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Research report

Carbon chain length and the stimulus problem in olfaction

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ABSTRACT

Understanding how odour quality perception is encoded in its molecular properties arguably poses one of the most significant problems in olfaction. Determining the odour structure–quality relationships of structurally similar odorants could provide a key tool to this problem. We tentatively explored whether a mixture of two molecules, differing only in carbon chain length (C), would yield the same percept as a single odorant with an intermediate carbon chain length, akin to colour vision, or be perceived as a different quality. Ability to discriminate between pairs of iso-intense solutions of n-butanol (4C), n-propanol (3C), n-pentanol (5C), and an intermediate 50/50 molecular weight mixture of n-propanol and n-pentanol (3C/5C) was assessed in 20 healthy young adults. We found that participants were able to discriminate 4C from the 50/50 molecular weight mixture of n-propanol and n-pentanol (3C/5C), and also from the other alcohols. In conclusion, we successfully replicated previous data demonstrating that participants are able to discriminate between structurally similar alcohols, and, more importantly, the present study shows that an odour mixture of two molecules differing only in carbon chain length is clearly distinguishable from a single odorant with an intermediate carbon chain length. These findings suggest that although carbon chain length matters to odour quality, carbon chain length is not a physical continuum within homologous series of substances that corresponds to a single qualitative dimension akin to the wavelength–hue relation for monochromatic light.

1. Introduction

Understanding how odour quality perception is encoded in an odorants' molecular properties poses a significant problem in olfaction. Whereas research in other senses, e.g., vision and audition, has been successful in solving this so-called stimulus problem, efforts in olfaction has hitherto had limited success in finding a theory for odour quality based on physical properties of the stimulus [24]. Many theories have been proposed on how olfactory coding works, however there is still no absolute consensus on the mechanisms behind odour quality perception. In the 1960s, Mozell suggested that olfactory discrimination was based on the absorption of odours along the mucosal receptor sheet [18]. Around the same time, Amoore sought the "clue to the olfactory code" by quantifying specific anosmias in order to understand or identify primary odours in terms of which it may be possible to describe all others [1]. More recently, it was proposed that olfactory receptors detect odorants by their molecular vibrations by means of inelastic electron tunnelling [25]. This vibration theory supposedly can calculate the quality of an odour from its molecular structure; this, however, has not been widely agreed upon [11]. The currently more prevailing view is the so-called 'lock-and-key' recognition of odorant molecules by the olfactory receptors [4], in which one OR can recognize multiple odorants, one odorant can be recognized by multiple ORs, and different odorants can be recognized by different combinations of ORs [2,17]. However, there is still a large gap remaining, from understanding how olfactory receptors recognized and bind odour molecules, to odour quality perception. Vis-a-vis, Khan et al. found that the primary axis of physicochemical properties reflects the primary axis of olfactory perception, pleasantness [12]. This can be regarded as a first step in the attempt to determine an odour's quality from its molecular properties.

One might hypothesize that there are a number of continua, each more or less orthogonally covering a small portion of the perceptual space. These *local continua* may not be easily found if the odorants chosen for investigation of the perceptual space are chosen randomly, or to represent a broad variety of odour qualities or odorant structures [3,5,14,21–23]. In other words, where it has been difficult to find quality dimensions in the perceptual space in experiments investigating a larger proportion of the space, several shorter vectors in this space may correspond to local continua. In line with the assumption of local continua is to use the odour structure–quality relationships of structurally similar odorants to address the stimulus problem (for review, see [9]). For example, Laska and Teubner

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studied the discriminability of aliphatic alcohols and aldehydes. They demonstrated that perceived odour quality differed between structurally similar odorants as a function of a change in the carbon chain length, as little as by one atom [14]. More importantly, the greater the difference in carbon chain length between two odorants, the better participants could discriminate between them. In addition, it has recently been shown that odorant receptive fields in mitral-tufted cells respond specifically to a specific carbon chain length and its closest neighbours [8], thereby implying an intimate relation between carbon chain length and odour quality.

Since discrimination ability reflects qualitative dissimilarity between odorants [27] the question arises whether perception of homologous series varying just in carbon chain length would follow local dimensions in the perceptual space. Such correspondences are common for several other senses, such as vision (wavelength and hue), audition (frequency and pitch) and the skin senses (temperature and warmth). Combining stimuli from different positions on these physical continua results in perceived qualities that are similar or seemingly identical to those of intermediate ones. In vision, for instance, humans can easily distinguish between two coloured monochromatic lights, e.g. those perceived as yellow and red. However, discriminating a mixture of yellow and red light, thus forming an orange mixture, from monochromatic orange light is not possible [6]. Although the different senses are quite different in terms of sensory physiology, these psychophysical correspondences have been a source of inspiration in pursuing the issue of stimulus continua also in olfaction.

Mixtures of two different odorants, in line with the example of additive mixtures of monochromatic light, typically adopt a quality in between those of its constituents [7,20,26]. Wise and Cain investigated the odour quality space for 15 odorant pairs. They measured discrimination errors and response latencies and found that the mixtures were harder to discriminate from the components than the components from each other. The authors concluded that “to the extent that latency to discriminate reflects similarity of quality, this finding suggests binary mixtures fall on a straight line connecting their components.” In the past, several experiments have investigated the quality of odour mixtures but none have organized the odour stimulus according to a physico-chemical dimension which could be hypothesized to exhibit linearity in perceptual space.

With this background, we hypothesized that if odour quality perception is to some extent regulated by carbon chain length in structurally similar odorants, as indicated by Laska and Teubner [14], and if odour mixtures follow rules similar to those of how coloured lights add up, then a mixture of two molecules differing only in carbon chain length would be perceived as indistinguishable from a single odorant with an intermediate carbon chain length. Such an outcome would suggest that odour quality is similar to what has been demonstrated for colour vision and suggest that carbon chain length could be regarded as a local continuum. Moreover, such a result would provide a theory for mimicking a target quality based on knowledge of physical properties. If, on the other hand, such an odour mixture would be perceived as fundamentally different in quality from its intermediate, determination of odour quality cannot be based solely on carbon chain length. Rather, other physical or perceptual properties would be the main determining factor. To the best of our knowledge, how we process odour mixtures based on this seemingly linear dimension of chemical structure [14] has not been previously studied. Therefore, in this study, we tested whether a mixture of n-propanol, an alcohol with a carbon chain of three atoms (3C) and n-pentanol, an alcohol with five atoms (5C) would mimic the odour quality of the four carbon alcohol n-butanol (4C; Fig. 1). As a prerequisite, we attempted to replicate Laska and Teubner’s results that participants can discriminate between iso-intense concentrations of these three odorants [14].

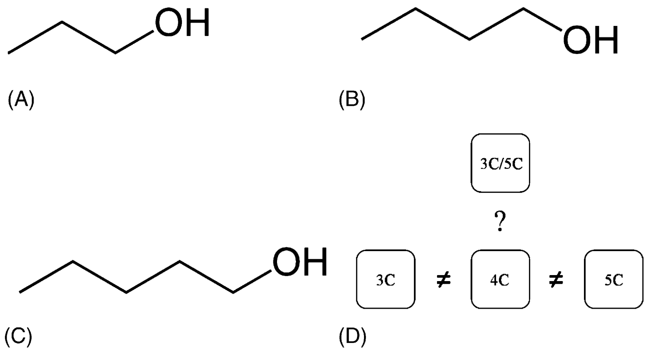

Fig. 1. Alcohols and study design. Structural formula of (A) n-propanol, (B) n-butanol, (C) n-pentanol, and (D) schematic representation of study design.

2. Methods

2.1. Participants

A total of 24 healthy participants (12 men; age range 18–37 years, mean 27.3, SD ± 4.9) were recruited for this study. None of the participants reported a history of nasal and neurological disorders known to affect olfactory function. Detailed information regarding the experiment was given to all participants before testing and written consent was obtained. All aspects of the study were performed in accordance with the University of Pennsylvania Internal Review Board.

2.2. Psychophysical testing

The odorants used were n-butanol (Fisher Scientific, >99% purity), n-propanol (Fisher Scientific, >99% purity), n-pentanol (Fisher Scientific, >99% purity), and an intermediate 50/50 molecular weight mixture of n-propanol and n-pentanol.

Participants took part in two sessions. Due to large inter-individual variance in intensity perception, we matched the stimulus intensities on an individual level by means of an intensity-matching task in the first session. Participants compared the intensities of various odorant concentrations to a target. For each participant, and each odour, the concentration rated closest to the target was used for the second session, to ensure that the three alcohols and the mixture were perceived as iso-intense. In the second session, participants performed a discrimination task where ability to discriminate between n-butanol (4C) and the other stimuli, n-propanol (3C), n-pentanol (5C), and the mixture of n-propanol and n-pentanol (3C/5C) was assessed (Fig. 1).

2.3. Session 1: intensity matching

A 10% (v/v) concentration of n-butanol (4C) in propylene glycol was chosen as a target, and rated for intensity by means of a labelled magnitude scale. In order to get an ‘intermediate mixture’ with an equal amount of carbon chains, we mixed a 50/50 molecular weight solution of n-propanol and n-pentanol (molecular weight n-propanol: 88.15 g/mol, density 0.811 g/ml; molecular weight n-pentanol: 60.10 g/mol, density 0.804 g/ml; resulting in a 4.075:5.925 n-propanol:n-pentanol volume ratio). We chose to use a 50/50 mixture of n-propanol and n-pentanol based on their respective molecular weights, and not on their volume. In this way, we ensured that the mixture contained the same theoretical amount of carbon chains per volume as the n-butanol stimulus. Participants had to rate the intensity of a range of concentrations (see Table 1) of n-propanol (3C), n-pentanol (5C), and the n-propanol and n-pentanol mixture (3C/5C) relative to the target, using a modified 100 mm VAS scale. The rating of the odour concentration for each odorant that best matched the target had to be within 10 mm away from the target that was indicated in the middle of the scale. Participants were excluded ($n = 4$) from the second part of the study if they were unable to match their intensity ratings to the target for any one of the individual odorants.

2.4. Session 2: discrimination

Twenty remaining participants (9 men; age range 22–37 years, mean 30.0, SD ± 4.6) participated in the second session of this study, a discrimination task. To ensure that all alcohols and the mixture were perceived as iso-intense, the concentrations of 3C, 5C and the mixture of 3C/5C that were rated closest to the target based on intensity, on an individual level, were used. In the alcohol discrimination test, participants were blindfolded and presented with a total of 54 odour triplets (9 repetitions of each combination) in a three-alternative, forced-choice test without feedback. Each triplet consisted of two identical and one aberrant alcohol. Participants were asked to select the odd odour out of the three odorants presented, without the need to recognize or name the odours. After each individual trial, participants were asked whether their choice was predominantly based on perceived

Table 1
Odorant concentrations used for discrimination task.

n-Propanol		n-Pentanol		Mixture of n-propanol and n-pentanol	
Concentration	*N*	Concentration	*N*	Concentration	*N*
5	1	2	2	2	0
10	6	5	4	5	5
15	1	10	7	10	3
20	2	15	3	15	7
25	2	20	4	20	5
30	8				

N: number of participants that rated the perceived intensity of this concentration closest to the intensity of the target (10% n-butanol). All concentrations are given as % (v/v) in propylene glycol.

difference in odour quality or intensity. After both the 18th and the 36th trial, participants were given a short break to prevent olfactory adaptation, during which the participant filled out short non-related questionnaires given as a distracter task. Presentation order of the odour triplets was pre-randomized in a pseudo-randomized order. All odorants used in the intensity matching and discrimination task were delivered using 100 ml amber glass bottles, containing a total of 10 ml of liquid each with no visual markers.

2.5. Data analysis

Unique odour combinations were paired with their inverse counterpart (e.g. an example triplet consisting of 3C, 3C, 4C measures the same discrimination ability as the triplet 3C, 4C, 4C; therefore these results were paired, creating a '3C–4C' combination), leading to 18 trials per alcohol combination. Trials where participants based their judgments predominantly on intensity were excluded from subsequent analysis to further constrain the analyses to quality discrimination performance.

Separate one-sample Students' *t*-tests were performed to determine if discrimination scores for each combination were above predicted chance level (33.3%). A repeated measures ANOVA was used to analyze possible differences in discrimination ability between the various combinations. Data were analyzed using SPSS 17.0 for Windows (Chicago, IL, USA).

3. Results

3.1. Intensity matching

Mean intensity rating of the target odour, 10% n-butanol, was 23.6 (SD ± 14.1). After exclusion of the 4 participants that did not perform the second part of the study, mean intensity rating of n-butanol was 24.6 (SD ± 14.7), indicating a mean intensity rating in between "moderate" and "strong" on the labelled magnitude scale. For each participant, an individual iso-intense concentration of both alcohols and the mixture was determined to be used in the discrimination task (results in Table 1).

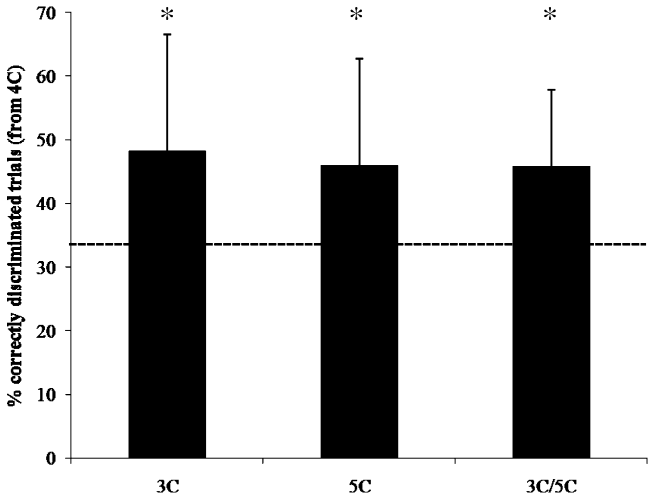

Fig. 2. Percentage correctly discriminated trials for each alcohol combination. 3C = n-propanol, 5C = n-pentanol, 3C/5C = 50/50 molecular weight mixture of n-propanol and n-pentanol. Dotted line is predicted chance level (33.3%); star indicates discrimination ability is significantly different from chance level.

3.2. Discrimination task

On average, 4.23 out of 18 trials per alcohol combination were excluded per participant (23.5%) since discrimination in these trials was based predominantly on perceived intensity rather than quality difference between the alcohols.

Mean percentage of correctly discriminated trials was 48.2% for the 3C–4C combination (statistically different from chance: $t_{19} = 3.64$, $p = .002$), 46.0% for the 5C–4C combination ($t_{19} = 3.39$, $p = .003$), and 45.8% for the 3C/5C–4C combination ($t_{19} = 4.64$, $p < .001$) (Fig. 2). There was no significant difference in the percentage of correctly discriminated trials between the three different combinations ($F_{2,38} = .14$, $p = .874$).

4. Discussion

In this study, we tested whether a mixture of two structurally similar substances differing only in carbon chain length would mimic a single odorant with an intermediate carbon chain length. This hypothesis was inspired by two observations: by mixing of monochromatic lights, the outcome is indiscriminable from the hue of a light that is intermediate in wavelength. In parallel, qualitative dissimilarity of homologous series of odorants is related to the difference in carbon chain length; odorants closer in carbon chain length tend to be harder to discriminate perceptually. Thus, our hypothesis was that a mixture of two odorants differing only in carbon chain length would mimic a homolog with an intermediate chain length.

First, we established that participants can discriminate iso-intense odour stimuli n-propanol (3C) and n-butanol (4C) from n-pentanol (5C). This testifies in line with several studies [13,14] to the importance of carbon chain length for odour quality. Second, we tested whether participants were able to discriminate between 4C, and a 50/50 molecular weight mixture of n-propanol and n-pentanol (3C/5C). Two results are notable: the 3C/5C mixture was not indiscriminate to the target odour, n-butanol (4C). Moreover, the mixture was clearly discriminable from the target. Indeed, this mixture was as discriminable from the target 4C as were 3C and 5C from 4C. Hence, the hypothesis that carbon chain length would represent a continuum along which odorants of homologous series could be mixed to resemble intermediate ones could not be supported.

Since carbon chain length, as measured by discrimination ability [14], is at least systematically related to odour quality, a substance of intermediate length (4C) should yield a quality roughly in between its neighbours (3C and 5C). Several studies have also suggested that odour quality of a mixture, independent of carbon chain length, has an intermediate quality to those of its constituents [20,26]. However, the olfactory bulb is able to separate binary mixture representations from the representations of both pure components, and this discrete classification of activity patterns may be reflected in the odour quality perception [19]. Altogether, the results suggest that these shifts in quality, from 3C to 5C, either via 4C or via the mixture 3C/5C, may occur along different axes in a multidimensional space, rather than along a single major axis implying that olfactory perception does not follow identical rules – when it comes to quality – as visual perception [16].

Hitherto, there is no physicochemical theory for mimicking a target odour by combining two or more other single odorants based on their molecular structure. Instead, the successful examples that are found in the literature are typically the result of skilled practitioners' ability to imagine how different odorant qualities would combine to a mixture percept that would yield a significant similarity to a target odour. For instance, the combination of ethyl isobutyrate + ethyl maltol + allyl-a-ionone gives rise to a mixture that smells more like pineapple than any of its components alone. The same thing goes for the mixture of 6 compounds (beta-ionone + damascenone + frambinone + ethyl acetate + isoamyl acetate + vanilline) in relation to the target odour of red cordial [15].

Although the current study rejects the hypothesis that carbon chain length would be a physical continuum for a unidimensional change of odour quality, this stimulus parameter remains intriguing and holds promise for future structure–quality research. Indeed, an interesting follow-up study in humans would be to test whether the mixture 3C/5C is more similar to 4C than it is to 3C and/or 5C. If so, this would give additional support to the notion that carbon chain length plays an important role in odour quality perception. Even though certain mitral-tufted cell odorant receptive fields respond preferentially to a specific carbon chain length and its closest neighbours [8], thus implying a relation between carbon chain length and odour quality, this receptive range can differ for other odour receptors. Different receptors may respond to different functional groups or other structural components [2,17], perhaps recognizing other components of the molecule than carbon chain length, which may enable the discrimination between 4C and 3C/5C. Guerrieri et al. [10] found that conditioning in honeybees led to a larger generalization to odorants of the same functional group that were close in carbon chain length. Interestingly, they also found that there was a larger generalization between functional groups for odorants of the same carbon chain length. They concluded that much of the perceptual quality of investigated odours can be explained by a three dimensional space. The most important axis spreads out scents according to carbon chain length, whereas the other two axes separate the odours according to functional group.

In conclusion, we confirmed that participants are able to discriminate between structurally very similar alcohols. Accordingly, humans have a highly developed and sophisticated olfactory system that is able to detect even minute differences in odorant structure. Although we found that carbon chain length does matter for odour quality, we have gained no support for carbon chain length being a physical continuum within homologous series of substances that correspond to a single qualitative dimension akin to the wavelength–hue relation for monochromatic light.

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