Published in final edited form as:

J Comp Neurol. 2006 December 1; 499(4): 519–532.

Differential responses to branched and unsaturated aliphatic hydrocarbons in the rat olfactory system

Sabrina L. Ho, Brett A. Johnson, Andrew L. Chen, and Michael Leon

Department of Neurobiology and Behavior, University of California, Irvine, CA 92697-4550 USA

Abstract

In an effort to understand mammalian olfactory processing, we have been describing the responses to systematically different odorants in the glomerular layer of the main olfactory bulb of rats. Previously, we have demonstrated chemotopically organized and distinct olfactory responses to a homologous series of straight-chained alkanes that consisted of purely hydrocarbon structures, indicating that hydrocarbon chains could serve as molecular features in the combinatorial coding of odorant information. To better understand the processing of hydrocarbon odorants, we now have examined responses to other types of chemical changes in this kind of molecules, namely branching and carbon-carbon bond saturation. To this end, we used the [^{14}C]2-deoxyglucose method to determine glomerular responses to a group of eight-carbon branched alkane isomers, unsaturated octenes (double-bonded), and octynes (triple-bonded). In contrast to the differential responses we observed previously for straight-chained alkanes of differing carbon number, the rat olfactory system was not particularly sensitive to these variations in branching and bond saturation. This result was unexpected, given the distinct molecular conformations and property profiles of the odorants. The similarity in activity patterns was paralleled by a similarity in spontaneous perceptual responses measured using a habituation assay. These results demonstrate again the functional relationship between bulbar activity patterns and odor perception. The results further suggest that the olfactory system does not respond equally to all aspects of odorant chemistry, functioning as a specific, rather than a general chemical analysis system.

Keywords

branched alkanes; isomers; double bond; triple bond; odor similarity; glomeruli; deoxyglucose; habituation

Various aspects of odorant chemistry are encoded by the rat olfactory system to produce chemotopically organized responses in the olfactory bulb (Imamura et al., 1992; Katoh et al., 1993; Joerges et al., 1997; Johnson et al., 1998, 1999, 2002, 2004, 2005a, b; Rubin and Katz, 1999; Johnson and Leon, 2000a, b; Uchida et al., 2000; Linster et al., 2001; Meister and Bonhoeffer, 2001; Igarashi and Mori, 2005; Ho et al., 2006). Comparable chemotopic organization also has been observed in other animal species (Kauer and Cinelli, 1993; Friedrich and Korsching, 1997, 1998; Sachse et al., 1999; Belluscio and Katz, 2001; Fuss and Korsching, 2001; Carlsson et al., 2002; Fried et al., 2002; Kent et al., 2003). Thus far, these observations have been obtained mostly through using odorants with oxygen-containing functional groups that are known to have a profound impact on perceived odors (Laska and Freyer, 1997; Laska and Teubner, 1999; Laska et al., 1999, 2001; Linster and Hasselmo, 1999; Laska and Galizia, 2001; Laska and Hübener, 2001; Cleland et al., 2002). Recently, we showed that the system

also was differentially responsive to a homologous series of straight-chained aliphatic alkanes consisting of purely hydrocarbon structures (Ho et al., 2006). Even though these alkanes could bind to olfactory receptors only by way of weak Van der Waals forces, they evoked highly specific glomerular response patterns that were found to be significantly different for alkanes of different carbon number. In addition, rats were able to discriminate spontaneously among pairs of these odorants that differed by as little as one carbon in the carbon chain, although humans report their odors to be quite similar.

Progressively changing carbon chain length across a homologous series is only one way to vary hydrocarbon structures systematically. More subtle chemical differences exist for isomeric chemicals, which possess identical molecular formulae, but which have different molecular arrangements that could result in distinct molecular shapes and properties that might be recognized by specific odorant receptors. Such structural variations include positional isomers where functional groups or alkyl groups are present at different locations along the carbon backbone of aliphatic molecules. Indeed, significantly different glomerular activity patterns were detected for some, but not all, isomeric aliphatic odorants that had the same oxygen-containing functional groups at different positions along the hydrocarbon chain (Johnson et al., 2005a). We also have observed small differences in glomerular responses to enantiomeric odorants (Linster et al., 2001). However, not all enantiomeric odorant pairs tested in that study were discriminated spontaneously by the animals (Linster et al., 2001), indicating that small differences in glomerular responses may contribute to the discrimination of only some odorants. Therefore, it is unlikely that all of the small differences observed in glomerular responses are equally relevant to all perceptual processes.

Another type of structural variation that has not yet been investigated systematically in studies of olfactory coding involves the nature of carbon-carbon bonds present in an odorant molecule. Double and triple bonds confer distinct bond angles and geometries (Fig. 1), reduce flexibility, and offer the potential of stronger interactions through hydrogen bonding (Fig. 2). In fact, unsaturated alkenes (double-bonded) and alkynes (triple-bonded) do have very different molecular properties (Fig. 2) and are chemically more reactive than their parent alkanes.

In the present study, we first examined both glomerular and perceptual responses to branched-chain isomers of octane (Fig. 1) to investigate systematically the impact of branching in pure hydrocarbon structures with no oxygen-containing functional groups. In addition, we studied responses to a group of eight-carbon unsaturated hydrocarbons to determine how the system may respond to changes in bond saturation. It should be noted that unsaturated bonds are considered to be functional groups, and we chose to examine their effects on olfactory responses without the influence of any other functional groups by using various eight-carbon octenes and octynes (Fig. 1). Although odorants with structures that are more closely related to each other have more similar molecular property profiles in general, as demonstrated by neighboring members in a homologous series such as the straight-chained alkanes (Fig. 2), individual odorants involving different branching and bond types display characteristic property profiles that are distinct from each other. Given that significantly different glomerular activity patterns and perceived odors were observed among odorants as molecularly related to each other as neighboring alkanes (Ho et al., 2006), we expected to find robust differences in neural and perceptual responses to the branched and unsaturated hydrocarbons that we tested in this study.

MATERIALS AND METHODS

Molecular conformations and properties

Images of odorants shown in Figure 1 were generated in Chem3D Pro version 5.0 (CambridgeSoft, Cambridge, MA). Most of the molecular properties included in Figure 2 were

estimates from Molecular Modeling Pro version 3.14 (ChemSW, Fairfield, CA). Reported values for log P, water solubility, and vapor pressure were medians of all unique values collected from Molecular Modeling Pro and the following additional sources: PhysProp Database from Syracuse Research Corporation (<http://www.syrres.com/esc/physdemo.htm>), the Chemical and Physical Properties Database from the Pennsylvania Department of Environmental Protection (http://www.dep.state.pa.us/physicalproperties/CPP_Search.htm), and ChemDraw Ultra version 6.0 (CambridgeSoft, Cambridge, MA). For each property, individual values were expressed as ratios of the sum across all odorants within a study.

Odorant Exposures

Table 1 contains detailed information regarding all odorants included in this study, which consisted of two independently conducted experiments. One experiment tested a group of eight-carbon branched alkanes isomeric to octane, whereas the other included octenes and octynes. Some of these compounds were commercially available only at a purity grade lower than what we typically use in our studies, and since we have shown that studies of olfactory coding can be affected by odorant contaminants (Johnson et al., 2004; Ho et al., 2006), we were particularly aware of looking for differences in responses that could be based on such factors. Even at a relatively low purity, the high cost of some of the odorants in the isomer study required us to dilute each odorant in mineral oil at 1/10 of the total volume, before 50 mL of which was placed in a 125-mL gas-washing bottle equipped with an extra-coarse porosity diffuser. For the experiment involving unsaturated hydrocarbons, we used 100 mL of each neat odorant.

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Odorant exposures were conducted as described in our previous studies (Johnson et al., 1998, 1999, 2002, 2004, 2005a, b; Johnson and Leon, 2000a, b; Ho et al., 2006). Seventeen- to nineteen-day-old Wistar rats, culled to eight per litter, were used for all experiments. In order to reduce odors carried over from the soiled housing cage, each litter was transferred to a clean cage with the dam at least one hour before an exposure. The first pup obtained from each litter was always exposed to the vehicle, which was mineral oil for the branched isomers and air for the unsaturated hydrocarbons. Prior to the introduction of an animal, the exposure system first was equilibrated with the odorant of interest for fifteen minutes. Highly pure research-grade nitrogen was passed through the odorant column at a rate of 250 mL/min (100 mL/min for branched alkanes). The resulting vapor was mixed with ultra-zero grade air, after which the mixture was delivered to the exposure chamber at a rate of 2 L/min (1 L/min for branched alkanes). All odorants in the bond saturation study were presented at a fixed concentration of 1000 ppm by differential dilution calculated from their vapor pressures. Due to dilution in mineral oil, the final vapor phase concentration of the eight-carbon alkanes could not be determined. No two rats from the same litter were exposed to the identical odorant condition, and the number of animals exposed to each odorant condition is shown in Table 1. The order of odorant presentation was pseudo-randomized across litters to avoid systematic relationships among odorants.

Individual pups were injected subcutaneously at the back of the neck with a dose of [^{14}C]2-deoxyglucose (2-DG) determined by body weights ($1.6\ \mu\text{L/g}$, $0.1\ \text{mCi/mL}$, $52\ \text{mCi/mmol}$, Sigma Chemical Company, St. Louis) before being placed in a clean, 2-L glass jar used as the exposure chamber. Each exposure lasted for forty-five minutes, with the odorant entering and exiting the glass jar through two vents in the jar lid, so that odorant concentration increased steadily at the beginning of the exposure. Animals' brains then were immediately removed, frozen in isopentane at -45°C , and stored at -80°C .

Measurement and analysis of 2-DG uptake

Histology, autoradiography, and activity mapping were performed as described in our previous studies (Johnson et al., 1998, 1999, 2002, 2004, 2005a, Johnson et al., b; Johnson and Leon, 2000a, b; Ho et al., 2006). Briefly, for each coronal bulb section, an appropriate selection from a set of polar grids was chosen according to the number of bulb sections between anatomical landmarks and was applied to the section's autoradiographic image overlaid with the image of the adjacent Nissl-stained section. The grids guided the collection of 2-DG uptake measurements from the glomerular layer throughout the entire olfactory bulb into standardized matrices. After the subtraction of measurements from vehicle, each individual data matrix was converted to z-scores. Matrices from the same odorant condition within a study were averaged to yield an average z-score pattern for that condition. However, for statistical purposes, individual z-score patterns, rather than average z-score patterns from a single experiment were subjected to ANOVA followed by false discovery rate analyses (Curran-Everett, 2000; Johnson et al., 2002, 2004, 2005a, b; Ho et al., 2006) to test for statistical differences among odorant-induced responses in predetermined areas termed glomerular modules (Johnson et al., 2002, 2004, 2005a, Johnson et al., b; Ho et al., 2006). The use of these predetermined modules in the present study does not imply their functional significance within the olfactory system. Rather, it serves as a tool both to ensure an unbiased simplification of the activity patterns for statistical analysis and to facilitate comparisons with previous studies that were analyzed with respect to the same modules.

Overall pattern similarity was analyzed by comparing averaged glomerular responses produced by the hydrocarbons to octane-evoked response obtained from the same experiment using Pearson correlation. Each average response pattern for an odorant actually represented a z-score array containing over 2,300 values. Using Pearson correlation (Johnson et al., 2002; 2004, 2005 a, b; Ho et al., 2006), point-to-point comparisons could be made between any two patterns, and the level of overall pattern similarity produced by two odorants was indicated by the correlation coefficient (r).

Olfactory discrimination

Perceived odor similarity was assessed using a habituation assay similar to that described in prior studies (Linster et al., 2001; Cleland et al., 2002; Ho et al., 2006). This discrimination paradigm depends on the reliable tendency of rats to investigate novel odorants in their environment. With repeated exposure, however, the novelty of the odorant dissipates and rats are less and less likely to investigate that odorant. Adult male Wistar rats were handled and shaped in the behavior testing apparatus for seven consecutive days prior to experimentation. The testing apparatus included a clean test cage similar to the home cages (30 cm L $\times$ 20 cm W $\times$ 19 cm H), and a clear, customized cage lid with evenly distributed 1-cm wide holes and a slit for a cage separator, which could be lowered vertically to separate the length of the cage into two compartments.

Animals were food-deprived for two days throughout behavioral testing, which was conducted under dim light during the dark phase of a reverse light-dark cycle. An animal was placed in a clean cage, and a plastic cap lined with clean filter paper was positioned over one of the holes in the cage lid. A ten-minute control period then began immediately, during which animals were allowed to investigate the cage. Upon the termination of the control period, the cage separator was lowered to confine the rat to one side of the cage. An odorized cap was then put on top of the other side of cage, and the first experimental trial started after the separator was lifted. Each experimental trial lasted for two minutes, followed by a ten-minute inter-trial period.

Octane was presented for the first three trials to familiarize and habituate animals to its odor. Test odorants then were alternated with the familiar odorant for the remaining trials. Individual experimental series were conducted with different odorant sets (Table 2). The presentation order of test odorants was varied across animals in such a way to avoid systematic relationships among odorants. To control for performance fatigue, the final trial used an odorant different from the experimental odorant set based on molecular properties and glomerular activity patterns. All trials were recorded on videotape for subsequent analysis.

While we have compared the glomerular response of young rats (PND 17-19) to that of adult rats, there is some reason to have confidence in this comparison. By PND 17-19, the rat olfactory bulb is mature anatomically (Brunjes and Frazier, 1986; Malun and Brunjes, 1996) and functionally (Astic and Saucier, 1982; Greer et al., 1982; Mair and Gesteland, 1982; Gregory and Pfaff, 1971; Fletcher et al., 2005). Moreover the glomerular response that we have observed in the glomerular layer has been replicated for other odorants in the lateral aspect of the glomerular layer of adult rats (Uchida and Mori, 2000). The glomerular response patterns also have been used successfully to predict the perceptual response patterns of adult rats for odorant enantiomers (Linster, et al., 2001).

Behavioral data analysis

The total amount of time each animal spent investigating the odorant during each trial was determined. Investigation was defined as active sniffing within 1 cm of the odorized cap. Animals were excluded from further analysis if they failed to maintain habituation to the familiar odorant during the experiment, or failed to investigate odorants during the first or the last trial. The maintenance of habituation was indicated by a lack of increase in the investigation time in subsequent octane trials from that observed during its first presentation. We imposed these criteria because habituation to octane was required for it to serve as the reference odorant to be compared with all subsequent test odorants, whereas a lack of investigation in the first or the last trial may indicate different kinds of behavioral problems. A general low level of interest in or motivation for the behavioral task may be indicated if there were no odorant investigation during the first odorant presentation. Meanwhile, performance fatigue may cause a lack of investigative behavior in the final trial. Investigation time was sorted by odorants and analyzed for statistical differences with an ANOVA performed for each experimental series, followed by Dunnett's *post hoc* tests.

RESULTS

Neurobehavioral responses to branched isomeric alkanes

To investigate the effects of isomeric changes in purely hydrocarbon structures, we studied a group of eight-carbon branched alkanes with systematic variations in branching, and compared the evoked patterns with that stimulated by the unbranched octane. We found that octane produced a glomerular activity pattern (Fig. 3) comparable to that observed in previous studies (Johnson et al., 2005b; Ho et al., 2006). Glomerular responses evoked by these isomers (Fig. 3) were found to be significantly different using ANOVA tests ($p < 0.05$) for uptake in previously defined regions of the bulb followed by false discovery rate analysis (Curran-Everett, 2000; Johnson et al., 2002, 2004, 2005a,b; Ho et al., 2006). The most obvious differences involved changes in glomerular regions that overlapped with octane's paired anterior foci (Fig. 3, circled in black). Differential effects were found in these glomerular regions depending on the number of methyl branches present in a molecule and their substitution positions along the carbon chain. In general, reduced activation in the outlined regions was associated with increasing number of methyl substitution and distance of substitution from either end of the hydrocarbon backbone.

The most dissimilar pairs of patterns were found in the comparisons of the unbranched octane to the most branched isomers (Fig. 3). However, Pearson correlation analysis of these response patterns revealed considerable similarity in the paired comparisons between octane and each of the branched alkanes (r values ranging from 0.61 to 0.73), despite significant differences observed in their patterns using ANOVA tests across glomerular modules.

The resemblance in the neural responses in this experiment was paralleled by perceived odor similarity. Using an odor habituation paradigm previously shown to be effective for testing spontaneous odor discriminability (Linster et al., 2001; Cleland et al., 2002; Ho et al., 2006), we examined odor similarity among the branched isomers as perceived by rats. After such habituation, animals will investigate a new odorant placed in their environment only if they regard the new odor as being different from the habituated odor. Behavioral results are presented in Figure 4A, with the measured investigation time sorted by test odorants included in this study. Except for the control odorants used for testing performance fatigue, no significant difference was observed in the investigation time for any of the isomers. That is, the odors of these branched alkanes were perceived to be so similar to the odor of octane that they were essentially indistinguishable, a result that corresponds with their high pattern similarity.

Unexpected neurobehavioral responses to unsaturated hydrocarbons

Another type of structural variation involving pure hydrocarbons that alters molecular conformations and properties even more profoundly than branching is changes in bond saturation (Figs. 1 and 2). The presence of a double or a triple bond provides hydrogen bonding opportunities that are not available with saturated alkanes (Fig. 2), which may allow unsaturated molecules to interact with receptors more strongly than they could through weaker Van der Waals forces only. To examine how these non-oxygen-containing functional groups may be processed by the system, we tested a group of eight-carbon octenes (double-bonded) and octynes (triple-bonded), as well as their saturated parent alkane, octane.

Again, the glomerular response evoked by octane (Fig. 5) agreed with our previous observations (Johnson et al., 2004; Ho et al., 2006). Using identical statistical analyses as with the branched isomers, glomerular responses evoked by this set of odorants also were found to be significantly different ($p < 0.05$), mostly involving parts of the glomerular layer overlapping with octane's paired anterior response foci (Fig. 5, black outlines) as well as more dorsal regions (Fig. 5, white arrows) that were defined as modules c and C previously (Johnson and Leon, 2000a; Johnson et al., 2002, 2004, 2005a,b; Ho et al., 2006). Differential effects were detected in these regions depending on the type, the position, the number, and the stereoconfiguration of carbon-carbon bonds present in a molecule (Fig. 5). Generally, decreased activation in the regions outlined in black (Fig. 5) was associated with decreasing bond saturation determined by the type and the number of unsaturated carbon-carbon bond. This observation was particularly evident for odorants possessing these bonds near the center of the aliphatic backbone, where such bonds may have maximal steric influence on interactions with receptors. The same bond variations that decreased activation of these more ventral areas increased activation in more dorsal regions of the bulb (Fig. 5, white arrows).

Despite the statistically significant differences in glomerular responses revealed by the modular ANOVA tests, the overall patterns evoked by individual unsaturated odorants were highly similar to the pattern evoked by octane according to Pearson correlation comparisons (r values ranging from 0.60 to 0.84). Moreover, the similarity in evoked activity patterns was paralleled by a similarity of the perceived odor as measured in the habituation assay described earlier (Fig. 4B). The amount of investigation time for all the octenes and octynes was not significantly different from that for octane, indicating that animals did not discriminate spontaneously among these odorants.

The overall sensitivity of the rat olfactory system to variations in hydrocarbon structure

In both of the present experiments on hydrocarbon branching and bond saturation, as well as in our prior study of alkane carbon number (Ho et al., 2006), we included octane so that it could serve as a common reference odorant. Figure 6 shows overall pattern comparisons using Pearson correlations between octane and each of the hydrocarbons we have tested thus far. The addition of methylene ($-\text{CH}_2-$) groups to a molecule produced the largest differences in glomerular responses, as indicated by lower values of correlation coefficients (r) for the five odorants through decane and tetradecane. There was comparatively very little effect of the rearrangement of methyl ($-\text{CH}_3$) groups in isomeric molecules on the glomerular response. Perhaps more surprisingly, the addition of an unsaturated carbon-carbon bond functional group also had very little effect on the glomerular response (Fig. 6). Indeed, despite very different molecular conformations and properties associated with double and triple bonds (Figs. 1 and 2), octane shared greater overlap in glomerular responses with these unsaturated odorants than with some of the more molecularly related branched isomers and homologous aliphatic alkanes (Fig. 6). It should be noted that even with the highly similar responses evoked by the branched and unsaturated odorants, the greatest differences were observed when the maximum length of the unbranched, saturated hydrocarbon chain was the most disrupted by structural changes (Figs. 3, 5, and 6). Interestingly, the paired glomerular regions included in octane's anterior response foci (Figs. 3 and 5), which also have been shown previously to be stimulated by aliphatic odorants that have similar carbon chain length but different functional groups (Ho et al., 2006), were the locations of differential activation evident between both groups of odorants and octane.

Furthermore, regression analyses of differences between individual molecular properties of all of the aliphatic hydrocarbon odorants we have studied to date versus similarity of the patterns with octane revealed that the most highly correlated property was molecular length (Fig. 7, Table 3). That is, the degree to which any odorant differed in length from octane predicted how different its evoked activity pattern was from octane's evoked pattern. Differences in other molecular properties, including the logarithm of vapor pressure, molecular volume, surface area, $\log P_{\text{O-W}}$, Hansen polarity, the logarithm of water solubility, and H bond acceptor strength also yielded significant correlations with the pattern similarity to octane (Table 3). It may be significant that differences in these other properties are themselves correlated with differences in molecular length, and the extent of their correlation with length was closely related to the extent of their correlation with pattern similarity (Table 3), suggesting that length-related differences might be the principal determinants of pattern differences for these hydrocarbons.

The inclusion of octane as a common reference odorant in each of the three series of hydrocarbons also allowed us to investigate the overall relationship between the similarity in evoked activity pattern and the similarity in perceived odor across the three series. In Figure 8, we have plotted the relative investigation time for each odorant following habituation to octane as a function of the similarity of the pattern evoked by that odorant to the pattern evoked by octane in the same experiment. The values were highly correlated ($r = -0.55$), and the correlation was significant ($F = 9.8$, $p = 0.005$), suggesting that there is an overall predictive relationship between patterns of 2-DG uptake and perceptual similarity across these hydrocarbon odorants. Further analysis of Figure 8 reveals that the unsaturated eight-carbon hydrocarbons were in general more similar in both pattern and perception to octane than were the branched eight-carbon hydrocarbons, a finding that is consistent with the greater differences in length within the latter series.

DISCUSSION

Unique processing of pure hydrocarbon structures

Given that we observed specific neural and perceptual responses to a homologous series of straight-chained alkanes despite their predicted weak interactions with olfactory receptors (Ho et al., 2006), we were interested in how other structural variations in pure hydrocarbon odorants would be processed by the system free of any influence from oxygen-containing functional groups. To this end, we now have compared eight-carbon isomeric branched alkanes, double-bonded octenes and triple-bonded octynes to the unbranched and saturated octane. We found highly similar but consistently different glomerular responses among these odorants. In addition, pattern similarity was also reflected in perceived odor similarity. Such similarity was surprising, especially when one considers that the molecular conformations (Fig. 1) and properties (Fig. 2) of some of these branched and unsaturated hydrocarbons are very different from those of octane. In particular, the unsaturated odorants present hydrogen bonding ability that could allow them to interact more strongly with olfactory receptors than saturated molecules. Regardless of these seemingly important differences, it appeared that many of the olfactory receptors recognizing octane were not particularly sensitive to these variations in structure, so that there were remarkable spatial overlaps in the glomeruli activated by these odorants. It is interesting to note that comparable effects have been reported for the rat I7 receptor, which has a high affinity for the eight-carbon saturated aldehyde, octanal, as well as other branched and unsaturated aldehydes of similar carbon chain length (Araneda et al., 2000). Therefore, it may be that olfactory receptors activated by saturated odorants also respond to unsaturated odorants bearing the same functional groups, as long as they share similar carbon chain length.

Moreover, neither high levels of glomerular pattern similarity nor the relative difficulty of rats to discriminate odors would have been predicted based on the odor descriptions reported by humans. Our personal observations indicated very different odors for octane, as compared to some of the branched alkanes, and most of the unsaturated odorants. In particular, unsaturated alkenes (double-bonded) and alkynes (triple-bonded) are not regarded generally as having the “gasoline-like” odor typical of saturated alkanes such as octane (see, for example, <http://hazmap.nlm.nih.gov/>). In fact, alkenes and alkynes often appear in the headspace analysis of meat (Ahn and Love, 1998; Ahn et al., 1999, 2000; Meynier et al., 1999; Gorraiz et al., 2002). Such differences between species in olfactory responses also have been observed previously. For example, within a homologous series of straight-chained alkanes, rats were able to discriminate spontaneously even between neighboring members when humans described them as having the same odor (Ho et al., 2006). In addition, differences in the responsiveness to isovaleric acid have been reported even among different strains of mice and populations of humans (Royet et al., 1987; Sicard et al., 1989). These reports also are consistent with a recent demonstration of different ketone binding thresholds for the mouse and human olfactory receptor orthologs despite similarities in their amino acid sequences (Hummel et al., 2005). Based on these results, it may be the case that meaningful functional relationships between stages of olfactory processing will be more valid when conspecific comparisons are made.

Nevertheless, small but consistent differences were found among these highly similar glomerular patterns. The greatest impact on the glomerular response was evident when the maximum length of an unbranched, saturated hydrocarbon chain, which should be distinguished from the overall molecular length of a molecule, was the most disrupted. With isomeric alkanes, increasing the number of methyl substitutions decreases the chain length of the hydrocarbon backbone, whereas the presence of a methyl substitution towards the center of the molecule drastically reduces the maximum length of the non-substituted carbon chain. In fact, weakened activation by these structural variations in the outlined glomerular regions

(Fig. 3) that are located somewhat ventrally in the bulb is consistent with the previously observed dorsal-ventral chemotopy associated with molecular length of odorants (Johnson et al., 1999,2004;Ho et al., 2006). Decreased activation in similar regions also was observed with octenes and octynes depending on the position of the unsaturated bond along the carbon backbone, which could disrupt the length of the saturated hydrocarbon chain available for receptor binding, rendering the molecules more difficult to be recognized by the octane-activated receptors that project to glomeruli within the regions. On the other hand, increased activation was evident in the dorsal regions indicated by arrows (Fig. 5). These areas appeared to be particularly responsive to small and hydrophilic molecules at high concentrations (Johnson and Leon, 2000a;Johnson et al., 2004;Ho et al., 2006), and may therefore respond more strongly to unsaturated odorants with decreased hydrophobicity (Fig. 2, Log P), a response pattern consistent with that predicted by Schoenfeld and Cleland (2005). However, such small differences among these glomerular responses may not impact the spontaneous discrimination of hydrocarbon odorants in rats, as suggested by the high level of perceived odor similarity. Indeed, we have shown differential importance of small glomerular pattern differences in their contribution to perceptual processing in a prior study (Linster et al., 2001).

Furthermore, significant correlations were obtained between glomerular pattern similarity and length-related properties only. Therefore, receptors responding to pure hydrocarbons structures may be particularly sensitive to these properties, which may be more relevant than other molecular features to evoke odorant distinct glomerular activity patterns and odor discrimination. It remains possible that the importance of molecular length is specific only for the coding of aliphatic hydrocarbons because they may rely mainly on weak Van der Waals force to interact with receptors. Other molecular properties, such as those associated with hydrogen bonding or polarity, may be much more critical for the processing of other odorants that bind receptors by way of other interactions. Hydrocarbon structures may be processed by the system in a unique way given that they constitute the molecular backbone of most aliphatic odorants, and consequently are shared among many molecules containing a variety of functional groups.

The olfactory system as a specific chemical analysis tool

Through the mechanism of combinatorial coding, it has been estimated that the olfactory system can process thousands of odorants (Polak, 1973; Buck and Axel, 1991). Indeed, chemotopically organized and distinct responses to changes in a variety of molecular features have been observed in many studies (Imamura et al., 1992; Katoh et al., 1993; Kauer and Cinelli, 1993; Friedrich and Korsching, 1997, 1998; Joerges et al., 1997; Johnson et al., 1998, 1999, 2002, 2004, 2005a, b; Rubin and Katz, 1999; Sachse et al., 1999; Johnson and Leon, 2000a, b; Uchida et al., 2000; Belluscio and Katz, 2001; Fuss and Korsching, 2001; Linster et al., 2001; Meister and Bonhoeffer, 2001; Carlsson et al., 2002; Fried et al., 2002; Kent et al., 2003; Igarashi and Mori, 2005; Ho et al., 2006). Despite the fact that the olfactory system responds to a very broad spectrum of odorants, the present data indicate that not all changes in odorant chemistry are resolved equally well. We also have found other evidence in prior studies that profound molecular modifications can lead to minor changes in glomerular activity patterns (Johnson and Leon, 2000b; Linster et al., 2001; Johnson et al., 2005b). In addition, odorants with very different molecular structures, such as benzaldehyde and cyanide, have been found to have odors that are not discriminable (Dreveny et al., 2002). Thus, the olfactory system probably does not function as a general chemical analysis system that is equally sensitive to all kinds of molecular variations. Rather, the system seems to display differential responsiveness for some odorants or odorant features that may have been critical in its evolution (Singer et al., 1996; Gilad et al., 2003a, b, 2004; Oleander et al., 2004). Differences in the array of olfactory receptors in humans and rodents that have been reported

(Young and Trask, 2002) are likely to underlie species differences. Furthermore, not only may certain molecular properties contribute more than others to the coding of odorants, differential levels of contribution from any one property to the coding of various classes of odorants also may occur (Polak, 1973). These complex interactions between odorants and the olfactory system may support further the reduced relevance of hydrogen bonding ability to the processing of pure hydrocarbons than of other odorants with oxygen-containing functional groups, as well as the diminished importance of stereoconfiguration for the coding of certain enantiomeric odorants relative to other odorants (Linster et al., 2001).

CONCLUSION

In contrast to a homologous series of straight-chained alkanes, which evoked chemotopically organized and highly distinct glomerular responses despite weak interactions with olfactory receptors, we found that the rat olfactory system was not particularly sensitive to molecular changes that do not significantly alter the length of unbranched, saturated carbon chains in pure hydrocarbon odorants. Such insensitivity was reflected in both glomerular and perceptual responses, and may indicate unique processing of hydrocarbon structures in the system. Our data further suggested interspecific differences in the responsiveness of the system to the same odorants. These data together indicate that the olfactory system may function as a specific, rather than as a general chemical analysis system that does not respond equally well to every aspect of odorant chemistry.

Acknowledgements

We thank the following individuals for their technical assistance: Jennifer Kwok and Zhe Xu for conducting 2-DG experiments; Joan Ong, Paige Pancoast, and Mickel Gerges for 2-DG activity mapping; Sakura Minami and Sepideh Saber for their work on behavioral shaping; and Espartaco (Spart) Arguello for the development and maintenance of a database for our activity patterns, analysis software, and our laboratory's website. We also thank an anonymous reviewer for insightfully suggesting additional analyses and illustrations.

This research is supported by grants DC03545 and DC006516 from NIDCD.

References

- Ahn, DU.; Love, J. Effect of irradiation on lipid oxidation and off-flavor development in cooked pork products with different fatty acids and packaging. Iowa State University Swine Research Report Food Safety ASL-R1607; 1998.
- Ahn, DU.; Jo, C.; Olson, DG. Analysis of volatile components and sensory characteristics of irradiated raw pork. Iowa State University Swine Research Report Food Safety ASL-R1711; 1999.
- Ahn, DU.; Nam, KC.; Du, M.; Jo, C. Volatile Production of Irradiated Normal, PSE, and DFD Pork. Iowa State University Swine Research Report Food Safety ASL-R694; 2000.
- Araneda RC, Kini AD, Firestein S. The molecular receptive range of an odorant receptor. *Nat Neurosci* 2000;3:1248–1255. [PubMed: 11100145]
- Astic L, Saucier D. Ontogenesis of the functional activity of rat olfactory bulb: autoradiographic study with the 2-deoxyglucose method. *Dev Brain Res* 1982;2:243–256.
- Belluscio L, Katz LC. Symmetry, stereotypy, and topography of odorant representations in mouse olfactory bulbs. *J Neurosci* 2001;21:2113–2122. [PubMed: 11245695]
- Brunjes PC, Frazier LL. Maturation and plasticity in the olfactory system of vertebrates. *Brain Res Rev* 1986;11:1–45.
- Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 1991;65:175–187. [PubMed: 1840504]
- Carlsson MA, Galizia CG, Hansson BS. Spatial representation of odours in the antennal lobe of the moth *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Chem Senses* 2002;27:231–244. [PubMed: 11923186]
- Cleland TA, Morse A, Yue EL, Linster C. Behavioral models of odor similarity. *Behav Neurosci* 2002;116:222–231. [PubMed: 11996308]

- Curran-Everett D. Multiple comparisons: philosophies and illustrations. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R1–R8. [PubMed: 10896857]
- Dreveny I, Kratky C, Gruber K. The active site of hydroxynitrile lyase from *Prunus amygdalus*: modeling studies provide new insights into the mechanism of cyanogenesis. *Protein Sci* 2002;11:292–300. [PubMed: 11790839]
- Fletcher ML, Smith AM, Best AR, Wilson DA. High-frequency oscillations are not necessary for simple olfactory discriminations in young rats. *J Neurosci* 2005;25:792–798. [PubMed: 15673658]
- Fried HU, Fuss SH, Korsching SI. Selective imaging of presynaptic activity in the mouse olfactory bulb shows concentration and structure dependence of odor responses in identified glomeruli. *Proc Natl Acad Sci U S A* 2002;99:3222–3227. [PubMed: 11854464]
- Friedrich RW, Korsching SI. Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. *Neuron* 1997;18:737–752. [PubMed: 9182799]
- Friedrich RW, Korsching SI. Chemotopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. *J Neurosci* 1998;18:9977–9988. [PubMed: 9822753]
- Fuss SH, Korsching SI. Odorant feature detection: activity mapping of structure response relationships in the zebrafish olfactory bulb. *J Neurosci* 2001;21:8396–8407. [PubMed: 11606628]
- Gilad Y, Man O, Paabo S, Lancet D. Human specific loss of olfactory receptor genes. *Proc Natl Acad Sci U S A* 2003a;100:3324–3327. [PubMed: 12612342]
- Gilad Y, Bustamante CD, Lancet D, Paabo S. Natural selection on the olfactory receptor gene family in humans and chimpanzees. *Am J Hum Genet* 2003b;73:489–501. [PubMed: 12908129]
- Gilad Y, Wiebe V, Przeworski M, Lancet D, Paabo S. Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol* 2004;2:E5. [PubMed: 14737185]
- Gorraiz C, Beriain MJ, Chasco J, Insausti K. Effect of aging time on volatile compounds, odor, and flavor of cooked beef from Pirenaica and Friesian bulls and heifers. *J Food Sci* 2002;67:916–922.
- Greer CA, Stewart WB, Teicher M, Shepherd GM. Functional development of the olfactory bulb and a unique glomerular complex in the neonatal rat. *J Neurosci* 1982;2:1744–1759. [PubMed: 7143049]
- Gregory EH, Pfaff DW. Development of olfactory-guided behavior in infant rats. *Physiol Behav* 1971;6:573–576. [PubMed: 5149448]
- Ho SL, Johnson BA, Leon M. Long hydrocarbon chains serve as unique molecular features recognized by ventral glomeruli of the rat olfactory bulb. *J Comp Neurol*. 2006in press
- Hummel P, Vaidehi N, Floriano WB, Hall SE, Goddard WA 3. Test of the binding threshold hypothesis for olfactory receptors: explanation of the differential binding of ketones to the mouse and human orthologs of olfactory receptor 912–93. *Protein Sci* 2005;14:703–710. [PubMed: 15722446]
- Igarashi KM, Mori K. Spatial representation of hydrocarbon odorants in the ventrolateral zones of the rat olfactory bulb. *J Neurophysiol* 2005;93:1007–1019. [PubMed: 15385587]
- Imamura K, Mataga N, Mori K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. *J Neurophysiol* 1992;68:1986–2002. [PubMed: 1491253]
- Joerges J, Küttner A, Galizia G, Menzel R. Internal representations for odours and combinatorial coding of odour mixtures visualized by optical imaging. *Nature* 1997;387:285–288.
- Johnson BA, Leon M. Modular representations of odorants in the glomerular layer of the rat olfactory bulb and the effects of stimulus concentration. *J Comp Neurol* 2000a;422:496–509. [PubMed: 10861522]
- Johnson BA, Leon M. Odorant molecular length: one aspect of the olfactory code. *J Comp Neurol* 2000b;426:330–338. [PubMed: 10982472]
- Johnson BA, Woo CC, Leon M. Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. *J Comp Neurol* 1998;393:457–471. [PubMed: 9550151]
- Johnson BA, Woo CC, Hingco EE, Pham KL, Leon M. Multidimensional chemotopic responses to n-aliphatic acid odorants in the rat olfactory bulb. *J Comp Neurol* 1999;409:529–548. [PubMed: 10376738]
- Johnson BA, Ho SL, Xu Z, Yihan JS, Yip S, Hingco EE, Leon M. Functional mapping of the rat olfactory bulb using diverse odorants reveals modular responses to functional groups and hydrocarbon structural features. *J Comp Neurol* 2002;449:180–194. [PubMed: 12115688]

- Johnson BA, Farahbod H, Xu Z, Saber S, Leon M. Local and global chemotopic organization: general features of the glomerular representations of aliphatic odorants differing in carbon number. *J Comp Neurol* 2004;480:234–249. [PubMed: 15514935]
- Johnson BA, Farahbod H, Saber S, Leon M. Effects of functional group position on spatial representations of aliphatic odorants in the rat olfactory bulb. *J Comp Neurol* 2005a;483:192–204. [PubMed: 15678475]
- Johnson BA, Farahbod H, Leon M. Interactions between odorant functional group and hydrocarbon structure influence activity in glomerular response modules in the rat olfactory bulb. *J Comp Neurol* 2005b;483:205–216. [PubMed: 15678471]
- Kato K, Koshimoto H, Tani A, Mori K. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. II. Aromatic compounds. *J Neurophysiol* 1993;70:2161–2175. [PubMed: 8294977]
- Kauer JS, Cinelli AR. Are there structural and functional modules in the vertebrate olfactory bulb? *Microsc Res Tech* 1993;24:157–167. [PubMed: 8457727]
- Kent PF, Mozell MM, Youngentob SL, Yurco P. Mucosal activity patterns as a basis for olfactory discrimination: comparing behavior and optical recordings. *Brain Res* 2003;981:1–11. [PubMed: 12885420]
- Laska M, Freyer D. Olfactory discrimination ability for aliphatic esters in squirrel monkeys and humans. *Chem Senses* 1997;22:457–465. [PubMed: 9279468]
- Laska M, Galizia CG. Enantioselectivity of odor perception in honeybees (*Apis mellifera carnica*). *Behav Neurosci* 2001;115:632–639. [PubMed: 11439452]
- Laska M, Hübener F. Olfactory discrimination ability for homologous series of aliphatic ketones and acetic esters. *Behav Brain Res* 2001;119:193–201. [PubMed: 11165335]
- Laska M, Teubner P. Olfactory discrimination ability for homologous series of aliphatic alcohols and aldehydes. *Chem Senses* 1999;24:263–270. [PubMed: 10400444]
- Laska M, Galizia CG, Giurfa M, Menzel R. Olfactory discrimination ability and odor structure-activity relationships in honeybees. *Chem Senses* 1999;24:429–438. [PubMed: 10480679]
- Laska M, Ayabe-Kanamura S, Hübener F, Saito S. Olfactory discrimination ability for aliphatic odorants as a function of oxygen moiety. *Chem Senses* 2001;25:189–197. [PubMed: 10781026]
- Linster C, Hasselmo ME. Behavioral responses to aliphatic aldehydes can be predicted from known electrophysiological responses of mitral cells in the olfactory bulb. *Physiol Behav* 1999;66:497–502. [PubMed: 10357440]
- Linster C, Johnson BA, Yue E, Morse A, Xu Z, Hingco EE, Choi Y, Choi M, Messiha A, Leon M. Perceptual correlates of neural representations evoked by odorant enantiomers. *J Neurosci* 2001;21:9837–9843. [PubMed: 11739591]
- Mair RG, Gesteland RC. Response properties of mitral cells in the olfactory bulb of the neonatal rat. *Neuroscience* 1982;7:3117–3125. [PubMed: 7162628]
- Malun D, Brunjes P. Development of olfactory glomeruli: temporal and spatial interactions between olfactory receptor axons and mitral cells in opossums and rats. *J Comp Neurol* 1996;368:1–16. [PubMed: 8725290]
- Meister M, Bonhoeffer T. Tuning and topography in an odor map on the rat olfactory bulb. *J Neurosci* 2001;21:1351–1360. [PubMed: 11160406]
- Meynier A, Novelli E, Chizzolini R, Zanardi E, Gandemer G. Volatile compounds of commercial Milano salami. *Meat Sci* 1999;51:175–183.
- Olender T, Feldmesser E, Atarot T, Eisenstein M, Lancet D. The olfactory receptor universe--from whole genome analysis to structure and evolution. *Genet Mol Res* 2004;3:545–553. [PubMed: 15688320]
- Polak EH. Multiple profile-multiple receptor site model for vertebrate olfaction. *J Theor Biol* 1973;40:469–484. [PubMed: 4754892]
- Royet JP, Sicard G, Souchier C, Jourdan F. Specificity of spatial patterns of glomerular activation in the mouse olfactory bulb: computer-assisted image analysis of 2-deoxyglucose autoradiograms. *Brain Res* 1987;417:1–11. [PubMed: 3620970]
- Rubin BD, Katz LC. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 1999;23:499–511. [PubMed: 10433262]

- Sachse S, Rappert A, Galizia CG. The spatial representation of chemical structures in the antennal lobe of honeybees: steps towards the olfactory code. *Eur J Neurosci* 1999;11:3970–3982. [PubMed: 10583486]
- Schoenfeld TA, Cleland TA. The anatomical logic of smell. *Trends Neurosci* 2005;28:620–627. [PubMed: 16182387]
- Sicard G, Royet JP, Jourdan F. A comparative study of 2-deoxyglucose patterns of glomerular activation in the olfactory bulbs of C57 BL/6J and AKR/J mice. *Brain Res* 1989;481:325–334. [PubMed: 2720385]
- Singer MS, Weisinger-Lewin Y, Lancet D, Shepherd GM. Positive selection moments identify potential functional residues in human olfactory receptors. *Receptors Channels* 1996;4:141–147. [PubMed: 9014237]
- Uchida N, Takahashi YK, Tanifuji M, Mori K. Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. *Nat Neurosci* 2000;3:1035–1043. [PubMed: 11017177]
- Young JM, Trask BJ. The sense of smell: genomics of vertebrate odorant receptors. *Hum Mol Genet* 2002;11:1153–1160. [PubMed: 12015274]

Figure 1.

Molecular structures of the eight-carbon isomeric branched alkanes, octenes, and octynes, some of which have greatly different molecular shapes from the unbranched and saturated octane. Carbon and hydrogen atoms are represented by gray and blue solid spheres, respectively. Dotted areas with corresponding colors illustrate Van der Waals forces surrounding each atom. Greater overlaps in such force fields, illustrated by increased dot density, would result in higher steric hindrance. The combination of molecular shape and Van der Waals force fields results in distinct steric environments among these chemicals. The differences in the diameter of like atoms in the same molecule give a perspective of depth as provided by the molecular modeling software.

Figure 2.

Stacked bar graphs showing that the molecular property profiles of individual hydrocarbon odorants are distinct from each other. Each molecular property of individual odorants was expressed as a ratio of the sum of values obtained from all odorants tested within an experimental series, so that longer bars correspond to relatively higher values. Molecular properties of a homologous series of straight-chained alkanes, which have been shown previously to evoke significantly different glomerular response patterns, also are included here. The most profound differences among these hydrocarbons were observed with water solubility, vapor pressure, dipole moment, Hansen polarity, hydrogen bond acceptor and donor strengths, whereas the variance was comparatively less substantial for Log $P_{(o-w)}$, molecular length,

width, depth, volume, and surface area. The absence of bars for polarity, H bond acceptor strength, and H bond donor strength for straight-chained and branched alkanes indicates that the values for these properties were zero for all of the alkanes. Absolute values for the molecular properties of all odorants can be obtained from our new laboratory website (<http://leonsolver.bio.uci.edu/>) and from a supplementary table available at

Figure 3.

Color-coded contour charts reflecting glomerular activity evoked by eight-carbon isomeric alkanes with methyl branching variations. These charts represent a 2-D projection of the glomerular layer centered on the ventral aspect of the olfactory bulb. The average glomerular 2-DG uptake pattern for each odorant was calculated across matrices from five individual rats that previously had undergone the subtraction of vehicle-induced activity and z-score transformation. The z-score values are given in the color scale at the bottom right, directly below the anatomical orientation. Both the color scale and the orientation are applicable to all 2-DG uptake patterns included in this article. The overall activity patterns evoked by these odorant isomers were found to be quite similar. The greatest differences among these activity

patterns involved the anterior paired response foci produced by octane, the outline of which is traced in black on all charts. Differential activation of the outlined glomerular regions was observed depending on the number and the position of methyl branches along a molecule's hydrocarbon backbone. In general, reduced activation was evident with increasing number of methyl branches, so that greater dissimilarity in response patterns was found between the unbranched octane and the most branched isomers. In addition, increasing distance of methyl substitution from either end of the hydrocarbon backbone disrupts the activation of the same glomerular regions more profoundly.

Figure 4.

Bar graphs showing the mean investigation time ($\pm$ standard error) of various hydrocarbon odorants after habituation to octane. **A:** Behavioral responses to the isomeric branched alkanes were not significantly different from octane, despite significant differences (asterisk) between octane and the performance fatigue control odorants (Table 2). These results indicate an odor similarity perceived by the animals that was consistent with the highly similar glomerular responses evoked by these isomers. **B:** Behavioral responses to eight-carbon unsaturated hydrocarbons also were not significantly different from octane, despite significant differences (asterisk) between octane and the performance fatigue control odorants (Table 2). These results

indicate that the highly similar glomerular responses evoked by these compounds accurately predicted perceptual similarity.

Figure 5.

Color-coded contour charts reflecting glomerular activity evoked by straight-chained, eight-carbon hydrocarbons. These charts represent a 2-D projection of the glomerular layer centered on the ventral aspect of the olfactory bulb. Glomerular response patterns evoked by the unsaturated octenes and octynes were unexpectedly similar to the saturated octane. However, depending on the type, the position, the number, and the stereoconfiguration of the carbon-carbon bond, differential activation was observed in two main paired glomerular regions. One pair involved the anterior response foci of octane outlined in black, which were stimulated less strongly with decreasing bond saturation determined by the type and the number of unsaturated carbon-carbon bond. This was particularly evident for odorants possessing these bonds near

the center of the aliphatic backbone, where such bonds may have maximal steric influence on interactions with receptors. The same molecular changes increased activation in more dorsally paired regions indicated by white arrows.

Figure 6.

Bar graph showing the similarities in glomerular activity patterns between octane and each of the various other hydrocarbon odorants we have studied. Pattern similarity is expressed as Pearson correlation coefficients between pairs of data matrices. The results indicate that the rat olfactory system is relatively more sensitive to changes in carbon number. The homologous series of straight-chained alkanes (Ho et al., 2006), the eight-carbon isomeric branched alkanes, and the unsaturated hydrocarbons are represented by white, gray, and black bars, respectively. Despite profound differences in the molecular conformation and properties between octane and the branched and unsaturated odorants (Figs. 1 and 2), the activity patterns were as similar

to octane's as were those evoked by alkanes neighboring octane in the straight-chained homologous series.

Figure 7.

Scatter plot showing the relationship between differences in activity patterns and differences in molecular length. For each animal's odorant-evoked activity pattern matrix, we determined the cell-by-cell Pearson correlation coefficients resulting from pair-wise comparisons with the octane-evoked activity patterns obtained in the same study. For each odorant, we then determined the mean value for these coefficients to establish a measure of the overall pattern similarity to octane. We then plotted these pattern similarity values as a function of the absolute values of the differences in molecular length between each odorant chemical and octane. Molecular lengths were calculated using Molecular Modeling Pro software. The line is the result of a linear regression. The calculated correlation coefficient was 0.81 ($p < 0.0001$). The value of overall pattern similarity for octane versus octane (solid circle) represents the mean across all three studies of correlation coefficients resulting from all possible pair-wise combinations of patterns from individual rats exposed to octane within the same study.

Figure 8.

Scatter plot showing the relationship between similarity of 2-DG uptake pattern and perceptual similarity. For animals tested in an odorant habituation assay that used octane as the habituated odorant, we expressed the relative investigation time as a ratio to the average time spent investigating octane. These ratios then were averaged across the different rats exposed to the same odorant. These average values were plotted as a function of the similarity of the patterns to octane-evoked patterns as detailed in the legend for Figure 7.

Table 1

Information and Exposure Conditions of Odorants.

Odorant	CAS #	Molecular Formula	Vendor	Purity (%)	Dilution in Air	Number of rats
Octane ^I	111-65-9	C ₈ H ₁₈	A	>99.0	1/8	5
2-Methylheptane ^I	592-27-8	C ₈ H ₁₈	A	99.0	1/8	5
3-Methylheptane ^I	589-81-1	C ₈ H ₁₈	Al	98.0	1/8	5
4-Methylheptane ^I	589-53-7	C ₈ H ₁₈	TCI	>99.0	1/8	5
2,2-Dimethylhexane ^I	590-73-8	C ₈ H ₁₈	Al	98.0	1/8	5
2,3-Dimethylhexane ^I	584-94-1	C ₈ H ₁₈	TCI	94.0	1/8	5
2,4-Dimethylhexane ^I	589-43-5	C ₈ H ₁₈	TCI	98.0	1/8	5
2,5-Dimethylhexane ^I	592-13-2	C ₈ H ₁₈	A	99.0	1/8	5
2,2,4-Trimethylpentane ^I	540-84-1	C ₈ H ₁₈	Al	99.8	1/8	5
2,3,4-Trimethylpentane ^I	565-75-3	C ₈ H ₁₈	L	99.0	1/8	5
Octane	111-65-9	C ₈ H ₁₈	A	>99.0	1/18	5
1-Octene	111-66-0	C ₈ H ₁₆	A	>99.0	1/23	5
Trans-2-octene	13389-42-9	C ₈ H ₁₆	A	98.0	1/16	5
Trans-4-octene	14850-23-8	C ₈ H ₁₆	AA	97.0	1/17	5
Cis-4-octene	7642-15-1	C ₈ H ₁₆	AA	97.0	1/11	5
1,7-Octadiene	3710-30-3	C ₈ H ₁₄	A	98.5	1/8	5
1-Octyne	629-05-0	C ₈ H ₁₄	A	99	1/18	5
2-Octyne	2809-67-8	C ₈ H ₁₄	L	>98.0	1/14	5
4-Octyne	1942-45-6	C ₈ H ₁₄	AA	99.0	1/15	5
1,7-Octadiyne	871-84-1	C ₈ H ₁₀	A	99.0	1/20	5

A: Acros; Al: Aldrich; TCI: TCI America; L: Lancaster; AA: Alfa Aesar. Values for purity shown here were those reported by the vendors.

^I The odorant was diluted first in mineral oil at 1/10 of the total liquid volume before vaporization and dilution in air.

Table 2
Odorants for Individual Series of Behavioral Experiments.

Odorants	Branched alkanes 1	Branched alkanes 2	Octenes	Octynes
Familiar	Octane	Octane	Octane	Octane
Test	Octane	Octane	Octane	Octane
	2-Methylheptane	2,2-Dimethylhexane	1-Octene	1-Octyne
	3-Methylheptane	2,3-Dimethylhexane	Trans-2-octene	2-Octyne
	4-Methylheptane	2,4-Dimethylhexane	Trans-4-octene	4-Octyne
	2,2,4-Trimethylpentane	2,5-Dimethylhexane	Cis-4-octene	1,7-Octadiyne
	2,3,4-Trimethylpentane		1,7-Octadiene	
Fatigue control	m-Anisaldehyde	m-Anisaldehyde or L-Menthone	m-Anisaldehyde or Methyl valerate	m-Anisaldehyde

Table 3Correlations between similarities in odorant molecular properties and similarities in patterns of 2DG uptake.¹

property	correlation with pattern			correlation with length
	r	F	p	r
molecular length	0.81	52.9	<0.0001	1.00
vapor pressure (log)	0.75	34.6	<0.0001	0.92
molecular volume	0.71	27.2	<0.0001	0.87
surface area	0.70	26.4	<0.0001	0.87
log P	0.57	13.0	0.0012	0.72
Hansen polarity	0.51	9.5	0.0047	0.53
water solubility (log)	0.50	9.1	0.0056	0.65
H bond acceptor	0.43	6.2	0.0188	0.50
dipole moment	0.22	1.4	0.24	0.31
H bond donor	0.20	1.2	0.29	0.28
molecular depth	0.14	0.6	0.46	0.09
molecular width	0.09	0.2	0.64	0.08

¹For each of the 29 hydrocarbon odorants in this study, we determined the difference between each molecular property and the corresponding property for octane. We also determined the correlation coefficient between each rat's activity pattern and the average activity pattern for octane, and we calculated the average across animals for each odorant. For each property, we then determined the correlation between the difference in the property and the pattern correlation coefficient. We also show the correlation between each property and molecular length, which was the property that was most correlated with the pattern differences.