

Behavioral Models of Odor Similarity

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Carbon chain length in several classes of straight-chain aliphatic odorants has been proposed as a model axis of similarity for olfactory research, on the basis of successes of studies in insect and vertebrate species. To assess the influence of task on measured perceptual similarities among odorants and to demonstrate that the systematic similarities observed within homologous odorant series are not task specific, the authors compare 3 different behavioral paradigms for rats (olfactory habituation, generalization, and discrimination). Although overall patterns of odorant similarity are consistent across all 3 of these paradigms, both quantitative measurements of perceptual similarity and comparability with 2-deoxyglucose imaging data from the olfactory bulb are dependent on the specific behavioral tasks used. Thus, behavioral indices of perceptual similarity are affected by task parameters such as learning and reward associations.

Elementary olfactory stimuli do not vary along a single dimension analogous to wavelength in vision or frequency in audition. Rather, the chemical structures and epitopes that activate primary olfactory neurons are hugely diverse, such that distance measures that might be used to measure similarity among odorants cannot feasibly be compressed into a tractable number of dimensions. However, selected series of molecules (termed *homologous series*) can be defined such that the constituent molecules differ structurally from one another in a limited and sequential manner, forming a candidate axis of odor variation (an arbitrary one-dimensional subspace) without any defined relation to other such potential axes. If it can be shown that these odor molecules are perceptually similar to one another to a degree that covaries with their relative positions in the series, and/or if some defined physiological odor response correlates with an odorant's position in the series, then the odor subspace defined by that series can serve as a credible axis of variation for studies of odorant similarity.

Carbon chain length has been proposed as a model axis for olfactory research in several classes of straight-chain aliphatic odorant molecules, on the basis of diverse studies performed in both insect and vertebrate species. Electrophysiological and imaging studies have shown that, within a given homologous series of straight-chain aliphatic odorants sharing the same functional group (e.g., *n*-aliphatic aldehydes, carboxylic acids, esters, or alcohols), odorants with carbon chains of neighboring lengths tend to activate substantially overlapping populations of olfactory glomeruli within both the insect antennal lobe and vertebrate olfactory bulb (OB) or, comparably, OB mitral cells (Belluscio & Katz, 2001; Doving, 1966; Imamura, Mataga, & Mori, 1992; Johnson & Leon, 2000; Johnson, Woo, Hingco, Pham, & Leon, 1999; Kaluza & Breer, 2000; Katoh, Koshimoto, Tani, & Mori, 1993; Meister &

Bonhoeffer, 2001; Mori, Mataga, & Imamura, 1992; Rubin & Katz, 1999; Sachse, Rappert, & Galizia, 1999; Sato, Hirono, Tonoike, & Takebayashi, 1994; Yokoi, Mori, & Nakanishi, 1995). Consequently, the spatial overlap between the activity patterns evoked in these structures by two straight-chain odorants with similar carbon chain lengths would be greater than that evoked by two such odorants with dissimilar carbon chain lengths. Behaviorally, if perceived odor quality is to directly correlate with these spatial activation patterns, then greater degrees of overlap between odorants' activation patterns should correlate with an increased perceptual similarity of those odorants. Indeed, behavioral data from a number of species (including rats, Linster & Hasselmo, 1999; squirrel monkeys, Laska & Freyer, 1997; Laska & Teubner, 1998, 1999; and honeybees, Laska, Galizia, Giurfa, & Menzel, 1999) support the utility of the straight-chain aliphatic homologous series as an axis for graded odorant similarity.

Although all of the above-mentioned behavioral studies were based on the same axis of odor similarity, each study used only a single behavioral paradigm to measure perceptual similarities between odorants, limiting the comparability of the data and raising the problem of the possible influence of task on each study's results. To assess the influence of task on measured perceptual similarities among odorants and to demonstrate that the systematic similarities observed within homologous odorant series are not task-specific, we compared three different behavioral paradigms for rats (olfactory habituation, generalization, and discrimination). Furthermore, to draw comparisons between these behavioral results and odor-evoked spatial activation data from the OB, we used a homologous odorant series of aliphatic carboxylic acids previously used by Johnson et al. (1999) to measure the dissimilarity of odor-evoked activity patterns in the rat OB glomerular layer by means of 2-deoxyglucose (2DG) staining. Imaging of the OB glomerular layer by 2DG staining enables the visualization and quantification of activity patterns across the entire structure, an advantage over *in vivo* dye imaging techniques, which typically permit observation of only a small portion of the dorsal surface of the OB and are consequently of limited utility for quantitative studies of spatial activation patterning (Belluscio & Katz, 2001; Lam, Cohen, Wachowiak, & Zochowski, 2000; Meister & Bon-

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hoeffer, 2001; Rubin & Katz, 1999; Sachse et al., 1999; Wachowiak, Zochowski, Cohen, & Falk, 2000). First, we showed that olfactory habituation, a task that does not involve reward associations and measures untrained, differential responses to odor stimuli, can measure graded similarities between aliphatic carboxylic acids and, furthermore, that these similarities are similar to calculated distance measures between the bulbar activation patterns evoked by these same odorants, as derived from 2DG data in unrewarded rats (Johnson et al., 1999). Second, olfactory discrimination, in which a reward association is made with one of two odorants, also could measure graded similarities if overtraining is avoided by designing the task to minimize the number of conditioning trials. Third, olfactory generalization, a task also involving reward association (Linster & Hasselmo, 1999), was able to measure the similarity between closely neighboring odorants but did not yield a usefully graded pattern of similarity across the five-carbon structural range used in this study. We conclude that, although rough odorant similarity patterns were consistent across all three of these paradigms, quantitative measurements of perceptual similarity are dependent on the specific behavioral tasks used; consequently, any conclusions pertaining to odor perception made on the basis of such experiments must be put carefully into context, with due consideration of the particular odorants, species, and behavioral paradigms involved.

Method

Subjects

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) served as subjects for the habituation, generalization, and discrimination experiments. Subjects were maintained on a 12-hr light–dark cycle in an environmentally controlled room. All behavioral training was conducted in the afternoon (1500–1900). During habituation experiments, food and water were continuously available; during generalization and discrimination experiments, water was continuously available, but subjects were maintained on a food-deprivation schedule designed to keep them at approximately 85–95% of their ad libitum body weight over the experimental period. All procedures were performed under the auspices of a protocol approved by the Cornell University Institutional Animal Care and Use Committee.

Experiment 1: Olfactory Habituation

Apparatus. All habituation experiments took place in an opaque, black Plexiglas box (38 cm long \times 38 cm wide \times 30 cm high; see Figure 1A) in which a 2.5-cm diameter hole had been drilled in the floor for secure placement of a small vial. The rats entered the box via a sliding door (10 cm \times 10 cm). Odors were diluted in 5 ml mineral oil (see Table 1 for dilutions of the individual odorants) in a 20-ml vial for presentation to the rats in the experimental apparatus.

Shaping. During shaping (learning of the basic task), rats were simply exposed to the habituation box over several days until they became accustomed to it. At first, an odor vial containing only mineral oil was introduced into the box; during subsequent days of shaping, vials containing odorants (different from those used in the experiment) were introduced into the box. For each rat, the shaping procedure was considered accomplished when the rat investigated a vial containing a novel odorant for several seconds and the duration of this investigation decreased with successive presentations of the same odor vial. Figure 1B shows a typical habituation curve measured during the shaping period.

Odor set. The odor set consisted of a series of aliphatic acids with unbranched carbon chains varying from two to six carbons in length (see Table 1); in addition, a chemically dissimilar odorant, *n*-amyl acetate, was used as a control odor. All odorants were diluted in mineral oil to approximate a theoretical vapor pressure of 0.2 Pa; the corresponding volume/volume dilutions in mineral oil are listed in Table 1. All test odors were encoded so that the experimenter was unaware of the identity of each odor.

Behavioral testing. In this experiment, we tested how well rats differentiate between aliphatic acids with different carbon chain lengths using an odor habituation paradigm. During each trial, the rat was introduced into the habituation box (into which an odor vial had been placed) for a maximum of 90 s, during which the experimenter recorded with a stopwatch how long the rat investigated the odor vial. *Investigation* was defined as active sniffing within 1 cm of the mouth of the odor vial. Because of the large number of test odors, the habituation experiment was divided into two sets of trials; each rat was trained on 2 separate days, separated by a week. Each training session consisted of the following succession of 10 trials, each separated by 10 min (also summarized in Figure 1C). In the first 2 trials, the rat was habituated to a vial containing 5 ml of plain mineral oil (no odor, null). These trials were followed by 3 trials in which the vial contained the habituation odor (O_{hab}), which was always the two-carbon acid in the series (i.e., acetic acid). Subsequently, test trials with one of the test odors ($O_{\text{test}1-6}$) and additional habituation trials with the habituation odor (O_{hab}) were alternated, three times each. Thus, on each training day, the response to three test odors was recorded. We also included the two-carbon habituation odor as one of the test odorants to measure the habituated odor response under the same masked conditions in which other test odor responses were measured. Investigation times were recorded in all trials except null trials. The test odors were presented in pseudorandom order over the 2 experimental days.

Data analysis. The primary data, as depicted on figure ordinates, consisted of odor investigation times during test trials. Only rats that investigated O_{hab} for at least 5 s during its first presentation (i.e., Trial 3) were included in the analysis. After analysis of variance (ANOVA) testing for differences in response levels among rats, with test odorant as a within-subject factor (i.e., differences of zero, one, two, three, or four carbons from the habituation odor), pairwise post hoc tests (Tukey's honestly significant difference [HSD]) were performed to determine whether the investigation time elicited by a test odorant was significantly different from that elicited by the habituated odorant or the control odorant. Correlations between the average investigation times of test odorants and the relative structural similarity of each test odorant to the habituated odorant (in terms of carbon chain length) were evaluated by calculating Pearson's *R* and tested for significance with *t* tests. All tests were two-tailed, and alpha was set to .05. All statistical analyses were performed with SPSS statistical software.

Experiment 2: Olfactory Generalization

Apparatus. All behavioral training took place in a transparent Plexiglas chamber (51 cm long \times 38 cm wide \times 25 cm high) divided into two subchambers by a sliding, opaque Plexiglas board (see Figure 2A). Ceramic dishes (9 cm in diameter, 4.5 cm high) were used for placement of the odorants and the reward. The end of a Q-tip was covered with fine plastic mesh that was taped to the bottom of the dish. At the beginning of each daily training set, a separate dish was prepared for the training odorant (O_{train}) and the test odorants ($O_{\text{test}1-6}$) by saturating the Q-tip with 0.1 ml of diluted odorant. The dish was then filled with bedding (Bed-O-Cobs 0.125-in. laboratory bedding, The Andersons, Maumee, OH). The reward, a bit of sweetened cereal (Kellogg's Froot Loops, reduced in odor by long exposure to air and heat), was buried in the bedding. The bedding in each dish was replaced after every trial.

Shaping. First, rats were taught to retrieve a reward by digging in dishes of bedding. At the beginning of each trial, the rat was placed in Subchamber A (see Figure 2A), with the divider between the two sub-

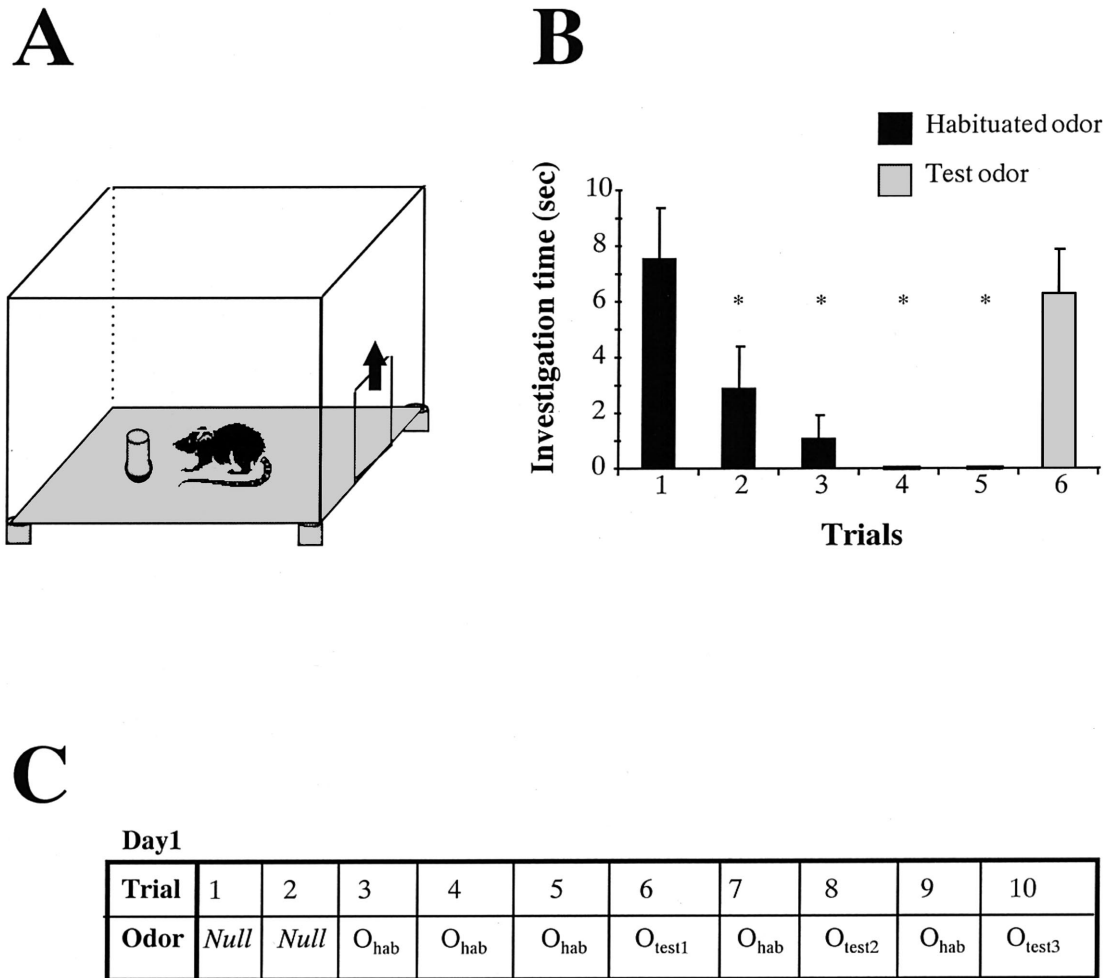


Figure 1. Experimental setup for the habituation study. **A:** Behavioral chamber. An opaque black Plexiglas box (38 cm long \times 38 cm wide \times 30 cm high) with a sliding door on one side was used in these experiments. A 20-ml vial containing either mineral oil or odorant diluted in mineral oil was placed into a 2.5-cm hole drilled in the bottom of the box. During each trial, the rat entered the box through the sliding door and was allowed to investigate the odor vial for no more than 90 s. **B:** Example of habituation to odors during the shaping period. The graph shows the average (\pm SEM) investigation times measured in response to five repeated trials with the habituated odor (almond oil, 0.1% vol/vol) at 10-min intertrial intervals, followed by the average response to a novel odor (methyl salicylate, 0.1% vol/vol) on Trial 6. Investigation times in Trials 2–5 were significantly different from the first trial ($p < .02$, Tukey's honestly significant difference [HSD] test), but the response to the novel odor was not different from that in the first trial (Tukey's HSD, $p > .90$). Asterisks denote significant differences in investigation time compared with Trial 1 ($p < .05$). **C:** Experimental protocol. Trials 1–2: habituation to a vial containing 5 ml mineral oil; Trials 3–5: habituation to the habituated odor (O_{hab}, acetic acid); Trials 6–10: alternate trials with a test odor (O_{test1}–O_{test6}) and O_{hab}. All trials were separated by 10-min intertrial intervals. *Null* indicates that no odorant was present.

chambers closed. Two dishes were then placed in Subchamber B: one containing both a reward and an odor, the other containing no reward and no odor. When the divider was removed, the rat entered Subchamber B and was allowed to dig in both dishes until it retrieved the reward. During the first few trials, the reward was placed on top of the odor-containing dish. After successful retrieval of the reward over several trials, the reward was buried deeper and deeper into the dish. Once the rat learned to dig to retrieve the reward, the dishes were moved around randomly in Subchamber B, such that odor was the only reliable predictor of which dish contained a buried reward. Shaping was considered complete when a rat would reliably identify the reward-containing dish and retrieve deeply

buried rewards, and dig in the odor-containing dish even in the absence of a reward (thus controlling for the possibility of rats simply smelling the reward). Initial shaping was performed with odorants dissimilar to those used in experiments.

Odor set. The odor set consisted of a series of aliphatic acids with unbranched carbon chains varying from two to six carbons in length (see Table 1); in addition, a chemically dissimilar odorant, *n*-amyl acetate, was used as a control odor. All odorants were diluted in mineral oil to approximate a theoretical vapor pressure of 1.0 Pa before application to the Q-tip; the corresponding volume/volume dilutions in mineral oil are listed in Table 1.

Table 1
Odors and Their Percentage (Vol/Vol) Dilutions as Used in Experiments 1, 2, and 3

Experiment	Odor					
	Acetic acid (2)COOH	Propionic acid (3)COOH	Butyric acid (4)COOH	Valeric acid (5)COOH	Caproic acid (6)COOH	<i>n</i> -Amyl acetate control
1	0.0014	0.0069	0.0214	0.2326	1.2866	0.0127
2	0.0070	0.0345	0.1070	1.1630	6.4330	0.0635
3	0.0140	0.0690	0.2140	2.3260	12.8660	0.1270

Note. Specific dilutions were selected to approximate a theoretically consistent vapor pressure for each of the different odorants (nominally 0.2 Pa in Experiment 1, 1.0 Pa in Experiment 2, and 2.0 Pa in Experiment 3). Vapor pressures of pure odorants were estimated with ChemSketch (2001) software and variously diluted in mineral oil to concentrations theoretically emitting the same partial pressure over each odorant. A formula weight estimate of 335 g/mol for mineral oil (Jefo Nutrition, St-Hyacinthe, Québec, Canada) was used for mole fraction calculations; as mineral oil is typically heterogeneous, this value should be considered an average. Solvent surface effects and other nonlinearities were neglected. These dilutions should be considered a reduction in the variance of odor concentrations rather than true gas-phase concentration matching as could be achieved by gas chromatographic measurements.

Behavioral testing. In this experiment, we used an odor generalization paradigm to test how rats generalize between aliphatic acids with different carbon chain lengths (see Figure 2B). Acetic acid ([2]COOH) was selected as the conditioned odorant (O_{cond}); the six test odorants included the two-to-six carbon straight-chain aliphatic acids and a control odorant (*n*-amyl acetate). Each rat was trained on O_{cond} over five conditioning trials in which it had a choice between a scented dish containing the reward and an unscented dish containing no reward (see Figure 2A). Subsequently, six unrewarded test trials (in which the rat was offered a choice between a dish scented with the test odorant and an unscented dish) were performed in a pseudorandom order; these test trials were separated by one or two rewarded conditioning trials to prevent extinction of the O_{cond} -reward association. During test trials, total digging times in the dish containing test odor were recorded with a stop watch. Each odorant was tested once. The experimenter was unaware of the identity of the test odors during performance of these experiments.

Data analysis. The primary data, as depicted on figure ordinates, consisted of digging times during test trials. Only rats that dug for at least 3 s in the dish scented with O_{cond} during unrewarded test trials were included in the analysis, to exclude rats that might have learned to detect the reward directly. After ANOVA testing for differences in digging times among rats, with test odorant as a within-subject factor (differences of zero, one, two, three or four carbons from O_{cond}), Tukey's HSD tests were performed to determine whether the digging time elicited by a test odorant was significantly different from that elicited by O_{cond} or the control odorant. Correlations between the average digging time in response to each test odorant and the relative structural similarity of each test odorant to O_{cond} (in terms of carbon chain lengths) were evaluated by calculating Pearson's *R* and tested for significance with *t* tests. All tests were two-tailed, and alpha was set to .05. All statistical analyses were performed with SPSS statistical software.

Experiment 3: Olfactory Discrimination

Apparatus and shaping. All behavioral training took place in the same Plexiglas chamber used for the generalization study (Experiment 2; see Figure 2A). All odors were prepared, and rats were shaped, in the same manner as they were in that study.

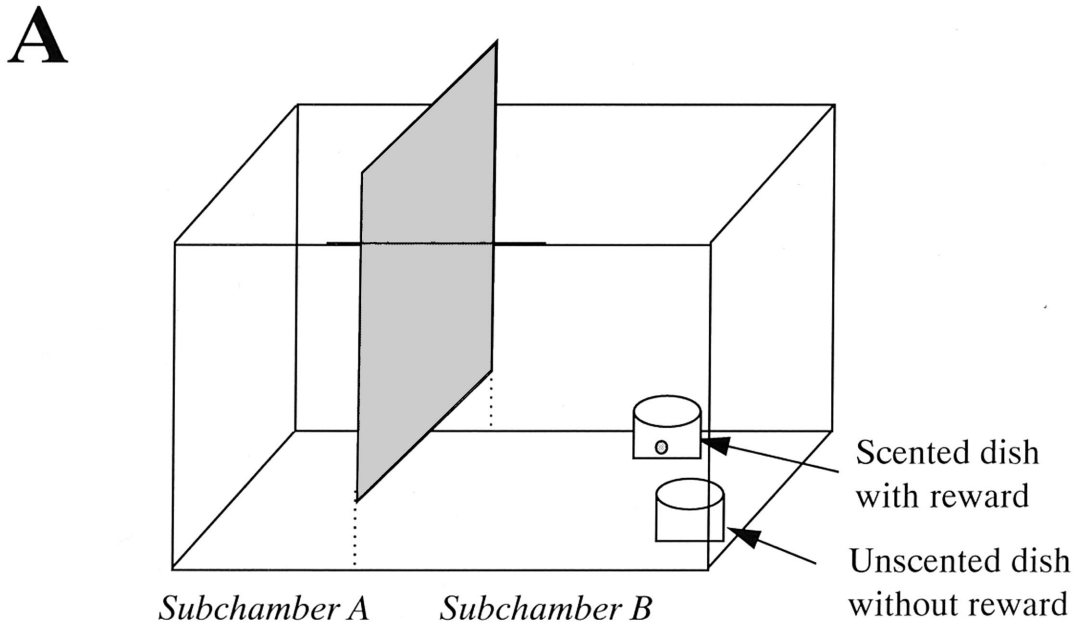
Odor set. The odor set consisted of a series of aliphatic acids with unbranched carbon chains varying from two to six carbons in length (see Table 1); in addition, a chemically dissimilar odorant, *n*-amyl acetate, was used as a control odor. All odorants were diluted in mineral oil to a theoretical vapor pressure of 2.0 Pa before application to the Q-tip; the corresponding volume/volume dilutions in mineral oil are listed in Table 1.

Behavioral testing. In this experiment, we investigated how rats learn to discriminate among aliphatic acids with different carbon chain lengths. Specifically, we tested how well rats could learn to discriminate between acetic acid (2-carbon) and test odorants (3–6-carbon), as well as a dissimilar control odorant (*n*-amyl acetate). Consequently, a total of five discriminations were tested over 3 days. On each day, half of the rats were randomly assigned to one of the five discrimination tasks; it was randomly determined which of the two odors in a given task was rewarded and which was unrewarded. Each rat was presented with a choice of two odorants (one rewarded, one unrewarded) for 20 trials. During each trial, the dish in which the rat dug first to retrieve the reward was recorded. Rats were allowed to self-correct after digging in the unrewarded odor. Every 5th trial, the reward was dropped on the dish only after the rat had started digging in the rewarded odor, ensuring the detection of rats that had learned to detect the reward directly rather than learning to discriminate the test odorants. Rats that failed to dig in any dish more than once during the unrewarded trials were eliminated from the analysis.

Data analysis. The primary data for the discrimination task consisted of the proportion of correct odor selections made by rats over 20 training trials (i.e., digging in the rewarded odor dish, as opposed to the unrewarded dish). After ANOVA testing for differences among odor pairs, Tukey's HSD tests were performed to determine whether the acquisition of a given odor pair differed from that of any other pair. Correlations between the average proportion of correct choices (across rats) and the relative structural similarities of the odor pairs (in terms of the difference in their carbon chain lengths) were evaluated by calculating Pearson's *R* and tested for significance with *t* tests. All tests were two-tailed, and alpha was set to .05. All statistical analyses were performed with SPSS statistical software.

Dissimilarity Index for Comparison With 2DG Data

To compare our behavioral results to odor-evoked spatial activation patterns measured in the OB (Johnson et al., 1999), we calculated a dissimilarity index between pairs of odorants for each experiment. To facilitate comparison with the dissimilarity index derived by Johnson et al. (1999) for their 2DG data, this index was designed so that a value of 1 represented very dissimilar odorants and a value of 0 represented very similar odorants. A separate dissimilarity index was calculated for each experiment, appropriate to the different behavioral measures used in each: for habituation, $D_{\text{hab}} = (1 - R_{\text{hab}}/R_{\text{test}})$, where *R* represents investigation time; for generalization, $D_{\text{gen}} = (1 - R_{\text{test}}/R_{\text{cond}})$, where *R* represents digging time; for discrimination, $D_{\text{disc}} = (1 - R_{\text{comp}}/R_{\text{test}})$, where R_{comp} was set to 50% to simulate a failure to discriminate (chance) and R_{test} represents the percentage of correct trials for a given odor pair.



B

Trial	1 - 5	6	7	8	9	10	11	12	13	14	15	16
Odor	O _{cond}	O _{test1}	O _{cond}	O _{test2}	O _{cond}	O _{test3}	O _{cond}	O _{test4}	O _{cond}	O _{test5}	O _{cond}	O _{test6}
Reward	X	-	X	-	X	-	X	-	X	-	X	-

Figure 2. Experimental setup for the generalization and discrimination studies. A: Behavioral chamber. A transparent Plexiglas chamber (51 cm long × 38 cm wide × 25 cm high) was separated into two subchambers by an opaque black Plexiglas sliding door. Two dishes (9 cm in diameter, 4.5 cm high), one containing odor and reward and the other containing only bedding, were placed in Subchamber B. At the beginning of each trial, the rats entered Subchamber B, retrieved the reward, and were then returned to Subchamber A. B: Experimental protocol. Rats were first conditioned to the conditioned odor (O_{cond}, acetic acid, Trials 1–5); over Trials 6–16 the rats were tested with each of the test odors (O_{test1}–O_{test6}) in the absence of reward (indicated by dashes). One to two rewarded trials (indicated by Xs) with O_{cond} were interspersed between each of the test trials to prevent extinction. The test odorants, O_{test1}–O_{test6}, were presented in a random order that differed for each rat.

Results

Experiment 1: Olfactory Habituation

Habituation to O_{hab}. After habituation to the odorless vial (Trials 1–2), rats were habituated to the habituation odor O_{hab} over three trials (Trials 3–5). As shown in Figure 3A, the duration of investigation of O_{hab} decreased with each presentation ($n = 11$ rats). No significant difference was measured in the overall response levels among rats, $F(10, 33) = 2.02, p > .05$. Response duration to presentation of O_{hab} depended significantly on the trial

number, $F(2, 20) = 20.47, p < .01$. Furthermore, the durations of investigation in Trials 4 and 5 were both significantly lower than that in Trial 3 (the first exposure to O_{hab}; Tukey’s HSD, $p < .01$), demonstrating that the rats successfully habituated to the odorant.

Investigation of test odors after habituation to O_{hab}. After habituating rats to the odorless vial (Trials 1–2) and to the habituating odor O_{hab} (Trials 3–5), responses to test odors were recorded on Trials 6, 8 and 10 on both experimental days (see Figure 3B, $n = 11$). These test trials were each separated by one habituation trial with O_{hab} to ensure that the rats remained habituated to

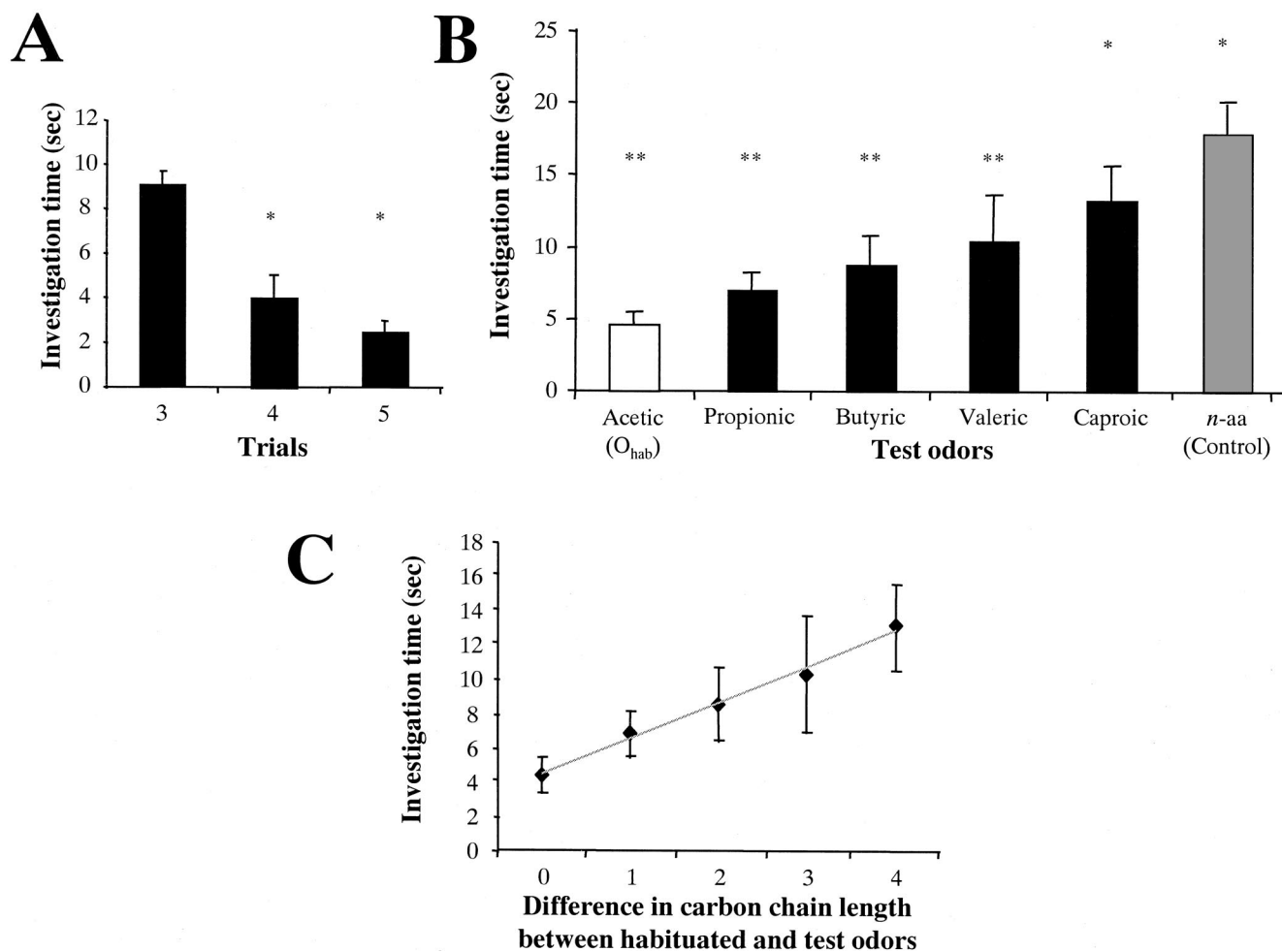


Figure 3. Results from Experiment 1 (habituation). A: Mean (\pm SEM) investigation times during odor habituation trials (Trials 3–5 in Figure 1C). The graph depicts the investigation times of 11 rats in response to O_{hab} (acetic acid). Asterisks denote a significant difference in investigation time compared with Trial 3 ($p < .05$). B: Mean (\pm SEM) investigation times in response to test odors during test trials. The graph shows the average responses of 11 rats to the habituated odor (O_{hab} , acetic acid), the other aliphatic acids in the series (propionic, butyric, valeric, and caproic acids), and the control odorant *n*-amyl acetate (*n*-aa). Asterisks denote a significant difference in investigation time compared with O_{hab} ($p < .05$); double asterisks denote a significant difference in investigation time compared with *n*-aa ($p < .05$). C: Mean (\pm SEM) investigation times as a function of the difference in carbon chain length between O_{hab} and each test odorant.

it. During test trials, the duration of investigation depended significantly on the odorant, $F(5, 50) = 8.08$, $p < .01$. The lowest investigation time was observed in response to the habituated odor, acetic acid ([2]COOH). The responses to caproic acid ([6]COOH) and to *n*-amyl acetate (control) were significantly different from the response to the habituated odor (Tukey's HSD, $p < .01$). The responses to acetic acid ([2]COOH), propionic acid ([3]COOH), butyric acid ([4]COOH), and valeric acid ([5]COOH) were significantly different from the response to the control odor, *n*-amyl acetate (Tukey's HSD: acetic acid, propionic acid, and butyric acid, $p < .01$; valeric acid, $p < .05$). As shown in Figure 3C, a highly significant correlation between the average investigation times and the structural similarity between the habituated and test odors was observed ($R = .995$, $p < .01$).

Experiment 2: Olfactory Generalization

After conditioning to O_{cond} (acetic acid), rats responded strongly to that odorant in the forced choice test procedure, in which they were presented with a choice between a dish scented with O_{cond} and an unscented dish. A total of 12 rats were conditioned to O_{cond} , 8 of which were included in the analysis. On average, the longest digging times were observed in response to O_{cond} ; when confronted with a choice between an aliphatic acid other than O_{cond} and an unscented dish (see Figure 4), rats showed average digging times that were lower for all these test odorants than for O_{cond} . Although individual rats did not differ from one another in their overall response levels to odors, $F(7, 35) = 0.46$, $p > .80$, there were significant differences in response levels to the

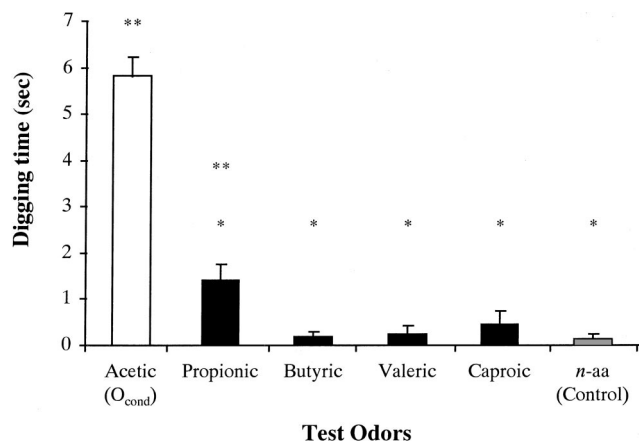


Figure 4. Results from Experiment 2 (generalization). Mean (\pm SEM) digging times of 12 rats during nonrewarded test trials in response to the conditioned odor (O_{cond}, acetic acid), the other aliphatic acids in the series (propionic, butyric, valeric, and caproic acids), and the control odor *n*-amyl acetate (*n*-aa). Asterisks denote a significant difference in investigation time compared with O_{cond} ($p < .05$); double asterisks denote a significant difference in investigation time compared with *n*-aa ($p < .05$).

different test odors, $F(5, 35) = 62.47$, $p < .01$. None of the test odors except propionic acid ([3]COOH) and O_{cond} itself ([2]COOH) elicited digging times significantly higher than those in response to the control odorant *n*-amyl acetate (Tukey's HSD, $p < .05$ for acetic and propionic acid only), indicating that rats generalized significantly from O_{cond} to propionic acid. No significant correlation between the average digging times and the structural similarity between the conditioned and the test odor was observed when carbon chain length differences from zero to four were included in the analysis ($R = .785$, $p > .10$).

Experiment 3: Olfactory Discrimination

In this experiment, we measured how well and how quickly rats can learn to discriminate among aliphatic acids of differing carbon chain lengths, using differences in discrimination learning rates (observable before acquisition of discrimination at asymptotic levels) that correlate with relative odorant similarities. Although all five discrimination tasks could be successfully learned (over 75% correct choices across Trials 15–20), the overall numbers of correct choices across all 20 trials differed significantly among the five odorpairs, $F(4, 24) = 10.40$, $p < .01$; see Figure 5A. Pairwise comparisons demonstrated that discriminations between acetic and propionic acids and between acetic and butyric acids were each significantly inferior to discrimination between acetic acid and the control odorant, *n*-amyl acetate (Tukey's HSD, $p < .01$ and $p < .05$, respectively). As shown in Figure 5B, a highly significant correlation between the average numbers of correct choices and the structural similarity between the discriminated odorants was observed across the range of carbon chain length differences depicted (one to four carbons difference; $R = .980$; $p < .05$). When analyzed in groups of 5 trials, a significant correlation between these two variables could be observed only during Trials 6–10 ($R = .964$, $p < .05$) and Trials 11–15 ($R = .977$, $p < .03$). During neither the first 5 trials nor the last 5 trials could a

significant correlation be observed; in the first 5 trials, the rats generalized between test odorants to an extent comparable to that of the untrained state, whereas in the last 5 trials they could reliably discriminate between any pair of odorants from the test set.

Discussion

The results from the behavioral experiments presented herein suggest that the perceptual similarity between pairs of odorants can be measured by a variety of olfactory behavioral tasks. Carbon chain length has been repeatedly used as an ad hoc axis for graded similarity among aliphatic odorants (Belluscio & Katz, 2001; Imamura et al., 1992; Johnson & Leon, 2000; Laska & Teubner, 1998; Linstler & Hasselmo, 1999; Meister & Bonhoeffer, 2001; Mori et al., 1992; Rubin & Katz, 1999; Yokoi et al., 1995); our results demonstrate that similarities in carbon chain length can indeed predict perceptual similarity between odorants when measured in three different behavioral paradigms. All three protocols

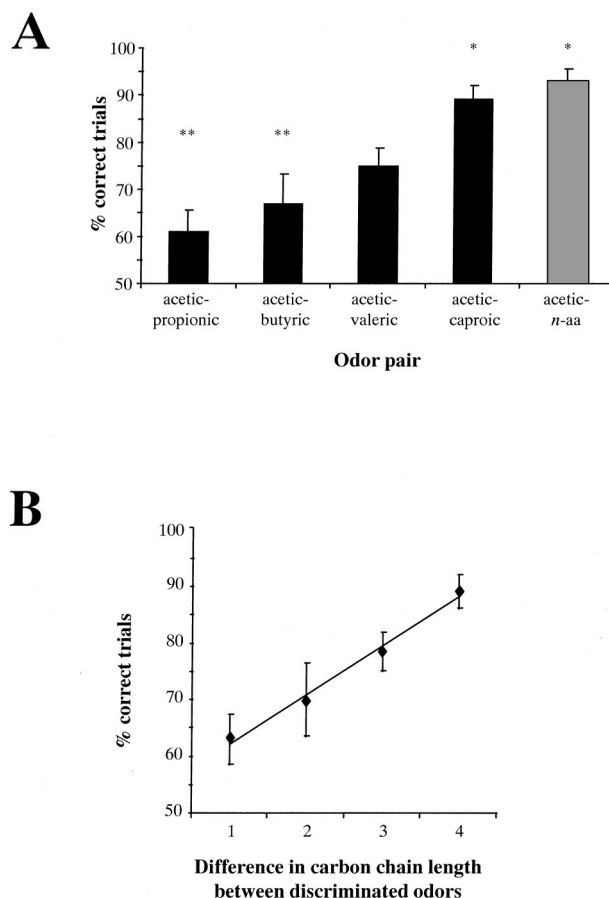


Figure 5. Results from Experiment 3 (discrimination). A: Mean (\pm SEM) percentage of correct trials (out of 20) for the five odor pairs used in the discrimination task. Asterisks denote performance significantly above chance levels ($p < .05$); double asterisks indicate discrimination significantly below that of the control odorant, *n*-amyl acetate (*n*-aa; $p < .05$). B: Mean (\pm SEM) percentages of correct trials as a function of the difference in carbon chain length between the odors in each pair.

demonstrate some utility in measuring perceptual similarities within the aliphatic acid odor series used, lending added weight to the legitimacy of this homologous series of odorants as a valid model system for studies requiring an axis of sequential odorant similarity. However, significant differences in the results derived from the three protocols also highlight their differential utility for addressing sensory and behavioral questions. In particular, the nature of the task and the presence or absence of motivation for associative learning were shown to influence results.

Olfactory habituation, a behavioral paradigm often used to investigate species-specific odor discrimination (Deiss, Feron, & Baudoin, 1999; Gouat, Patris, & Lalande, 1998; Guan, Blank, & Dluzen, 1993a, 1993b; Paolini & McKenzie, 1993; Petruilis, DeSouza, Schiller, & Johnston, 1998; Winslow & Camacho, 1995), measures the perceived similarity between odorant stimuli without

requiring an association between the odor and a reward. Consequently, this task is well suited for comparing behavioral results with imaging data depicting odor-evoked neural activity in naive, untrained animals. Indeed, the present habituation results (Experiment 1) can be predicted by the quantitative dissimilarity in bulbar spatial activation patterns evoked in rats by using the same odor set (Johnson et al., 1999; see Figure 6B, present study). In this behavioral experiment, responses to test odorants gradually changed as their carbon chain lengths became more dissimilar from that of the habituated odor, as indicated by the high correlation ($R = .995$, $p < .01$) between behavioral responses and differences in carbon chain length across the zero-to-four carbon difference range used (see Figure 3C). Furthermore, the response to caproic acid ([6]COOH) was not significantly different from that to the control odor (*n*-amyl acetate), showing that when structural differences between two odorants differ by more than a

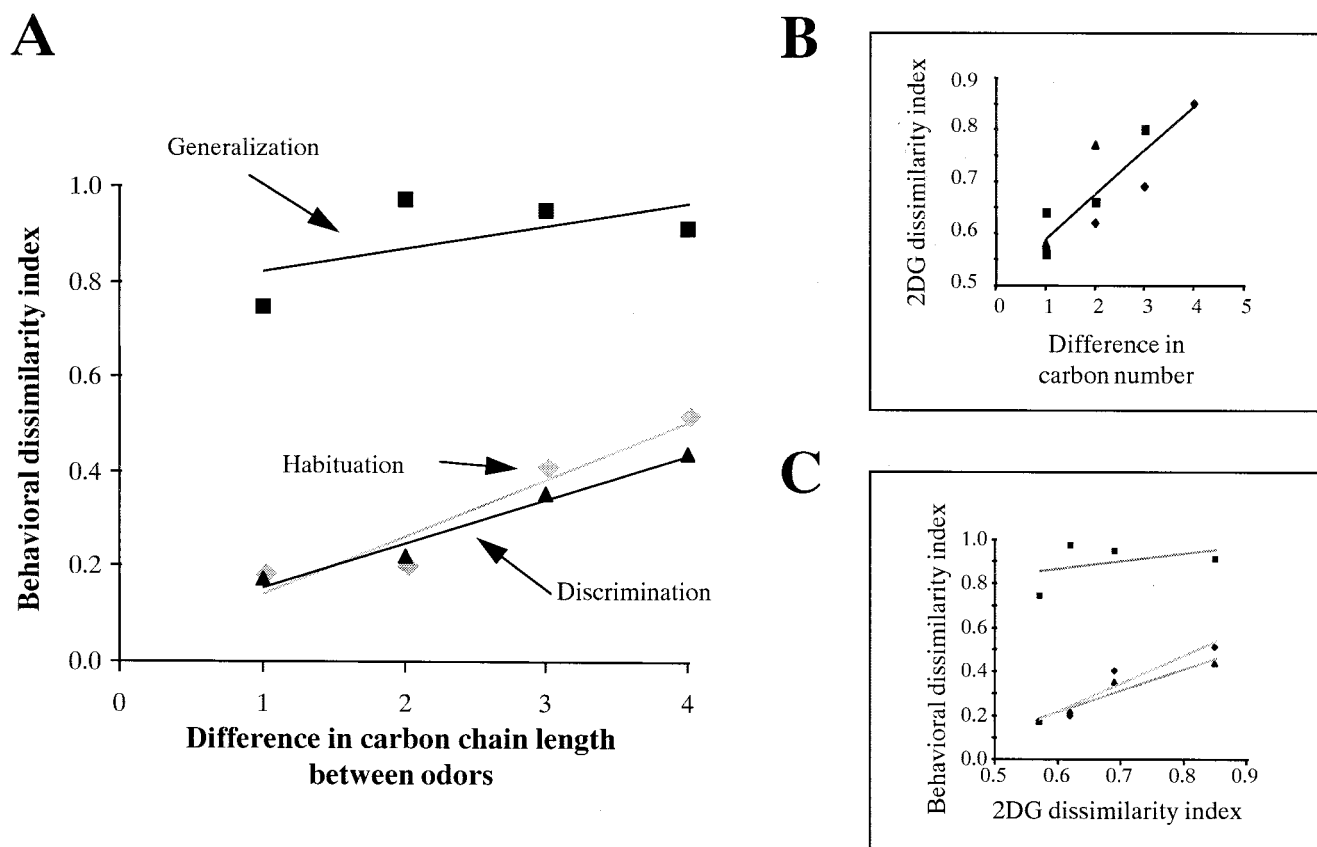


Figure 6. Comparison of behavioral results to each other and to imaging data. **A:** For comparison with 2-deoxyglucose (2DG) imaging data (Johnson et al., 1999), a dissimilarity index was calculated between pairs of odorants for each of the experiments (see the Method section). The figure depicts the dissimilarity indices between habituation, generalization, and discrimination odorant pairs, measured behaviorally as a function of the difference in carbon chain length between the odors in each pair. The habituation and discrimination test results are linear across the zero-to-four carbon chain length differences in odorants used in this study, whereas the generalization results are not. For comparison, Panel B depicts the dissimilarity index calculated from olfactory bulb activity patterns measured with 2DG imaging (From "Multidimensional chemotopic responses to *n*-aliphatic acid odorants in the rat olfactory bulb," by B. A. Johnson, C. C. Woo, E. E. Hingco, K. L. Pham, & M. Leon, 1999, *Journal of Comparative Neurology*, 409, pp. 529–548. Copyright © [1999, John Wiley & Sons]. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.), and Panel C depicts the behavioral data from Figure 6A replotted as a function of the average 2DG dissimilarity index.

limited degree (in this case, three unbranching carbons), the two odorants are not confounded (see Figure 3B).

In the generalization study (Experiment 2), rats learned to associate the conditioned odor with a reward; this behavioral task was based on training and reward-motivated digging rather than innate and unmotivated exploration behavior. In this task, rats' responses to the conditioned odor were always at least 3 times greater than to test odors (see Figure 4), presumably due to expectation of reward. In this paradigm, rats were not required to discriminate O_{cond} from any O_{test} ; rather, all odors were presented sequentially, each paired with an unscented dish. The generalization to each O_{test} in this paradigm thus reflects the degree to which subjects judged that stimulus as likely to share the learned contingency of O_{cond} . Although the generalization data clearly indicate that the rats confused propionic acid ([3]COOH) with the conditioned odorant, acetic acid ([2]COOH), their responses to all other test odors were not significantly different from those to the control odorant, *n*-amyl acetate. The appropriate distance across which a gradual decrease in confusion can be measured had thus substantially narrowed with respect to that seen in the habituation task. Whether these data indicate a task-dependent change in the perceived similarities of odorants or a change in the rats' responses to odorants of fixed similarity is, we propose, a terminological issue; the relative contributions of such factors to perception are not discriminable by the behavioral techniques used herein. The same set of odorants can be shown to evoke different behavioral measures of similarity as a function of task.

Olfactory discrimination paradigms condition subjects to discriminate between a rewarded and a nonrewarded odor, presented simultaneously, between which the subject must choose to obtain a reward. In this paradigm, the rate of acquisition can be used to evaluate how similar a pair of odorants smells to the subject, on the basis of the hypothesis that it is more difficult to learn to discriminate between similar odorants than between dissimilar odorants. As we have previously shown, this paradigm enables rats to learn odor discriminations within very few trials (10–20; Linster & Hasselmo, 1999); in contrast, paradigms using automated olfactometer approaches typically require large numbers of repeated trials (Bodyak & Slotnick, 1999; De Rosa & Hasselmo, 2000; De Rosa, Hasselmo, & Baxter, 2001; Doty, Bagla, & Kim, 1999; Lu & Slotnick, 1998; Rubin & Katz, 2001), potentially obscuring relevant data that may be best measured during acquisition. In Experiment 3, significant differences in the acquisition of pairs of aliphatic acids were observed (see Figure 5A): discrimination learning was significantly more difficult for odor pairs that differed by one or two carbons in carbon chain length than for the control pair (acetic acid vs. *n*-amyl acetate). Analyzing the changes in rats' performance over time (temporally grouping the 20 trials into four groups of 5) revealed that, in this particular task, a significant correlation between structural similarity and discrimination performance could be observed only within Trials 6–10 and 11–15. This illustrates how large numbers of repeated trials, during which rats become highly overtrained on a given odor discrimination, could obscure the presence of such correlations. Defining a discrimination index as the proportion of correct choices across 20 sequential training trials for each rat, we observed a strong correlation between this index and the difference in carbon chain lengths between paired odorants across a range of one to four carbons (see Figure 5B). The behavioral dissimilarity index de-

rived from this task, like that calculated from habituation data, was similar to that derived from the 2DG data (see Figure 6A).

The most striking difference in behavioral performance across the three paradigms is the observed variation in the olfactory confusion distance (from the habituated, conditioned, or rewarded odorant, acetic acid). Once the raw data were independently normalized by calculation of a behavioral dissimilarity index (see the Method section), the habituation and discrimination results are similar in their depiction of a graded reduction in perceived odor similarity across a structural difference range of one to four carbons (see Figure 6A), whereas generalization did not show a graded pattern of dissimilarity as a function of difference in carbon chain length. That is, although all three tasks measure degrees of odor similarity, the conclusions as to which odors are perceived as similar by the rats differ as a function of the particular behavioral paradigm. We propose that perceived similarities between odorants arise in part from the spatial overlap in odor-evoked neural activation patterns within the OB, and that these similarities can be affected by task parameters such as learning and reward associations. For example, although a given pair of odors may be perceived as similar within a task context involving no reward associations, subjects could quickly learn the discrimination given appropriate motivation, suggesting that learning to discriminate similar odorants necessitates the enhancement of differences between the two response patterns.

Our results show that similarities in carbon chain length can indeed predict perceptual similarities among straight-chain aliphatic acid odorants when measured in three different behavioral paradigms; consequently, the present behavioral results support the utility of systematically varied carbon chain lengths of aliphatic odorants as model systems for sequential odor variation. We have previously shown, using a variant of the generalization task described in this study, that rats generalize between aliphatic aldehydes differing by one or two carbons in their unbranched carbon chains (Linster & Hasselmo, 1999). In addition, we have obtained very similar results to those described in this study when testing rats on a generalization task using aliphatic acids, aldehydes, and alcohols in the context of a study investigating the effect of cholinergic lesions on odor generalization in rats (Linster, Garcia, Hasselmo, & Baxter, 2001). Extensive research by Laska and colleagues using a foraging-type discrimination task for squirrel monkeys has shown that, with low numbers of trials, a significant negative correlation between discrimination performance and differences in odorant carbon chain lengths could be observed with several different chemical groups (acetic esters, carboxylic acids, aliphatic alcohols, aldehydes, and ketones; Laska & Freyer, 1997; Laska & Teubner, 1998; Laska, Trolp, & Teubner, 1999). These same researchers have also shown that, in honeybees, a comparable negative correlation between odorant structure and discrimination performance could be observed using a free-flying discrimination paradigm, again with low numbers of trials (Laska, Galizia, Giurfa, & Menzel, 1999). Together, this body of research strongly supports the utility of such homologous series of odorants for studies requiring sequential similarities among odor stimuli, such as those investigating the effects of neuromodulators on olfactory memory and the discrimination between very similar olfactory stimuli.

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