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Does odour cross contamination alter olfactory thresholds in certain odours?*

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SUMMARY

Objectives: *To determine if:*

1) there is cross contamination between odours tested on thresholds achieved,

2) a delay period is necessary between testing different odours.

Methods: *Thirty–five subjects underwent threshold testing with phenethyl alcohol (PEA), ethyl-mercaptan (MER), acetic acid (ACE), and eucalyptol (EUC) using serial logarithmic dilutions. On separate occasions subjects were exposed to high concentrations of PEA, ACE and EUC in random order for two minutes, and thresholds for all four odours re–tested. Pre– and post–high concentration odour thresholds were compared.*

Results: *Exposure to high concentrations of PEA, ACE and EUC does not alter olfactory thresholds by more than 10^{-2} for the other odours except in specific circumstances with ACE and EUC.*

Conclusions: *There is limited cross contamination with ACE and EUC, which is avoided by specifying presentation order as: PEA, MER, ACE, EUC. Odours PEA, MER, ACE and EUC are recommended for olfactory testing.*

Key words: olfactory test, olfactory thresholds, odours, adaptation

INTRODUCTION

Olfactory disturbances are not uncommon and represent a significant impact on people's quality of life [1–3]. In order to assess a complaint such as hyposmia, validated olfactory tests are currently available and include the University of Pennsylvania Smell Identification Test (UPSIT) [4,5], Sniffin' Sticks [6] and the Combined Olfactory Test [7]. These tests use either (or in some cases both) qualitative or quantitative means to assess olfactory performance and although the Sniffin' Sticks tests assess olfactory discrimination; none of the tests consider potential interaction between odours because those that do test a threshold do so only for one odour. This factor is particularly important if olfactory threshold testing is to be performed for more than one odour.

An olfactometer, using a 'staircase technique' of threshold detection, has been designed by the Leicester otorhinolaryngology research team [8] with which the aim is to use established representative smells, which are easily detected, and do not significantly interact with each other or significantly alter olfactory thresholds of the other odours tested. These odours should represent key areas of the olfactory spectrum, similar to the different frequencies in audiological testing.

The aim of this study is to assess if there is any influence on one of these odours after smelling another odour at a supra–threshold concentration, as this may dictate the need to leave a time interval between testing the odours to allow for clearance, or dictate the order of testing.

SUBJECTS AND METHODS

Approval of the study was obtained from a local ethics committee. Thirty–five normal volunteers were chosen (23 Female: 12 Male, with a mean age of 36 years and age range 20 years – 65 years) without any rhinological disease in a controlled study. The four odours tested were phenethyl alcohol (PEA, Sigma–Aldrich Company Ltd., Gillingham, Dorset, UK), ethyl-mercaptan (MER, Sigma–Aldrich), glacial acetic acid (ACE, Fisher Scientific UK Ltd., Loughborough, Leicestershire, UK), and eucalyptol (EUC, Sigma–Aldrich), representing the smell of roses, propane gas, vinegar and eucalyptus, respectively.

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Table 1. Concentration of chemicals on the rack.

PEA	MER	ACE	EUC
10^{-2}	10^{-5}	10^{-1}	10^{-1}
10^{-3}	10^{-6}	10^{-2}	10^{-2}
10^{-4}	10^{-7}	10^{-3}	10^{-3}
10^{-5}	10^{-8}	10^{-4}	10^{-4}
10^{-6}	10^{-9}	10^{-5}	10^{-5}
10^{-7}	10^{-10}	10^{-6}	10^{-6}
10^{-8}	10^{-11}	10^{-7}	10^{-7}
10^{-9}	10^{-12}	10^{-8}	10^{-8}
10^{-10}	10^{-13}	10^{-9}	10^{-9}

PEA – Phenethyl Alcohol ($C_8H_{10}O$)
MER – Ethylmercaptan (C_2H_5SH)
ACE – Acetic Acid Glacial (CH COOH)
EUC – Eucalyptol ($C_{10}H_{18}O$)

These chemicals were diluted ten–fold in mineral oil [9] except acetic acid which was diluted in sterile water) using 28 ml cylindrical glass vials [10] (VWR International, Lutterworth, Leicestershire, UK) and arranged sequentially in a rack from weak to strong (Table 1) giving nine concentrations of each odour. The vials contained 5 ml of solution, leaving a 23 ml column of air above it, allowing the solution to vaporise and fill this space. These odours were chosen due to their apparent distinctiveness from each other and, as aforementioned, represent specific entities within the human olfactory range [11]. The odours also had no discernable trigeminal effect at the concentrations used in the study. Logarithmic steps were found necessary to cover the potential olfactory range of subjects [8].

Each subject's threshold for each of the above four odours was determined by starting with the weakest concentration for each odour and moving up to the next strongest concentration (comparing it to the mineral oil solvent only), in a stepwise manner until the subject could convincingly detect two successive concentrations of the odour in question. The lowest concentration at which this recognition was reached was recorded as the threshold. Each subject was then taken to a separate room and given a facemask with one of the odours (0.5 ml of the strongest concentration solution on the rack of phenethyl alco-

Table 2. Results of subject's threshold shifts.

SUBJECT	PEA IN MASK				EUC IN MASK				ACE IN MASK			
	PEA	MER	ACE	EUC	PEA	MER	ACE	EUC	PEA	MER	ACE	EUC
1	6	2	0	0	1	1	1	2	1	2	2	0
2	1	0	1	0	1	0	0	2	0	0	3	0
3	2	0	0	0	0	0	0	0	0	1	2	0
4	0	0	1	0	0	0	1	0	0	0	0	0
5	OFF SCALE	0	0	0	0	–1	0	0	0	0	2	0
6	6	0	0	0	0	0	0	1	0	0	3	0
7	1	1	0	0	0	0	0	2	1	1	1	1
8	OFF SCALE	0	–1	0	0	0	0	1	0	0	1	0
9	2	0	1	0	1	0	0	1	0	0	3	1
10	2	0	0	2	0	0	1	0	–1	2	1	0
11	2	2	0	0	0	1	0	0	0	1	1	1
12	2	0	2	0	2	1	1	1	2	1	1	0
13	2	1	0	0	0	0	0	1	0	0	2	0
14	OFF SCALE	0	0	0	0	0	0	1	–1	0	0	0
15	0	0	–1	–1	0	0	0	1	0	0	1	0
16	1	1	–1	–1	1	0	0	1	1	1	0	0
17	OFF SCALE	1	OFF SCALE	0	0	0	0	1	1	0	2	0
18	2	0	1	0	0	0	1	0	0	0	2	0
19	1	0	1	0	0	0	0	0	0	0	1	0
20	2	0	0	0	0	1	0	1	0	0	2	0
21	–1	0	1	0	1	1	–1	0	0	1	2	0
22	OFF SCALE	0	1	0	2	0	0	0	0	0	2	1
23	OFF SCALE	1	2	0	0	0	0	0	0	0	2	0
24	2	0	1	1	0	0	2	0	0	1	1	0
25	0	0	0	0	2	0	1	4	1	0	1	0
26	2	0	0	0	OFF SCALE	0	1	0	–1	0	1	0
27	2	–1	0	2	1	2	1	1	0	0	2	0
28	2	1	1	0	0	0	0	0	0	0	2	1
29	3	0	0	0	1	0	0	0	0	0	2	0
30	2	0	0	0	0	0	1	0	1	0	0	0
31	3	0	0	0	0	0	1	1	0	0	2	0
32	4	0	1	0	0	0	1	1	0	0	1	0
33	2	1	0	1	0	0	1	1	4	0	1	0
34	0	–1	0	0	0	0	–1	1	0	0	0	0
35	–1	2	0	0	2	0	0	1	0	0	0	2

hol, acetic acid or eucalyptol) pipetted and impregnated onto the inside of a dust mask (Cromwell Tools, Leicester, UK). Ethylmercaptan was not used in this way due to its unpleasantness when used at strong concentration. This mask was then held over the subject's nose for 2 minutes, after which it was safely discarded and the patient asked to return immediately to the original room for re-testing of their thresholds for the same odours in the same way as before. The order in which the odours were presented was varied in a few of the subjects to ensure that this did not have any effect on the results. The before and after threshold values were then analysed using paired Wilcoxon tests to allow for the non-normal distribution of the data and 95% confidence intervals for the difference in medians were calculated (Stata SE for Windows – Version 9.1, Texas, USA). The odour was considered to have had a significant effect on the threshold values if the 95% confidence interval contained a difference of two or more logarithmic steps. This was based on a preliminary study to look at the effect of an empty mask placed over the nose on the thresholds derived afterwards that showed variations of one logarithmic step either side of the pre-mask threshold (Table 3).

RESULTS

Table 3a. Olfactory threshold shifts using a blank mask.

Odour	Subject Number	Mean	SD	Min-Max
PEA	101	−0.1	0.46	−2, 1
MER	101	−0.2	0.56	−3, 1
ACE	101	−0.1	0.37	−2, 1
EUC	101	−0.1	0.47	−3, 1

Table 3b. T-test analysis.

Odour	Subject Number	Mean difference	p-value	95% CI
PEA	101	−0.10	0.0323	−0.19 to −0.01
MER	101	−0.23	0.0001	−0.34 to −0.12
ACE	101	−0.08	0.0318	−0.15 to −0.01
EUC	101	−0.11	0.0210	−0.20 to −0.02

The results, as displayed in Table 4, show that phenethyl alcohol (PEA) had no cross contamination effect on the other odours tested, except for on PEA itself, which is the expected effect of adaptation. The results with acetic acid (ACE) and eucalyptol (EUC) were less clearly defined. There did not appear to be an adaptation effect of EUC on itself, but there was a possible effect of EUC on PEA, which would need a greater number of subjects to clarify. With ACE this also appeared to show a possible cross contamination effect on PEA as well as having an adaptation effect on itself. However, as neither EUC nor ACE showed any significant threshold shift following supra-threshold PEA, any potential for cross contamination can be avoided by ensuring that PEA is the first odour to be tested, and thus no time delay should be required between the four threshold tests. When the order of odours presented was reversed no significant cross-contamination was shown.

Table 4. Results of Paired Wilcoxon Tests for Threshold Shifts.

Odour in mask	Odour tested	p-value	95% confidence interval	Cross contamination effect
PEA	PEA	<0.0001	−3 to −2	yes
	MER	0.02246	−2 to 0	no
	ACE	0.002808	−1.5 to −1	no
	EUC	0.3438	−2 to −1	no
EUC	PEA	0.000488	−3 to −1	unclear
	MER	0.0153	−2 to −1	no
	ACE	0.006531	−1.5 to −1	no
	EUC	<0.0001	−1.5 to −1	no
ACE	PEA	0.03125	−2.5 to −1	unclear
	MER	0.003906	−2 to −1	no
	ACE	<0.0001	−2.5 to −1	unclear
	EUC	0.3125	−2 to −1	no

DISCUSSION

The results show that by testing first with phenethyl alcohol there is no significant interaction between the four odours. It is not surprising that being exposed to a strong odour leads to adaptation to that same odour when tested afterwards and thus alters the olfactory threshold achieved. Ideally a larger sample size should be studied to clarify the effect of eucalyptol and acetic acid on phenethyl alcohol, but this potential effect can be avoided by testing the odours in the order phenethyl alcohol, ethylmercaptan, acetic acid and eucalyptol. Although strong concentrations of the odours were used in the mask, when the odours are tested under normal circumstance with the olfactometer, lower concentrations are generally used to determine thresholds [8,12,13]. Evidence from an older study confirms that cross-adaptation only occurs with high concentrations of odours [14].

Unlike other areas of otorhinolaryngology, such as with otology and audiology, little attention is given to olfactory testing in the UK. Therefore, there is a need for a cheap, quick, accurate, repeatable test that can be performed in the clinic setting to assess smell. By defining distinct odour groups for testing, key odours can be targeted for olfactory assessments to minimise the number of odours for inclusion. The findings from this study can now be utilised with the olfactometer being developed in our centre [8,12,13] to provide a comprehensive olfactory test battery for clinical purposes.

Previous studies by Carrie and Dawes on the smell map [15,16] have looked at the similarity of odours in a relative fashion and this has enabled a greater understanding of certain odour groups, although this is by no means definitive. Classically odours have been categorised into various groups, dating back to the botanist Linnaeus who cited camphoraceous, musky, floral, peppermint, ethereal, pungent and putrid as the groups for odour classification in the 1700s [17].

Work by Pierce et al. on odour interaction between androstenone and 5 similar compounds, found a cross adapta-

tion effect where there is a decrease in sensitivity to one odour after exposure to a different odour. This occurred with odour similarity, both perceptual and structural. This cross adaptation effect was even seen on an odour following exposure to a similarly structured but odourless compound. However further testing found cross adaptation effects with both perceptually and structurally different compounds, thus emphasizing the difficulties in predicting odour to odour interaction [18]. Other studies have observed significant psychological aspects to the perception of odour intensity and familiarity [19,20]. The cross–adaptation effect of dimethyl disulfide and hydrogen sulfide after exposure to mixtures of the compounds was found in work by Berglund and Engen to be small [21].

CONCLUSIONS

The odours phenethyl alcohol, ethylmercaptan, acetic acid and eucalyptol can be recommended for olfactory threshold testing and are useful in the clinical setting for their range across the olfactory spectrum, their importance (ethylmercaptan representing a household gas odour and a potential hazard), and their familiarity. The appropriate order of presentation should be phenethyl alcohol, ethylmercaptan, acetic acid and eucalyptol, to avoid a theoretical cross contamination effect when tested at threshold levels.

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