

Dissociable Codes of Odor Quality and Odorant Structure in Human Piriform Cortex
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1. Supplemental Experimental Procedures

a. Complementary Study (Group x Carbon Chain Length)

In order to test the robustness of our findings in the main study, namely, that neural representations of odor quality and odorant structure are coded within dissociable piriform subregions, we extended our hypothesis to another set of 8 odorants that systematically varied in molecular functional group and carbon-chain length: 4 alcohols (C-4 butanol, C-6 hexanol, C-8 octanol, C-10 decanol) and 4 aldehydes (C-4 butanal, C-6 hexanal, C-8 octanal, C-10 decanal) (Sigma-Aldrich) (**Fig. S3A**). Note this study was conducted by the same experimenters, using the same MRI scanner and equipment.

In this complementary study, an independent group of 18 healthy right-handed subjects (mean age, 25 years; 12 women) provided informed consent to participate in an olfactory paradigm of fMRI cross-adaptation that was approved by the local ethics committee. The experimental design was very similar to the main study. On each trial, subjects were cued to make sequential sniffs, smelling different combinations of odorants that varied in functional group and carbon

chain length. Stimulus pairs were arranged into six condition types: (1) same group/same length (corresponding to the identical-pair condition); (2) same group/2-carbon length difference; (3) same group/4-carbon length difference; (4) different group/same length; (5) different group/2-carbon length difference; (6) different group/4-carbon length difference. This conformed to a 2 x 3 factorial design (**Fig. S3B**), with the factors group (2 levels: same; different) and chain-length difference (3 levels: 0 carbon; 2 carbon; 4 carbon). The design was fully balanced, whereby each of the 8 odorants appeared equal numbers of times as 1st and 2nd stimulus in each of the 6 conditions, so that the effects could not be attributed to mere stimulus differences between odorants (e.g., odor intensity, valence, familiarity). There were 16 trials in each condition, with a stimulus-onset asynchrony of 28.6 s, for a total experimental duration of 46 minutes, divided into 2 fMRI sessions for subject comfort and to minimize olfactory fatigue.

The complementary study differed from the main study in terms of behavioral task: subjects provided odor quality ratings for each pair of odor stimuli (rather than making odor detection judgments), using a keypad to move a cursor along an online visual analog scale (anchors “extremely dissimilar” and “extremely similar”). Such a task enabled us to approximate the neural effect of odor quality, using each subject’s similarity ratings as parametric fMRI regressors of interest. The rating scale appeared with a random jitter (3.6 s – 14 s) following the second sniff of each trial and was displayed for 6.3 s on-screen. Note also that in contrast to the main study, the “no second odor” (blank) condition was not used, since the task was not contingent on the presence or absence of the 2nd odorant. For purposes of behavioral analysis (**Fig. S4**), the online similarity ratings for each stimulus pair were averaged across conditions and subjects, and then submitted to non-parametric statistical analysis in Matlab.

The fMRI data were acquired on the same MRI scanner and with the same sequence parameters that were used for the main study. Image pre-processing included spatial realignment, spatial normalization, and smoothing (6-mm full-width half-maximum). The event-related fMRI data were then analyzed in SPM2, following procedures outlined for the main study. The onset-times vectors for the six critical conditions, along with a 7th vector containing all 1st-odorant event onset times, were converted into delta functions, which were subsequently convolved with a canonical hemodynamic response function (HRF) (and its temporal and dispersion derivatives) to comprise the main regressors of interest. In addition, for each subject, a vector containing the onset times for all 2nd-odorant events was convolved with the canonical HRF and then parametrically modulated by the trial-specific odor similarity ratings (effectively, a trial-by-rating interaction). This regressor was entered into the fMRI model as a user-defined covariate in order to characterize the parametric effect of perceived odor quality. The effect sizes (betas, or parameter estimates) for each of the different event types were then estimated in SPM2 according to the General Linear Model (Friston et al., 1995).

In a subsequent step, we performed a region-of-interest (ROI) analysis of this data set, specifically based on the predictions (from the main study) that odor quality and odorant functional group would be coded in posterior and anterior piriform cortex, respectively. To this end, ROIs for the right anterior and left posterior piriform cortex were drawn using MRICro (Rorden and Brett, 2000), with reference to a brain atlas (Mai et al., 1997), by outlining these regions on each subject’s normalized T1-weighted high-resolution structural scan. These anatomical ROIs were functionally restricted to odor-responsive voxels from the main effect of

odor (at $P < 0.1$ uncorrected), on a subject-by-subject basis (after: Anderson et al., 2003; Winston et al., 2005). Finally, the condition-specific parameter estimates were averaged (by taking the arithmetic mean) across all voxels within each ROI for each subject. To test the effects of odorant structure, the parameter estimates for the six critical conditions were entered into a two (functional group)-by-three (carbon chain length difference) ANOVA. In turn, to test the effect of odor quality, the parameter estimate for the similarity regressor was entered into a two-tailed t test. Significance was set at $P < 0.05$. Note that in an ROI analysis of this type, there is no activation “coordinate” to report, since the effect is based upon the entire region of interest.

Finally, in order to illustrate the piriform response time-courses for the effects of functional group and carbon chain length (**Figs. 8A** and **8B**), we used an FIR basis set in a manner identical to that in the primary study (see Experimental Procedures, main text). On the other hand, as the effect of odor quality was defined parametrically (rather than as a canonical main effect), the corresponding time-course plots of quality (**Figs. 7B** and **7C**) were estimated using a separate FIR model, in which all of the odor trials, irrespective of group or length, were divided into quartiles of increasing perceptual similarity (that is, from most *dissimilar* to most *similar*). This approach yielded 24 events per quartile. Following data estimation, the stimulus-led responses for each quartile were plotted, and the mean area differences (2nd vs. 1st odorant) for each quartile were tested in a one-way ANOVA, following procedures outlined in the main text.

2. Supplemental Data: Complementary Study (Group x Carbon Chain Length)

a. Potential Influence of Odor Quality on the Effect of Functional Group

Because “different group” and “same group” conditions were perceived as qualitatively different (**Fig. S4**), it is possible that the effect of group is partially due to odor quality differences, rather than to group. While this cannot be completely discounted, we think it is unlikely for the following three reasons:

- 1) Subject-specific ratings of odor quality similarity were included in the fMRI model as user-defined regressors. The inclusion of this behavioral regressor should render the main effect of functional group independent of linear effects of odor quality, exemplified by the fact that as shown in **Fig. 7**, it effectively captured the neural effect of quality in posterior (but not anterior) piriform cortex. Importantly, as indicated in **Figs. 8A** and **8B**, the neural effect of functional group was identified in anterior (but not posterior) piriform cortex. The demonstration of an anatomical double dissociation verifies that this complementary fMRI study is able to distinguish odor quality and odorant structure (group) coding, irrespective of any possible co-linearity between these two factors.
- 2) If the comparison of “different group” and “similar group” actually reflected a quality-based difference between conditions, then we would have expected to see significant cross-adaptation in the “quality” area of posterior piriform cortex. **Fig. 8B** (top) shows that this was not the case. In fact, if anything, the condition associated with greater perceptual similarity (“same group,” red line) appears to evoke slightly higher activity than the less perceptually similar “different group”

condition (blue line), opposite to the predicted neural profile if quality were driving the effect. In like manner, similarity ratings of odor quality were greatest for odorant pairs that did not differ in carbon-chain length, yet the “0-C length difference” condition (black line) was associated with higher neural activity than either the 2-C or 4-C condition (**Fig. 8B**, bottom).

3) Since the predominant difference in odor quality similarity was carried in the “same group/0-C length” condition (corresponding to identical odorant pairs), we re-tested the group x length ANOVA in the absence of the 0-C conditions. This left 4 conditions (same group/2-C; same group/4-C; different group/2-C; and different group/4-C) for statistical analysis. These results are essentially identical to the main ANOVA, in that there was still a significant effect of functional group ($F_{1,17} = 4.64$; $P < 0.05$), but no effect of carbon-chain length difference ($F_{1,17} < 1$; $P = 0.81$) or group-by-length interaction ($F_{1,17} < 1$; $P = 0.99$). These findings indicate that the highly similar “same group/0-C condition” was not a principal factor underlying the effect of functional group in anterior piriform cortex.

Thus, while it remains possible that the main effect of molecular functional group might be partially attributed to differences in perceived odor quality between “different group” and “similar group” conditions, the above considerations make this less plausible.

b. Effect of Carbon Chain Length

Based on the available data from animal models of odor coding in olfactory receptor neurons and olfactory bulb, it was somewhat unexpected not to identify an effect for carbon-chain length in either anterior or posterior piriform cortex. To determine whether there were length-dependent effects outside of the predefined piriform ROIs, we also conducted a random-effects fMRI analysis across the whole brain. This approach identified significant cross-adapting responses only at reduced statistical threshold: in the left lateral amygdala (-30, -2, -28; $Z = 2.56$; $P = 0.005$ uncorrected) and at the border between olfactory tubercle and nucleus accumbens on the right (10, 4, -6; $Z = 2.41$; $P = 0.008$ uncorrected). While activity plots from these regions suggest that odorant pairs of the same length (0-C difference) elicited greater adaptation than pairs differing by 2-C in length (2-C difference), and pairs differing by 2-C in length elicited correspondingly greater adaptation than pairs differing by 4-C in length (4-C difference), we are hesitant to draw any conclusions from these findings, since they appear at such low threshold and in areas that were not predicted a priori.

There are several ways of interpreting this “negative” result. It remains possible that carbon-chain length is coded in piriform cortex, only in such a sparse way that fMRI is insensitive to this molecular feature, despite the use of adaptation techniques. Similarly, there may be much greater overlap in the neuronal populations activated by variations in carbon-chain length, so that fMRI cross-adaptation is simply unable to dissociate this parameter effectively. Alternatively, it is possible that length is a less critical structural determinant for odor coding at the level of olfactory cortex. This is not to say, however, that length necessarily plays no role in the perceptual basis (or the neural underpinnings) of odor quality – indeed, as shown in **Fig. S2**, the influence of carbon-chain length differences on the subjects’ similarity ratings of odor quality implies that this factor can contribute to olfactory discrimination. Perhaps the absence of a length effect in anterior piriform ultimately reflects the fact that from olfactory bulb to cortex, this particular structural feature is combined into a more complex representation, such that

length-based elements of the original stimulus are no longer accessible. It goes without saying that all of these suggestions are highly speculative and will require further investigation in future studies.

3. Supplemental Data: Main Study (Quality x Group)

a. Possible Effects of Quality on Structure Coding in Anterior Piriform Cortex

In the cluster analysis (**Fig. 3A**), it is notable that across functional group, the "lemon" odors are all more similar in quality than are the "vegetable" odors. Thus, fMRI effects in anterior piriform cortex evident in the comparison of *different* group to *same* group (main effect of odorant structure) could be partially due to the fact that in the *different* group condition, there is a high degree of *qualitative* dissimilarity. Therefore, a new fMRI model was estimated in which all *different* group/similar quality trials were divided into either lemon-containing trials (pairs 1:3, 1:4, 2:3, 2:4, and reverse – see **Fig. 2** for key) or vegetable-containing trials (pairs 5:7, 5:8, 6:7, 6:8, and reverse), yielding eight trials per condition. The prediction is that if perceptual quality is confounding the “structure” effect, then there should be more fMRI adaptation for *different* group/similar quality *lemon* pairs than for *different* group/similar quality *vegetable* pairs (notwithstanding item-specific differences).

At the anterior piriform “structure” peak (18, 10, -16), there was no significant difference in the magnitude of cross-adaptation between lemon pairs and vegetable pairs ($P = 0.26$) (**Fig. S2A**). This finding suggests that any difference in odor quality perception is not a driving factor in the effect of molecular functional group. Interestingly, at the posterior piriform “quality” peak (-22, 2, -22), there was a significant difference in fMRI cross-adaptation ($P = 0.035$), with the lemon pairs evoking greater response decrement than the vegetable pairs (**Fig. S2B**). This latter result provides a positive control for the anterior piriform analysis, by demonstrating that with as few as eight trials per condition, there was sufficient statistical power to detect a significant adaptation effect. Also, it once more confirms that odor quality is a critical factor influencing neural response properties in posterior piriform cortex.

b. fMRI Model of Molecular Properties

The experimental design of our primary experiment was fully balanced, such that any irrelevant molecular, chemical, or physicochemical determinants should effectively cancel out, leaving only the factor of interest (that is, molecular functional group). Nevertheless, we conducted a supplementary fMRI analysis to address the possibility that molecular determinants other than functional group could partially explain our main findings. For example, differences in membrane solubility or diffusion at the nasal epithelium, as a function of odorant hydrophobicity, could theoretically influence the extent to which the olfactory receptors show peripheral adaptation.

This analysis was identical to the primary fMRI model, except for the inclusion of three molecular “similarity” estimates as user-defined nuisance covariates. Molecular length (Å),

volume (\AA^3), and hydrophobicity ($\log P$) were calculated for each of the 8 odorants using Molecular Modeling Pro (ChemSW, Fairfield, CA). Differences in length, volume, and hydrophobicity were then computed for each odorant pair, on a trial-by-trial basis, followed by temporal convolution with a canonical HRF to form three event-related molecular covariates. These regressors were used to model out linear effects of length, volume, or hydrophobicity on the observed neural responses. Our motivation to include these particular molecular properties as linear regressors was based directly on the 2-DG data of Johnson and Leon ((Johnson and Leon, 2000); cf. their Fig. 4A), which show linear relationships between (a) the location of odorant-activated centroids in rodent olfactory bulb and (b) odorant molecular length, volume, and hydrophobicity. Therefore, we reasoned that if coding was similarly arranged in regions of piriform cortex, then a linear model would be sensitive to such an organization.

Despite the inclusion of these three “molecular” parametric regressors in the primary fMRI model, significant cross-adaptation was still detected in response to the main effects of quality repetition (posterior piriform: -20, 2, -22; $Z = 3.66$; $P < 0.05$ small-volume corrected [SVC]) and group repetition (anterior piriform: 18, 10, -16; $Z = 3.25$; $P < 0.05$ SVC). Thus, while the absence of linear effects does not prove these determinants might not have complex, higher-order effects on coding, these supplementary findings suggest that systematic variations in hydrocarbon features (besides functional group) do not easily explain the repetition effects of quality or structure in piriform cortex.

c. Test of Laterality Differences

As our main findings suggested prominent laterality differences between neural representations of quality (left piriform) and functional group (right piriform), we conducted hemisphere x condition analyses for both contrasts. In posterior piriform cortex, there was a significant hemisphere x condition (main effect of quality) interaction ($F_{1,15} = 14.67$; $P < 0.005$), whereas for anterior piriform cortex, the hemisphere x condition (main effect of group) interaction was not significant ($F_{1,15} < 1$; $P = 0.93$). These statistical findings were borne out by visual inspection of the contrasts at lower threshold ($P < 0.05$ uncorrected), which showed no indication for right posterior piriform activity in the main effect of quality, but did show left anterior piriform activity for the main effect of structure. This additional analysis suggests that structure codes are represented bilaterally, but that quality codes have a left-hemisphere bias. The latter finding is in keeping with the idea that quality-based information in left piriform cortex may depend upon inputs from higher-order brain areas, and in particular, upon cortical networks underlying language processing and verbal memory (functions that are typically left-lateralized).

d. Simple Comparisons between Alcohols/Aldehydes, Lemons/Vegetables

Our experimental design was fully balanced and all odorants appeared an equal number of times as 1st and 2nd stimulus across all trial types. This organization ensured that item-specific differences in perceived odor intensity, valence, familiarity, and pungency could not confound the main effects of odor quality and odorant structure (functional group). Nonetheless, it is interesting to ask whether simple comparisons between the differently categorized odorants (irrespective of the cross-adaptation effect) would also elicit dissociable codes of odor quality and odorant structure in piriform cortex. In a follow-up fMRI model, we divided the 1st-odorant

trials into four categories (lemon alcohols, lemon aldehydes, vegetable alcohols, vegetable aldehydes) and estimated these effects in SPM2 at the random-effects level (all alcohols vs. all aldehydes, and all lemon vs. all vegetable). Because this model disrupts the balanced nature of the original factorial design, the analysis is presented with the important qualification that the results could be confounded by item-specific perceptual differences across the comparisons of interest. The data indicate that even when examined at liberal thresholds ($P < 0.1$ uncorrected), there was no evidence for a tendency of fMRI spatial coding for odorant functional group (alcohols - aldehydes, or aldehydes - alcohols) at the 'structure' peak in anterior piriform cortex, nor for odor quality (lemon - vegetable, or vegetable - lemon) at the 'quality' peak in posterior piriform cortex. Although we are hesitant to draw any strong conclusions from these null results, these findings support our contention that fMRI cross-adaptation techniques provide a more sensitive means of delineating sensory coding mechanisms in human olfactory cortex.

4. Supplemental Figures

Figure S1

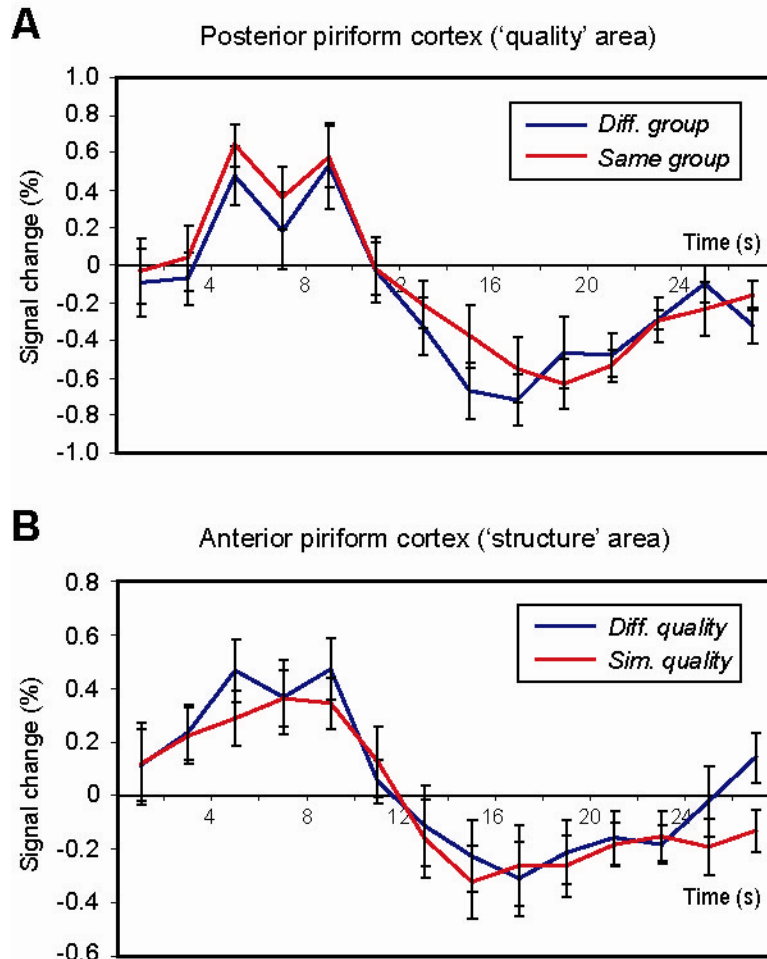


Figure S1. Additional response time-courses in piriform cortex, from the main experiment of quality vs. group. **(A)** The fMRI time-courses for different (diff.) and similar (sim.) structure are plotted from the 'quality' area in posterior piriform cortex and illustrate highly overlapping temporal response profiles. Confer **Fig. 4C** (main text) for the plots of different and similar quality from this region. These time-courses show that structure (functional group) has minimal (if any) impact on cross-adaptation in posterior piriform cortex. **(B)** In contrast, the fMRI time-courses for different and similar quality are plotted from the 'structure' area in anterior piriform cortex and also indicate a high degree of overlap between the waveforms. Confer **Fig. 6C** (main text) for the plots of different and similar structure from this region. These responses demonstrate that quality has little effect on cross-adaptation in anterior piriform cortex. Note, the area differences between 2nd and 1st odorants did not significantly differ between the conditions depicted in either **A** or **B**.

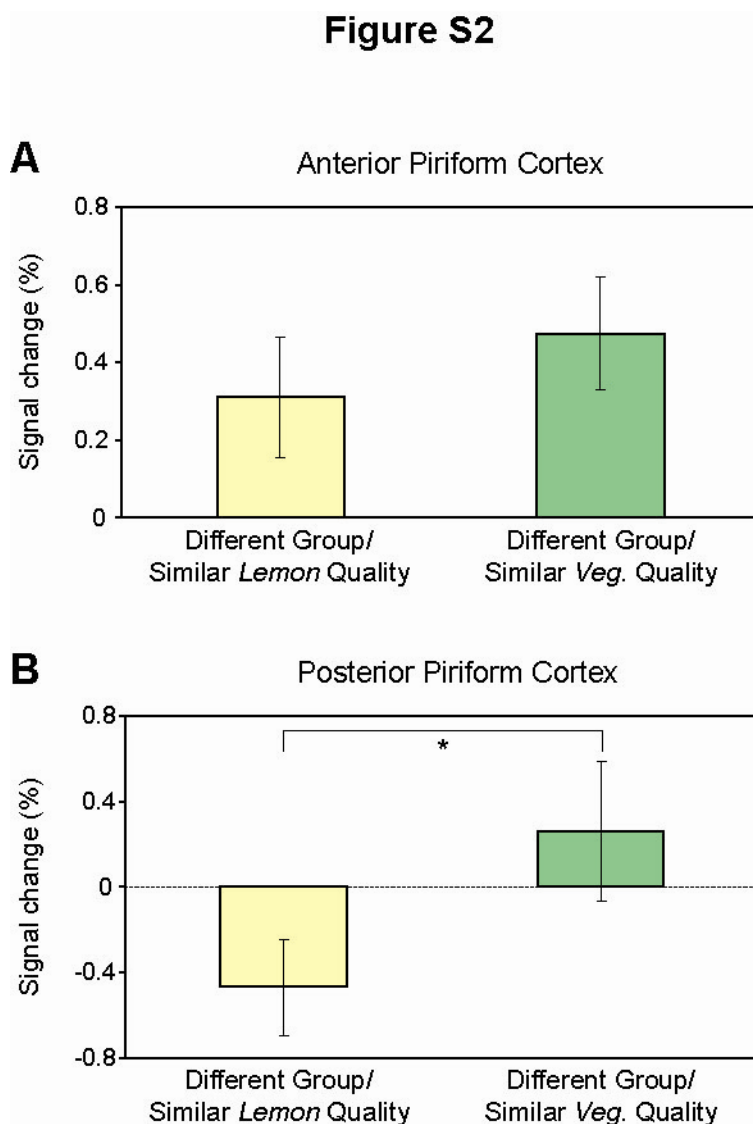


Figure S2. Odor quality is not a significant factor in odorant structure coding, from the main experiment of quality vs. group. **(A)** Plots show that the magnitude of fMRI cross-adaptation in anterior piriform cortex does not differ between the different group/similar *lemon* quality and the different group/similar *vegetable* quality conditions, despite the fact that the latter condition is perceptually more dissimilar, on the basis of the cluster analysis shown in Fig. 3A. Thus, odor quality does not appear to underscore the structure-based responses in anterior piriform cortex. **(B)** In contrast, in posterior piriform cortex, the different group/similar *vegetable* quality condition evokes significantly greater fMRI cross-adaptation than the *lemon* counterpart, consistent with the role of this region in odor quality coding. *, significant at $P < 0.05$.

Figure S3

A

		Functional Group	
		alcohol	aldehyde
Carbon Chain Length	4-C	butanol (4-OL)	butanal (4-AL)
	6-C	hexanol (6-OL)	hexanal (6-AL)
	8-C	octanol (8-OL)	octanal (8-AL)
	10-C	decanol (10-OL)	decanal (10-AL)

B

		Functional Group (of 2nd Odorant)			
		same		different	
Carbon Chain Length Difference (of 2nd Odorant)	0-C Δ	4-OL : 4-OL	4-AL : 4-AL	4-OL : 4-AL	4-AL : 4-OL
		6-OL : 6-OL	6-AL : 6-AL	6-OL : 6-AL	6-AL : 6-OL
		8-OL : 8-OL	8-AL : 8-AL	8-OL : 8-AL	8-AL : 8-OL
		10-OL : 10-OL	10-AL : 10-AL	10-OL : 10-AL	10-AL : 10-OL
	2-C Δ	4-OL : 6-OL	6-OL : 4-OL	4-OL : 6-AL	6-AL : 4-OL
		8-OL : 10-OL	10-OL : 8-OL	8-OL : 10-AL	10-AL : 8-OL
		4-AL : 6-AL	6-AL : 4-AL	4-AL : 6-OL	6-OL : 4-AL
		8-AL : 10-AL	10-AL : 8-AL	8-AL : 10-OL	10-OL : 8-AL
	4-C Δ	4-OL : 8-OL	8-OL : 4-OL	4-OL : 8-AL	8-AL : 4-OL
		6-OL : 10-OL	10-OL : 6-OL	6-OL : 10-AL	10-AL : 6-OL
		4-AL : 8-AL	8-AL : 4-AL	4-AL : 8-OL	8-OL : 4-AL
		6-AL : 10-AL	10-AL : 6-AL	6-AL : 10-OL	10-OL : 6-AL

Figure S3. Experimental design of the complementary experiment of group vs. carbon chain length. **(A)** The eight odorants systematically differed in molecular functional group (alcohol or aldehyde) and carbon chain length (4-, 6-, 8-, or 10-carbon length). **(B)** Odorants were arranged into discrete pairs, conforming to a two-way factorial design, with the factors of functional group (two levels: same; different) and carbon chain length difference (three levels: 0; 2; 4). Importantly, each odorant appeared equal numbers of times as 1st and 2nd stimulus in each condition, such that the design was fully balanced. Note that in order to fulfill this criterion, certain odorants were never paired with each other (e.g., 4-ol with 10-ol).

Figure S4

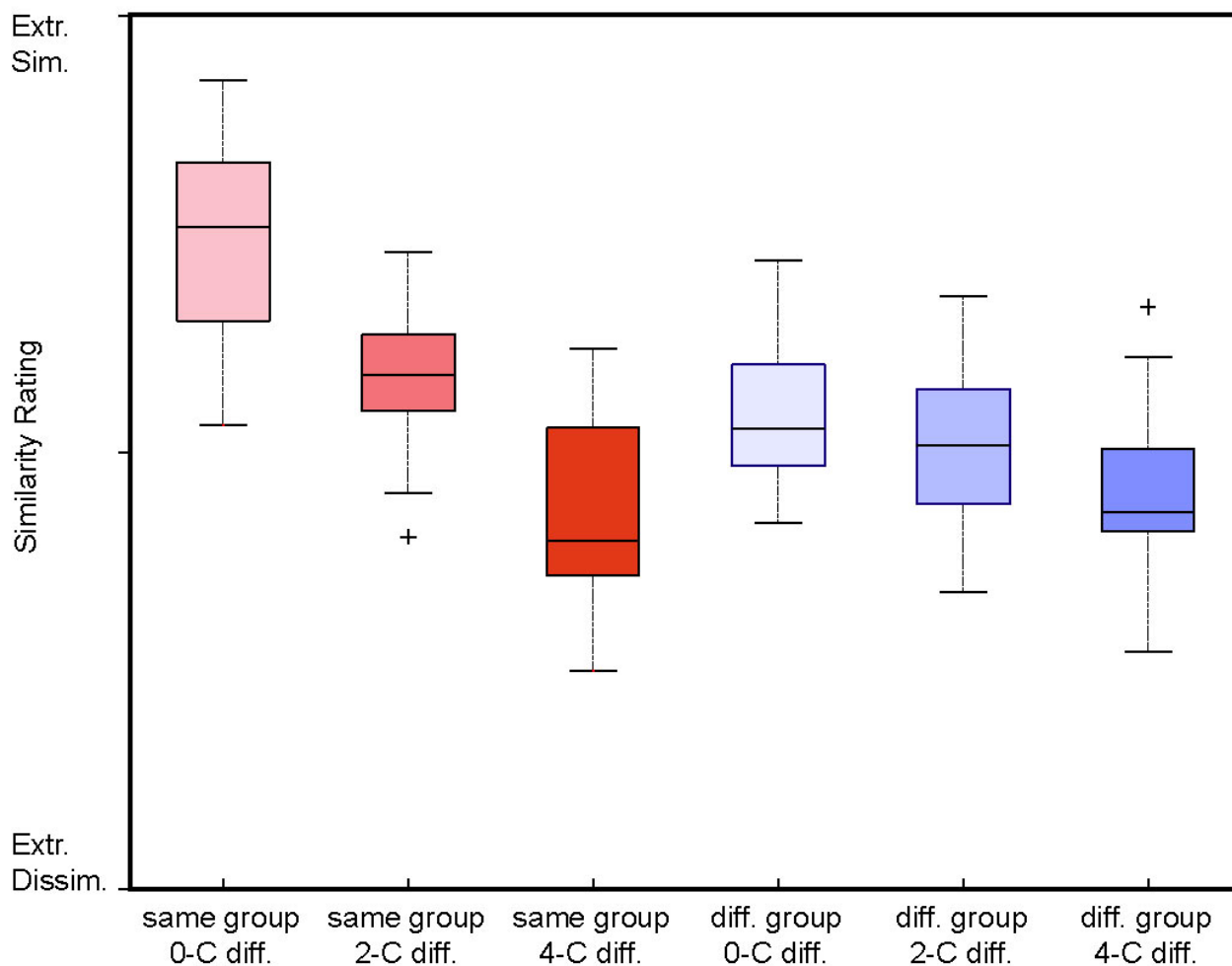


Figure S4. Behavioral ratings of odor quality similarity, from the complementary experiment of group vs. carbon chain length. Condition-specific similarity ratings of odor quality are depicted as boxplots. Boxes have lines at the lower quartile, median, and upper quartile. The whiskers extending from the ends of the boxes show the extent of the data between 10th and 90th percentiles, and the ‘+’ symbols denote additional outlier responses. Plots indicate that same-group pairs were perceived as more similar than different-group pairs, and that similarity ratings progressively decreased with increasing differences in carbon chain length between odorant pairs. Identical odorants (“same group/0-C difference”) were predictably rated as most similar in quality.

5. Supplemental References

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