimA = 0.596 (0.526, 0.666); plimB = 0.51 (0.412, 0.608); but (butanone) = 0.113 (0.049, 0.177). Filtered: mix (+lim/+lim) = 0.98 (0.96, 1.000); plimA = 0.596 (0.561, 0.631); plimB = 0.51 (0.461, 0.559); but (butanone) = 0.113 (0.057, 0.169).

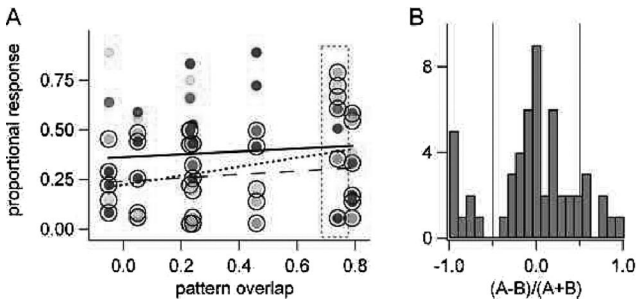


Figure 3. Generalization magnitude compared with pattern overlap. A. Generalization strength ([average response to components A and B]/ [response to the trained mixture]) is plotted against pattern overlap. Symbols indicate the eight rats. The solid fitted line is for the complete set of points ($n = 53$), and the correlation is not significant ($r = .09$, $p = .53$). The dotted line is the fit to the circled points, for which the difference between responses to components A and B is within 1 standard deviation of the average response, which is close to zero (see part B). This correlation is significant but small ($r = .32$, $p = .04$) and in the opposite direction from the prediction. Removing the odor set that appears to drive the correlation (Odor Set 1, overlap 0.74, (+)-limonene/ (–)-limonene, outlined by dashed rectangle) erases the weak correlation ($r = .14$, $p = .44$; dashed line). B. Distribution of A/B response differences is estimated from the differences measured by (response to component A – response to component B)/ (response to A + response to B). The average is −0.03, and vertical lines show the 1 standard deviation cutoff.

ically similar odorants that have opposite effects at the I7 receptor (Araneda, Kini, & Firestein, 2000). These effects predicted that, in combination with the primary ligand (octanal), they would have opposite perceptual effects, which was obtained in behavioral tests (Kay et al., 2003). Higher order mixture responses have recently been studied in anterior olfactory nucleus and piriform cortex unit responses (Kadohisa & Wilson, 2006; Lei, Mooney, & Katz, 2006) and in orbitofrontal cortex fMRI responses (Grabenhorst, Rolls, Margot, da Silva, & Velazco, 2007). In all of these studies, nonlinear effects have been seen when comparing binary mixture to component responses. Because in some of the cases we tested in this study overlap of mixture components was sufficient to predict behavioral effects, it is likely that inhibitory effects at the periphery may be negligible or that higher order areas may be simple linear processors of the olfactory bulb input patterns in these cases.

The logical extension of the overlap theory predicts the absurdity that an odorant combined with itself should not smell like itself. We tested this prediction and found that although rats responded significantly to the "component" odorants ((+)-limonene/(+)-limonene), they also responded significantly more to the "mixture," which had twice the concentration of the "components." So, why do perceptually or chemically similar odorants sometimes produce synthetic mixtures? It is possible that some results from mixtures of very similar odorants may be influenced by concentration effects, such that the mixture does smell qualitatively like the components but is significantly stronger than each of the components alone. Also, depending on the statistical measure (comparison to the mixture vs. an unrelated odorant), different conclusions may be drawn.

It should be noted that not all of the 2DG patterns were derived from the same airborne concentrations as our odorant samples. Because absolute concentration can play a role in odor perception (McNamara et al., 2007), it is possible that this is a factor in our results. However, the glomerular layer can normalize activation patterns, equalizing the effects of concentration in monomolecular patterns (Cleland, Johnson, Leon, & Linster, 2007), and our study tested whether these monomolecular patterns can predict the quality in combination. It is therefore possible that concentration effects on odor mixture quality may be driven by inhibitory effects at the receptor, glomerular, or higher order areas. When testing mixture components, a decision must also be made whether to examine objective (concentration) or subjective (perceptual threshold) odorant intensities. Human odor psychophysics often rely on the latter, testing fewer odor sets (Cometto-Muniz et al., 2005). Animal studies rely on the former but test many odor sets, primarily because threshold studies are extremely difficult in animals. Future studies should seek to reconcile these two types of methodologies.

In conclusion, we have shown that the current overlap theory is too simplistic to predict binary mixture qualities from individual odorant input patterns, which is consistent with results from intrinsic signal imaging of the dorsal surface of the olfactory bulb (Grossman et al., 2008). However, the individual component patterns may still be useful for some odor mixture questions, such as helping to predict inhibitory responses at the olfactory receptor level in combination with imaging of the mixture responses.

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