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Relationship between Molecular Structure, Concentration and Odor Qualities of Oxygenated Aliphatic Molecules

D.G. Laing, P.K. Legha, A.L. Jinks and I. Hutchinson

Centre For Advanced Food Research, College of Science, Technology and the Environment, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW, Australia, 1797

Correspondence should be sent to: Professor David G Laing, Centre For Advanced Food Research, College of Science, Technology and Environment, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW, Australia, 1797. e-mail: d.laing@uws.edu.au

Abstract

Increasing the concentration of an odorant increases the number of receptor cells and glomeruli in the olfactory bulb that are stimulated, and it is commonly acknowledged that these represent increased numbers of receptor types. Currently, it is not known whether a receptor type is associated with a unique quality and a unique molecular feature of an odorant, or its activation is used by the brain in a combinatorial manner with other activated receptor types to produce a characteristic quality. The present study investigated the proposal that a molecular feature common to several aliphatic odorants and known to be the key feature required to stimulate the same mitral cells in the olfactory bulb results in a quality that is common to the odorants. Since the common structural feature may activate a specific receptor type possibly at a similar concentration, the qualities of the odorants were determined at seven concentrations where the lowest and highest concentrations were the detection threshold (DT) and 729DT of each subject. A list of 146 descriptors was used by 15 subjects to describe the qualities of each odorant at each concentration. The results indicate that each of the five odorants was characterized by different qualities and the qualities of four of the odorants changed with changes in concentration. Importantly, no quality common to each of the odorants that had the same molecular feature could be identified and it is proposed that identification of the odorants occurs via a combinatorial mechanism involving several types of receptors.

Key words: humans, olfaction, odour profiles, thresholds, coding

Introduction

Relationships between the molecular structure and qualities of odorants have been sought by many workers (Guillot, 1948; Amoore, 1952, 1967; Wright, 1954). However, to date no structure–activity theory or model has been proposed that accounts for the wide range of odors encountered. In more recent times the advent of technologies based on genetics, molecular biology and computers, and new techniques for monitoring the responses of olfactory cells both at the periphery and centrally, have created new avenues for studying the relationship between molecular structure and odor qualities. Most prominent of the new discoveries of relevance to the present study are that humans have 500–1000 types of olfactory receptors (Buck and Axel, 1991), each receptor cell may carry only one receptor type (Chess *et al*., 1994), cells containing a particular receptor type project their axons to two glomeruli in the olfactory bulb (Mombaerts *et al*., 1996), and that the responses of the output neurons of the bulb, the mitral cells, reflect the responses of the receptor cells to which they are connected via synapses in the glomeruli (Imamura *et al*., 1992). Importantly, many of the recent studies have attempted to be systematic in their approach to studies of molecular structure and odors, and have used both homologous and analogous groups of aliphatic odorants. These studies have provided an insight as to how the olfactory system may code odors both at the level of the receptor cells and the various structures within the olfactory bulb. Araneda *et al.* (Araneda *et al.*, 2000), for example, showed that the length and branching of the carbon chain and the type of functional group were critical for activating a receptor that responded best to octanal. Indeed molecules without an aldehyde group failed to activate this receptor. In contrast, Imamura *et al*. (Imamura *et al*., 1992) found mitral cells in the dorso-medial region of the olfactory bulb that responded best to aliphatic oxygenated molecules with a similar carbon chain length. The oxygen-containing moiety in the stimulating odorants included aldehyde, ketone, ester or acid functional groups, with alcohols rarely activating these cells. Interestingly, each of the different types of oxygenated aliphatic molecules of similar chain length have very different odors. Since the responses of mitral cells are reported to reflect the responses of the corresponding

receptor cells and a receptor cell is reported to contain only one type of receptor, the different oxygenated odorants must activate at least one other type of receptor for them to be discriminated and identified by their own characteristic quality(s). This argument is supported by the finding of a receptor type by Araneda *et al*. (Araneda *et al*., 2000), as described above, that did not respond to oxygenated aliphatic molecules other than aldehydes with a similar carbon chain length. It is also supported by recent studies by Johnson and Leon (Johnson and Leon, 2000), who showed in studies of 2-DG activity in the olfactory bulb that aliphatic aldehydes were characterized by activation of glomeruli in the lateral and medial regions.

The latter findings raise an interesting question regarding whether an odor quality arises from the activation of a single receptor type or from an odor molecule interacting with two or more receptor types where it may adopt different conformations to be successful. Thus, in the case of the receptor that responded to an aliphatic aldehyde, ketone, ester or acid of similar carbon chain length (Imamura *et al*., 1992), these molecules must have adopted a similar conformation to activate the receptor. If activation of a single type of receptor produces a specific odor quality then all the molecules that activated the latter receptor may have a quality that is common to all. However, since each odorant must activate another receptor(s) type to allow each to be discriminated and identified, each must have been able to adopt a different and unique conformation that could not be achieved by the other odorants, but that clearly involved their only unique feature, their functional group. Accordingly, an aim of the present study is to determine whether aliphatic oxygenated odorants of similar chain length but having a different functional group (Figure 1) share a common quality that could reflect activation of a common receptor type(s). If this proposal is correct each odorant should also be characterized by at least a second non-common quality suggesting that two or more receptor types were activated. Failure to find a common quality should support the view that odor quality and identification of an odorant is achieved by a combinatorial mechanism that produces a composite of the pattern of activation in memory (Malnic *et al*., 1999). To date no study has investigated the possibility that an odor quality may arise from activation of a single receptor type. The exception to this could be studies with pheromones particularly in insects, where it is not unusual for an insect to have a specialist receptor cell type that has a high sensitivity to a single odorant, a pheromone, and presumably a single quality is registered by the insect.

The present study also examines another aspect of odor quality, namely, the relationship between quality and concentration. Electrophysiological studies of aliphatic odorants have shown that an olfactory receptor cell can have a very narrow response spectrum at low concentrations which enlarges substantially as the odorant concentration is increased (Sato *et al*., 1994). Thus, it was demonstrated that a receptor cell may respond to only one odorant at near threshold concentrations, e.g. a C7 alcohol, but to gradually increase its response spectrum to increasingly shorter or longer chain aliphatic alcohols in a systematic manner as the concentrations of the odorants are increased. This finding indicates that the single C7 odorant had a structure that best fitted the receptor and consequently had the higher probability of activating the receptor than related molecules used. Accordingly, if activation of a single receptor type is sufficient to produce a characteristic odor quality, it is possible that the molecular feature of heptanal, for example, that activated specific mitral cells (Imamura *et al*., 1992) and the corresponding receptor type, is the same as that shared by the other carbonyl-containing aliphatic odorants, i.e. ketone, ester and acid with a similar chain length. A second aim of this study, therefore, is concerned with determining the odor qualities of the latter odorants over a range of concentrations from the detection threshold (DT) to high levels to ascertain whether commonality of odor quality occurs at a specific concentration as a result of a common receptor type(s) being activated. Commonality at near threshold levels would support the proposal that a single receptor type had been activated to produce a single quality, whilst if it occurred at high concentrations activation of a common set of receptor types in a combinatorial mechanism for the production of an odor quality(s) would be more likely. To determine the importance of concentration on the odor quality(s) of the oxygenated aliphatics, the detection threshold of each subject was established before profiling of the odor qualities of each odorant over a range of concentrations was undertaken. Establishing the thresholds of subjects allowed a comparison of the quality(s) each subject perceived at equivalent supra-threshold concentrations. With one exception where only one supra-threshold concentration was used (Stevens and O'Connell, 1991), to our knowledge this 'equivalence' approach to describing odor qualities has not been reported.

In summary, the study has two main aims. First, to determine whether aliphatic oxygenated odorants having the same hydrocarbon chain length and a carbonyl group in an equivalent position (Figure 1) share a common quality that could reflect activation of a common receptor type. If this proposal is correct, to be discriminated from each other, each odorant should also be characterized by at least a second non-common quality indicating that two or more receptor types were activated. A second and complementary aim is concerned with determining the odor qualities of the five test odorants over a range of concentrations from the detection threshold to high levels to ascertain whether commonality of odor quality occurs at a specific concentration as a result of a common receptor type(s) being activated.

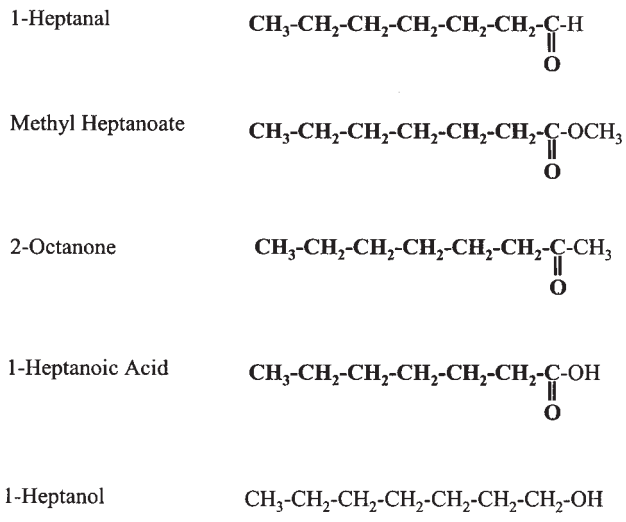

Figure 1 Molecular structures of the test odorants. Bold type indicates the common structural feature of the carbonyl-containing odorants.

Materials and methods

Subjects

Overall, 37 subjects (33 female and 4 male) aged between 18 and 47 years of age (mean ± SD = 29.7 ± 11.3 years) who were staff or students at the university or were residents from local suburbs, participated in the study. During the measurement of the detection threshold of a single odorant and its quality profile 15 of these subjects participated. Some of the subjects had experience in sensory studies and all received payment for participating. The study was conducted within the air-conditioned sensory facilities of the University.

Odorants

The main test substances (Fluka, Switzerland) and their purities were the oxygenated aliphatic odorants 1-heptanal (pract > 95%), 2-octanone (purum > 97%), 1-heptanoic acid (puriss > 99%), methyl heptanoate (puriss > 99%) and 1-heptanol (purum > 99%). The diluent used was 1,2-propandiol (99.5%, Aldrich, Sydney, Australia).

Training and test procedures

Thresholds

The detection threshold of a subject for an odorant was determined at a single test session using the staircase method (Cornsweet, 1962). Eleven concentrations of each odorant with a dilution factor of 2 between each concentration were prepared and presented in 250 ml polyethylene squeeze bottles (20 ml aliquot in each) equipped with a flip-top spout. To minimize the possibility of spillage each bottle contained odor-free cotton wool which absorbed the odorant. Subjects were instructed as to the manner of sampling, and at the start of the first session were allowed time to familiarize themselves with the handling of the bottles and the sampling technique. Care was taken that the flip-up spout was only a short distance (1–2 cm) from the nasal septum during sampling of an odorant in order to maximize the chance that the stimulus entered both nostrils. In the two-alternative forced-choice method used for presenting the samples, one bottle contained the odorant and solvent, the other only the solvent. At each test trial a subject was asked to sniff both bottles in the order prescribed and to identify 'which of the two bottles produced the stronger sensation/smell'. Between each pair of samples there was an inter-trial interval of at least 20 s. The sequence of presenting each pair of bottles was systematically varied between sessions and individual subjects. No feedback regarding the correctness of a response was given. As is usual in the staircase procedure, the test stimulus in the first trial was always the middle dilution level. If the response was incorrect, the next stimulus was two concentration steps higher. If the responses to two trials at that concentration were correct, the concentration presented in the next trial decreased by one step. Two correct trials at that concentration initiated counting of reversals. The criterion used for establishing a threshold was that six consecutive reversals had to occur within a range of three dilution steps. The detection threshold of a subject was calculated as the geometric mean of the six reversals.

Odor profiles

As in the threshold tests, sniff bottles were used to present the odorants. Subjects attended one short familiarization session before two test sessions (replicates) with an odorant. During the familiarization session subjects were given a list of 146 odour qualities (Dravnieks, 1985) where each quality had a 9-point category rating scale ranging from zero (no smell) to nine (extremely strong smell), and a sniff bottle that contained a weak to moderate strength of iso-amyl acetate (50 000 p.p.m. extra pure, banana/fruity; Reidel Haen AG, Germany). The familiarization odorant was changed to 2-phenyl ethanol (25 000 p.p.m., purum > 99%, floral, rose-like; Fluka) before the test session with methyl heptanone since the latter odorant and the acetate were characterized by fruity qualities and subjects may have tended to use descriptors of the acetate as a result of their exposure to it immediately before the test session. Subjects were told that a sample bottle may have as many as 146 odour qualities or none, and that they should use only odor qualities which appeared relevant to the stimulus being assessed. If they did not find any appropriate odor qualities the scoresheet should be left blank. If an odor quality was chosen they should indicate the strength of the odor on the rating scale.

During each of the two test sessions with a particular odorant, a subject assessed the odor qualities and perceived intensity of 14 samples. These consisted of seven concentrations of the test odorant, e.g. heptanal, at concentrations

which were 1, 3, 9, 27, 81, 243 and 729 times the detection threshold (DT) of that particular subject, six 'distracter' odorants of moderate perceived intensity and a sample of the solvent propandiol. The 'distracters' were odorants that had very different odor qualities to those of the five test odorants and were included because it was observed during pilot studies that subjects very quickly became demotivated to profiling the same odorant at several concentrations. Accordingly, to maximize the chance that subjects attended fully to the qualities of the test stimuli the order of presenting the six 'distracters', seven samples of the test stimulus and the solvent was randomized. Furthermore, to assist attention and minimize olfactory adaptation, the 14 samples were presented in two blocks of seven, 30 min apart. The seven samples always contained three of the 'distracters' randomly arranged with the other four samples. Each subject received different randomized sequences which varied across odorants and sessions. There was a 1 min interval between the assessment of each of the seven samples and subjects could sniff a sample as many times as necessary to produce a profile. The order of assessing the 146 odor qualities was also randomized across subjects and sessions. The 'distracters' were anisole (1000 p.p.m., purum > 99%; Fluka), eugenol (25 000 p.p.m., purum > 99%; Fluka), butanol (25 000 p.p.m.; Fluka, for UV spectroscopy), triethylamine (1000 p.p.m., 99%; Aldrich), furaneol (50 000 p.p.m., 15% in propylene glycol; Dragoco, Sydney, Australia), and galaxolide (250 000 p.p.m., 50% in diethylphthalate; International Flavour and Fragrances, Sydney, Australia).

Results

Odor thresholds

The distributions of thresholds of the individual subjects for each odorant are given in Figure 2. As expected, subjects differed widely in their sensitivities for each odorant, the difference between the most and least sensitive subjects with each of the odorants was heptanal (×92), methyl heptanoate (×140), octanone (×488), heptanoic acid (×46.2) and heptanol (×48). Such differences in sensitivity vindicates the approach adopted in this study to obtain the responses of individual subjects to the qualities of equivalent concentrations of an odorant derived from the detection thresholds of the individuals. Although individuals may also differ in their intensity–concentration response functions, and alter the perceptual equivalence of concentrations, it has been shown that subjects with lower thresholds provide higher intensity estimates of concentration than those with high thresholds (Laing *et al.*, 1978), suggesting the present equivalence assumption is a reasonable one. As regards comparing the mean thresholds with those in the literature, no thresholds for these odorants in propandiol could be found. Thresholds for the odorants have been reported in water to be substantially lower than found here (Fazzalari, 1978). However, this is not surprising given the different solvent/air partition coefficients that produce higher headspace concentrations when the odorants are in water compared to propandiol.

Odor qualities

For each of the 14 stimulus conditions (seven concentrations of the test odorant plus one solvent and six distracters), the relative contribution of a descriptor was based on a weighted score that was dependent on the frequency of use of a descriptor and perceived intensity ratings (Jinks and Laing, 2001). In these calculations the data from all subjects at a particular level relative to the detection threshold ('equivalent concentration') of each subject were combined. The relative contribution of a descriptor, therefore, was calculated by (i) examining the data of individual subjects separately and adding the two replicate perceived intensity ratings of each descriptor (if a descriptor was not selected it was given a score of zero), e.g. if a descriptor was chosen twice by a subject and given ratings of 5 and 6, a value of 11 would be obtained; (ii) multiplying the sum of the ratings for a descriptor by the number of times a non-zero rating was chosen, e.g. $11 \times 2 = 22$; (iii) adding the totals across all subjects for individual descriptors relative to the detection threshold at the specific level; and (iv) multiplying the summed value for a descriptor with the total number of times the descriptor was chosen over all subjects. The two multiplication steps allowed descriptors that were chosen consistently by the same subject and by many subjects to achieve high scores, thereby indicating the relative contribution of the descriptor to the overall quality of the stimulus. The highest possible weighted score was 16 200. The weighting system, therefore, ensured that both the quality and intensity of an odorant contributed to the estimation of its importance, and reduced the possibility of a descriptor being considered important on the basis of having a high-perceived intensity but low selection frequency. The final weighted score for a quality was obtained by subtracting the value recorded for the solvent, from the weighted score calculated for the odorant. The data included all descriptors used by any subject, even if used only once by only one subject. Applying similar criteria to that used previously (Jinks and Laing, 2001) only the descriptors with the five highest weighted scores for each odorant at each concentration were used as indicators of odor quality (Figure 3). The most striking features of the data in Figure 3 are as follows.

(i) For each odorant there is a clear trend to different descriptors as the concentration changed from low to high levels. Even at a concentration which was 27DT when an odorant can clearly be perceived, the main quality(s) was not necessarily the one(s) that dominated at higher concentration levels. For example, although 'oily' and 'pineapple' are the main descriptors of heptanal and methyl heptanoate at 729DT, respectively, only 'oily' is included as one of the

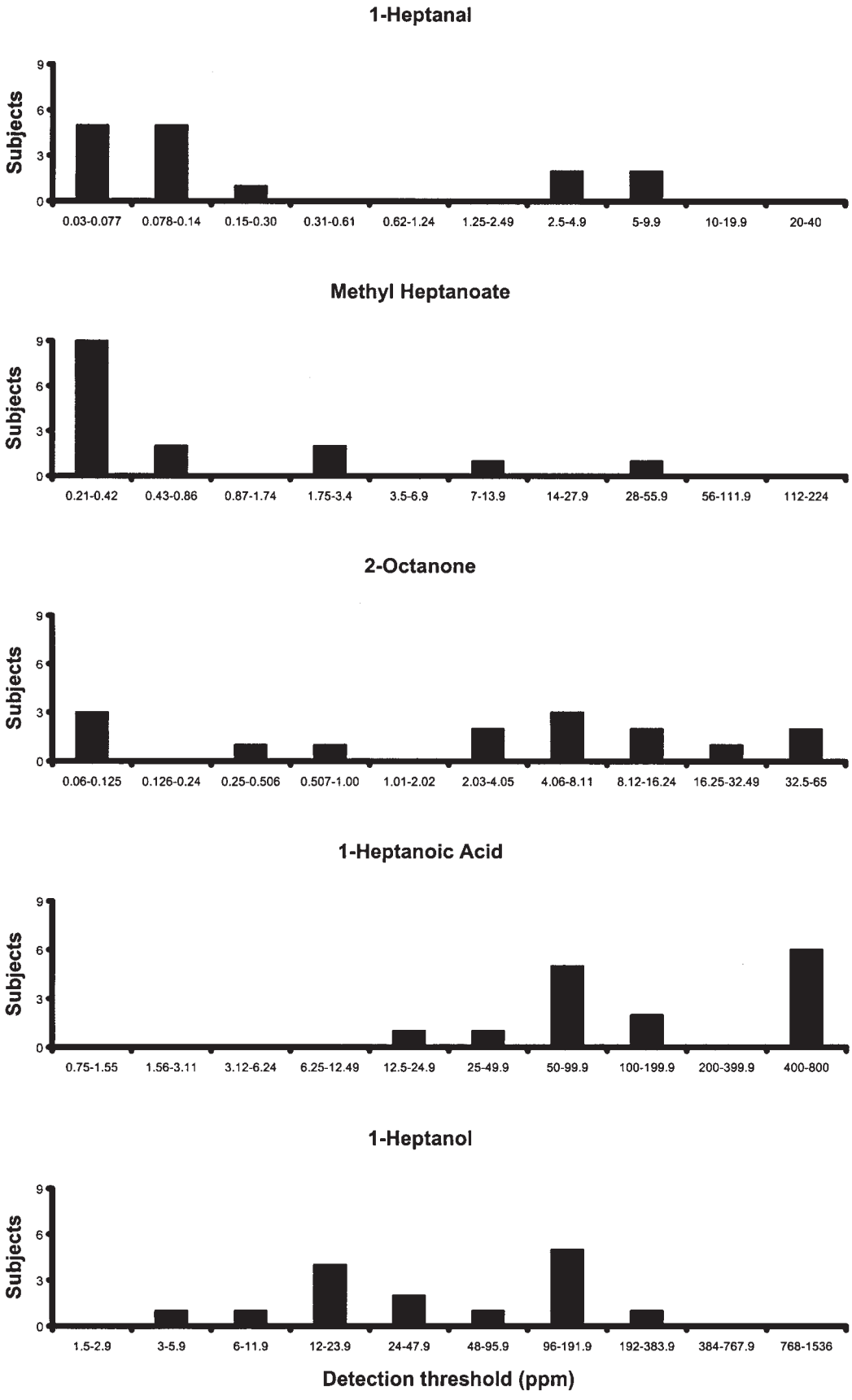

Figure 2 Frequency histograms of the distributions of subjects' thresholds for each odorant.

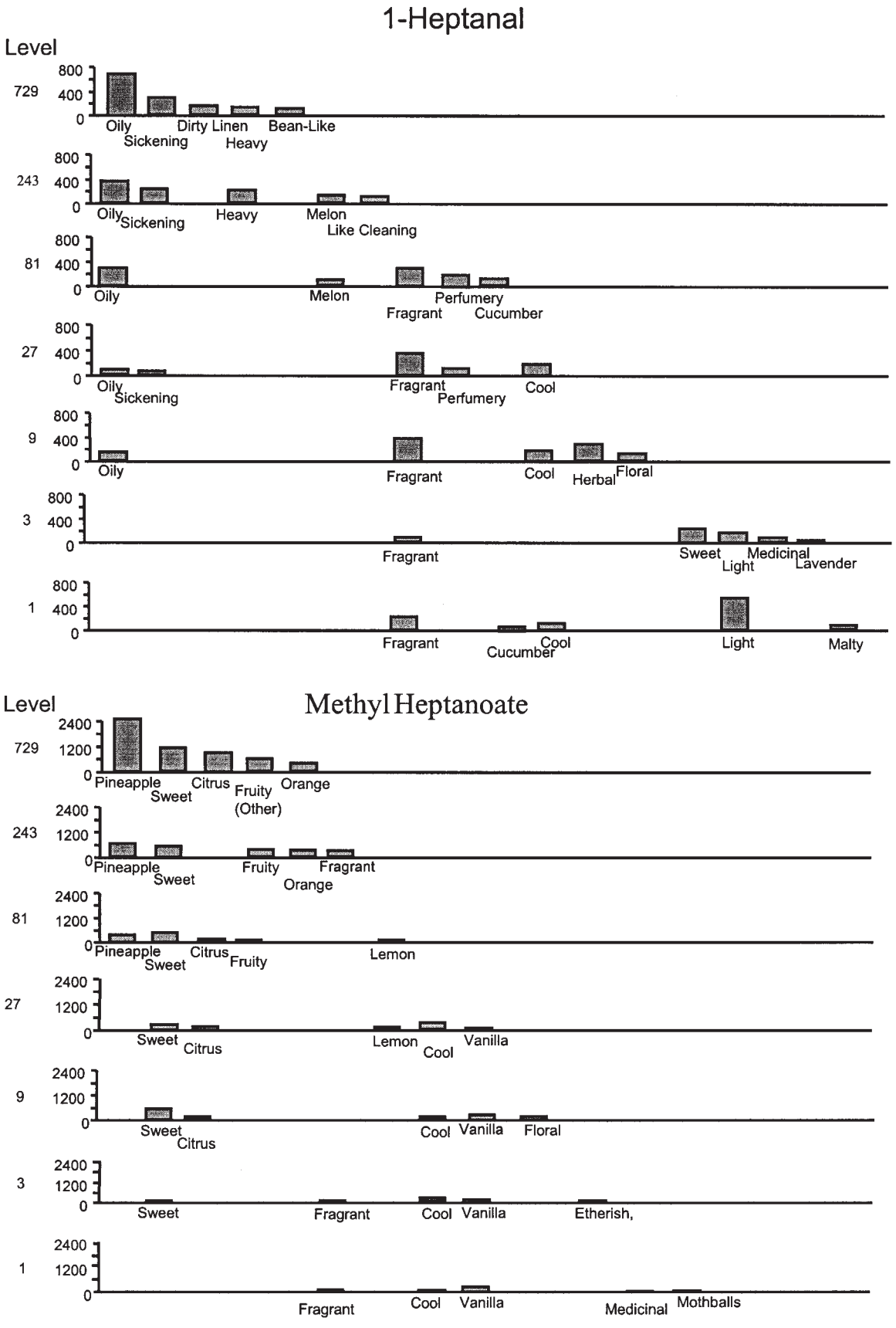

Figure 3 Part 1.

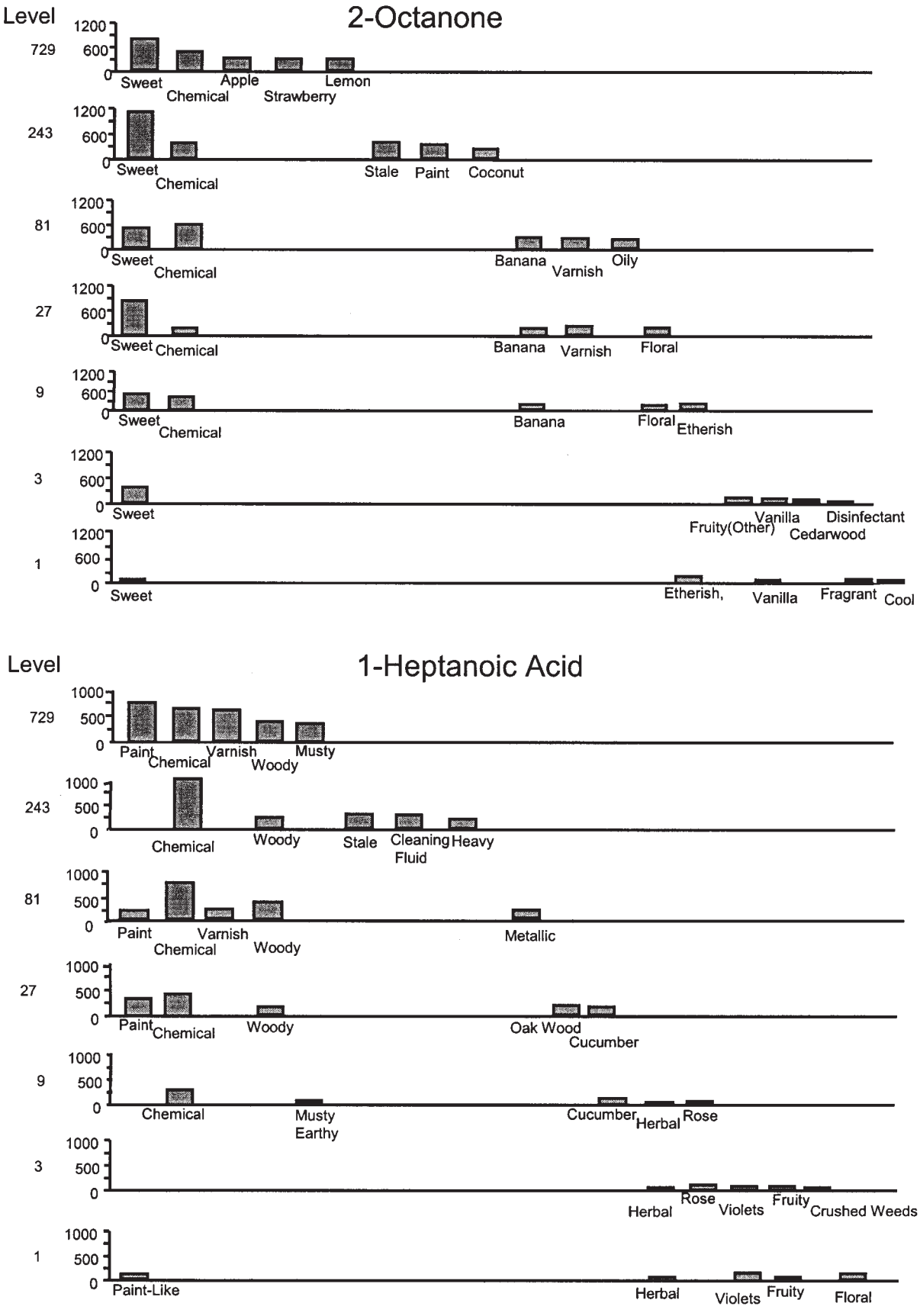

Figure 3 Part 2.

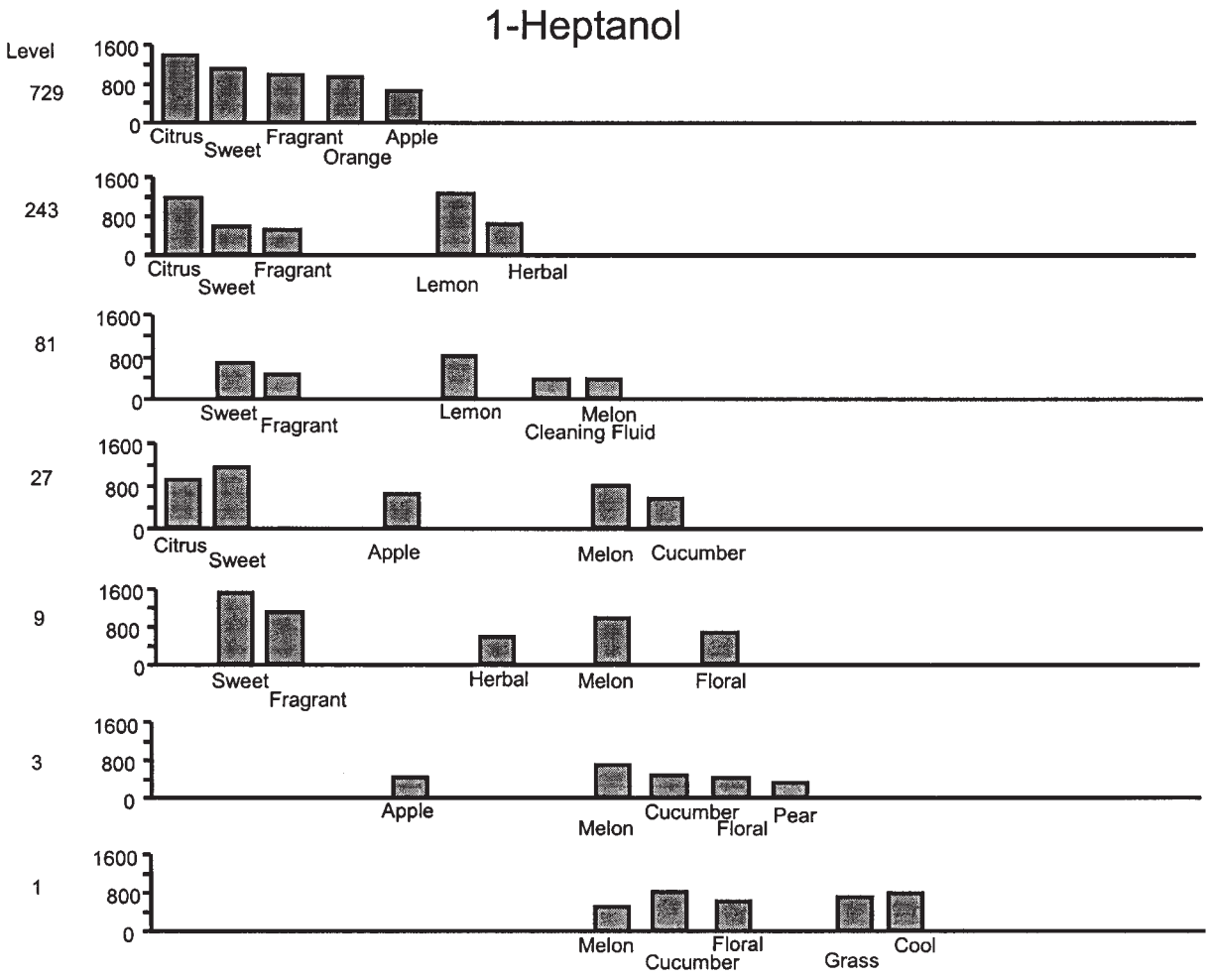

Figure 3 Odor quality profiles. The values in the column labeled 'Level' refer to the concentration above the detection threshold of each subject. Values on the ordinate are the calculated weighted contributions of each descriptor to the quality of an odorant. Words on the abscissa are the descriptors with the five highest weighted scores.

five main descriptors of heptanal at 27DT, but is not the dominant one. Furthermore, examination of the profiles of each of the five odorants shows that even at 81DT which is substantially above the detection threshold, no quality which is the dominant one at 729DT is the dominant one at this lower level.

(ii) The odorants differ in the set of descriptors each has at the highest concentrations indicating that they smell differently as is found during the casual sampling of each. Heptanal was characterized by oily, unpleasant qualities, methyl heptanoate was like pineapple, sweet and citrus, octanone was sweet, chemical and non-citrus, heptanoic acid was like paint and chemical, and heptanol was citrus and sweet. Interestingly, although the concentration range was substantial, i.e. 729DT, the change in some of the qualities from 243DT to 729DT suggests that an asymptote in perceived intensity was not reached with any of the odorants. Other evidence (Berglund *et al.*, 1978) supports this suggestion since no asymptotes were reached with ranges of 891 and 5238 with the odorants butanol and hydrogen sulphide, respectively.

(iii) Unlike some odorants which have a single dominant quality and smaller secondary qualities, e.g. carvone is largely spearmint, naphthalene is mothballs, *cis*-3-hexenal is cut-grass, methyl salicylate is wintergreen, none of the five odorants had a weighted score for a quality as high as found in an earlier study with four odorants that were chosen because one quality dominated at moderate to high concentrations (Jinks and Laing, 2001). This suggests that subjects in the present study found it difficult to find a single descriptor that adequately described these fairly nondescript odors so that across the subject group a variety of descriptors were chosen with few being selected by more than about half the subjects. Consequently, they made use of several complementary descriptors to describe the qualities they perceived. Thus, with heptanoic acid the descriptors 'paint'

and 'varnish' and possibly 'chemical' may have been the closest descriptors to a particular quality that did not have a descriptor in the list that provided an exact description. With heptanal, almost all the main descriptors at the two highest concentrations were of unpleasant sensations and again may have been the closest ones to the quality(s) perceived. Importantly, the main descriptors found here were similar to those reported by Dravnieks (Dravnieks, 1985). For example, 'oily/fatty' and 'sickening' were common to both studies for heptanal, and 'citrus/lemon/orange' and 'fragrant/sweet' were common for heptanol.

(iv) At the lower concentrations the main descriptors were generally of pleasant qualities. With heptanal, methyl heptanoate and octanone, each had several main descriptors of the group fragrant/floral/sweet/vanilla/cool/light suggesting that they may share some quality(s) at low concentrations. In contrast, the descriptors of low concentrations of the non-carbonyl odorant heptanol suggested green/floral/fruity qualities may exist, as is suggested for heptanoic acid, the latter being linked to the green qualities cucumber/herbal/crushed grass and floral qualities of rose/violets/floral.

Since the results above suggested there was a change in the quality(s) of each odorant as the concentration increased above the detection threshold, a principal component analysis (PCA) was conducted to explore the factor structure of each odorant and concentration and to compare the factor structures amongst the stimulus conditions. In brief, the analysis provided information about the relative similarity of each of the descriptor profiles and a more quantitative assessment of the existence of a quality change. Thus, a low similarity between the profiles of an odorant at low and high concentrations would indicate a change in quality had occurred, whilst high similarity would indicate there was little change in the qualities. In this analysis all the data from the list of 146 descriptors were included for each odorant and concentration, but not for the distracters. Since moderate correlations were obtained between some factors (<0.45) a non-orthogonal rotation of the components was conducted to provide the best solution. This analysis found nine rotated components had an eigen value of >1. To ascertain the extent of the similarities between odorants and between the seven concentrations of an odorant, the components with the greatest loadings (>0.2) for an odorant at each concentration were tabulated (Table 1). This showed that for heptanal the components at 3DT were different to those at 9–81DT which were different to the single common component at 243 and 729DT; for methyl heptanoate the same component at DT and 3DT differed in part from those at 9–27DT, following which there was a transition in the components which stabilised at 81 and 243DT, changing again as the concentration was increased to 729DT; for octanone the same main component was present at concentrations from 3 to 729DT; for heptanoic acid the main component was the same at DT and 3DT but changed to a new one at 9DT, which was the main component for all concentrations above this; for heptanol the two lowest concentrations had the same single component, there was a transition of components over the range 9–81DT, and a single main common component at 243 and 729DT. Since the data used in the PCA were composed of odor quality and intensity information, it seems likely that the changes in components that occurred as the concentration changed primarily represented changes in the quality(s) perceived. Indeed, there was no indication that any of the components specifically represented intensity or hedonics. Accordingly, quality changes appear to have occurred with each odorant except octanone, with the data suggesting that three changes in quality may have occurred with heptanal, methyl heptanoate and heptanol, and a single change with heptanoic acid.

As regards the components of the odorants at and near to the level of the detection threshold, no component was common across the odorants indicating that a common quality for several of the odorants at these levels did not exist. The finding that each odorant at the lowest concentration had a unique component or set of components (2-octanone) indicates that over the two replicates individuals varied in their sensitivity sufficiently to sense odorants on at least one of these occasions. If their sensitivity had not varied it would be expected that for all five odorants no component would have been recorded since by definition no quality can be perceived at the detection threshold.

The data in Table 1 also show that there is no evidence for commonality of quality across the odorants at any of the concentration levels. Different components were important to different odors. For example, at the highest concentration no component was common across the odorants. These results are in agreement with the quality profiles in Figure 3 that suggest each odor has its own unique set of profiles across the concentrations used, that is different from that of the other odorants.

The five main qualities of each of the distracter odorants when these odorants were included in a session with each of the test odorants are given in Table 2. The two most important findings are that (i) the main qualities of the distracters appear to be largely unchanged regardless of the test odorant used. If not listed in the top five, the main qualities were almost always within the top ten (not shown here); and (ii) when the qualities of the test odorants and distracters are compared, there appears to be no influence of one on the other. Thus, no descriptor common to one odorant or distracter appeared in a profile of the other because one or the other had just been assessed. In other words, the strategy of incorporating distracter odorants to assist subjects in attending to the qualities of a particular test odorant that was presented several times at different concentrations was successful. Other evidence for this is the changing qualities of the test odorants as their concentrations were altered.

Table 1 Main component loadings in PCA for each of the stimuli

	Component loadings[a]								
Odorant	1	2	3	4	5	6	7	8	9
1-Heptanal									
1[b]					0.939				
2					0.952				
3			0.690		0.453				
4			0.875						
5			0.771		0.200				
6	0.644					0.603			
7						0.932			
Methyl heptanoate									
1						0.981			
2	0.289							0.683	
3	0.578							0.490	
4				0.658				0.419	
5	0.658			0.476					
6				0.944					
7				0.857					
2-Octanone									
1	0.810	0.255							
2	0.840								
3	0.706	0.436							
4	0.893								
5	0.815	0.344							
6	0.813			0.281					
7	0.404		0.355	0.448					
1-Heptanoic acid									
1		0.788							
2		0.919							
3		0.941							
4		0.831							
5		0.878							
6							0.869		
7							0.832		
1-Heptanol									
1								0.763	
2								0.925	
3								0.685	0.301
4	0.265								0.515
5	0.449		0.249						0.409
6									0.923
7									0.742

[a]Only loadings of 0.200 and above are included.

[b]Odorant concentrations were: 1, 729DT; 2, 243DT; 3, 81DT; 4, 27DT; 5, 9DT; 6, 3DT; 7, DT.

Discussion

The present study investigated two proposals. First, whether four odorants that were characterized by a seven-membered hydrocarbon chain with a carbonyl group in an equivalent position shared a common odor quality (s) that is absent in the non-carbonyl analogue heptanol. Secondly, if a common quality(s) was shared by the carbonyl odorants, whether this was perceived at a specific concentration(s) relative to the detection threshold. The results indicated that no quality could be identified as common to the carbonyl-containing odorants, the five odorants differed in their major qualities (Figure 3, Table 1), and changes in qualities occurred with four of the odorants as their concentration was varied (Table 1).

The absence of a quality common to each of the carbonyl-containing odorants does not support the proposal that a structural feature of a molecule rather than the whole molecule can produce a quality. The driving force for this

Table 2 Top five odor qualities in descending order (based on weighted score) of odorous chemicals used as distracters for profiling with the five test odorants

	Test odorant				
Distracter	1-Heptanal	Methyl heptanoate	2-Octanone	1-Heptanoic Acid	1-Heptanol
Butanol	chemical sickening like cleaning fluid nail polish remover varnish	chemical varnish nail polish remover turpentine (pine oil) sharp, pungent, acid	nail polish remover sickening varnish chemical paint-like	chemical like cleaning fluid paint-like nail polish remover sickening	chemical nail polish remover alcohol-like sharp, pungent, acid medicinal
Furaneol	sweet caramel burnt milk molasses burnt, smokey	caramel sweet burnt milk burnt, smokey malty	caramel sweet burnt milk molasses burnt, smokey	sweet caramel vanilla-like burnt milk maple (as in syrup)	sweet caramel burnt, smokey maple (as in syrup) burnt milk
Eugenol	clove-like cinnamon spicy fragrant etherish, anaesthetic	clove-like spicy aromatic cinnamon fragrant	clove-like spicy cinnamon medicinal perfumery	clove-like spicy cinnamon aromatic fragrant	clove-like cinnamon spicy aromatic sweet
Anisole	nail polish remover varnish chemical paint-like turpentine (pine oil)	varnish nail polish remover chemical paint-like etherish, anaesthetic	varnish paint-like chemical like petrol solvent nail polish remover	paint-like turpentine (pine oil) chemical nail polish remover like cleaning fluid	chemical nail polish remover like petrol solvent like cleaning fluid sharp, pungent, acid
Galaxolide	perfumery fragrant rose-like cologne sweet	perfumery fragrant sweet floral cologne	perfumery floral fragrant light sweet	perfumery fragrant cologne soapy floral	perfumery sweet fragrant cologne floral
Triethylamine	sickening putrid, foul, decayed urine-like cat-urine-like like ammonia	sickening putrid,foul, decayed like ammonia urine-like cat-urine-like	urine-like. cat-urine-like sickening putrid, foul, decayed like ammonia	stale putrid,foul,decayed urine-like cat-urine-like sickening	putrid,foul, decayed sickening sharp, pungent, acid like cleaning fluid rancid

proposal were the findings by Imamura *et al.* (Imamura *et al.*, 1992) that (i) a single mitral cell could be activated by the four different types of carbonyl-containing odorants used here, whose common structural feature was the same hydrocarbon chain length and a carbonyl group in the equivalent position in each; and (ii) the responses of a mitral cell reflected those of the corresponding receptor. Since each of the odorants is characterized by different odor qualities, and provided as assumed here that the human olfactory system operates similarly to other mammals, it now seems more likely that activation of a common mitral cell(s) provides only part of the spatial code that is used to identify each odorant. As mentioned in the introduction, each odorant must activate at least one other type of receptor to be identified suggesting that a combination of inputs from several receptor types is required.

One clear outcome of the present study was the change in the type of odor qualities that occurred with four of the odorants as their concentration was increased. Qualities that were reported at low concentrations were usually not sensed at high concentrations and vice versa. Such changes have been reported by perfumers and flavorists for a small proportion of odorants (Arctander, 1969). Indeed, some odors which have been described as unpleasant at high concentrations are different and pleasant at low levels. For example, indole is putrid at high concentrations and has a floral odor at low concentrations. Gross-Isseroff and Lancet (Gross-Isseroff and Lancet, 1988) also reported that

changes in quality occur as the concentration is changed. Recently an insight to the underlying mechanisms of quality change was provided by Johnson and Leon (Johnson and Leon, 2000), who reported that the patterns of glomeruli in the rat which were activated by pentanal and 2-hexanone, homologues of two of the odorants used here, shifted as the concentrations of the odorants increased. The shifts in location were described as being as large as those found between the locations of glomeruli for different odorants. Both of these odorants were chosen by Johnson and Leon because they have been reported by humans to have different qualities at different concentrations (Arctander, 1969). In contrast, the other odorants used in their study, namely, pentanoic acid, methyl pentanoate and pentanol, all homologues of the odorants used here, showed no shift in location of the glomeruli, simply increases in the number of glomeruli in the regions activated by low concentrations of the odorants. The results of the present study where changes in quality occurred as the concentration was altered suggests that shifts in the position of activated glomeruli would occur if they were the stimulating odorants.

The most obvious differences in quality found here between the odorants occurred at the highest concentrations. This was not unexpected since informal sampling at these concentrations had indicated that all five are easily distinguished, and reported descriptions also indicate that they are different. The ease of discrimination is in accord with the greater probability that more receptor types would be activated at the higher concentrations as is indicated by the corresponding increase in the number of glomeruli activated (Johnson and Leon, 2000). Since each odorant is characterized by a different functional group, they could be expected to activate different types of receptors which may represent different qualities, or the receptor outputs could be combined to produce a single or several qualities characteristic of each odorant. As regards the present data, even allowing for semantic redundancy, e.g. 'paint' and 'varnish' ((heptanoic acid), 'pineapple', 'sweet' and 'fruity' (methyl heptanoate), it seems likely that each odorant is represented by more than a single quality arising from more than a single combinatorial mechanism (Malnic *et al.*, 1999).

The greatest similarity between the main qualities of the odorants occurred at the lower concentrations. Whether this similarity was a consequence of the perceived intensities of the odorants being low resulting in subjects using descriptors that tended to represent qualities which were generally pleasant *per se* is not known. However, the data from the PCA do not support the existence of a common quality across the odorants that have a common structural feature, at these or any other concentration.

Another interesting finding of the study was that the dominant quality at the highest concentration was sometimes identified at low concentrations, though not as the dominant one. Since there is a greater chance that a quality sensed at low concentrations is activating a single receptor type (Sato *et al.*, 1994), the identification of 'oily' (heptanal) and 'sweet' (octanone) at concentrations of 9DT and DT, respectively, suggests these major distinguishing qualities may each activate a highly specific receptor type. This is particularly so for octanone since no quality change was detected by the PCA. In contrast, the 'pineapple' quality of methyl heptanoate which was not sensed until 81DT is more likely to have resulted from the combined inputs of several receptor types with no specific receptor signaling this quality.

Finally, the absence of descriptors that indicated trigeminal stimulation particularly at the higher concentrations, was unexpected. The trigeminal nerve has been shown to modulate, namely inhibit, receptor cell activity (Bouvet *et al.*, 1987), which could be expected to reduce or block perception of one or more of the odor qualities of an odorant. Furthermore, although human trigeminal thresholds can occur at substantially higher concentrations than olfactory thresholds e.g., the trigeminal thresholds of methyl ethyl ketone and furfural were reported to be about 2 log units higher than the olfactory thresholds (Doty, 1975), the difference can be much less as occurs with acetic acid, propionic acid and amyl acetate where the differences were 30, 50 and 200 times (Walker and Jennings, 1991). Both of these studies, therefore, suggest that at least with the three highest concentrations used in the present study, trigeminal stimulation and possibly confounding of qualities could have occurred. However, there is little evidence that this happened. Although many trigeminal descriptors such as 'sharp', 'acid', 'vinegar', 'pungent', 'cool', 'ammonia', 'alcohol-like', 'etherish', 'eucalyptus', 'spicy', 'kerosene', 'petrol' were included in the list of 146 used to profile the odorants, none appeared as major quality contributors. Importantly, none of the main descriptors for heptanal, methyl heptanoate, or heptanol appear to describe trigeminal activation. The only possibilities occurred with octanone where the descriptor 'chemical' was used and with heptanoic acid where three of the main descriptors were 'paint', 'chemical' and 'varnish', all of which are candidates. However, with both octanone and heptanoic acid 'chemical' was used with all stimuli from as low as nine times the detection threshold (9DT). Since it seems unlikely that these odorants would have stimulated the trigeminal nerve at a concentration as low as this, there is little evidence that stimulation of the trigeminal nerve produced qualities that may have confounded the descriptions of olfactory-relevant qualities for each of the odorants.

In summary, the data obtained in the present study indicate that the four carbonyl-containing odorants that have a common structural feature have qualities which make them readily distinguishable from each other and heptanol. No common quality for these odorants was identified that could be attributed to their common structural feature. Four of the odorants exhibited changes in quality(s) as their concentration was increased above the detection threshold,

suggesting that the recruitment of new receptor types occurred. Finally, the data suggest that a combinatorial mechanism is used by the olfactory system for coding the identity of each of the odorants.

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