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Odor Perception Phenotypes: Multiple, Specific Hyperosmias to Musks

Avery N. Gilbert[1] and Sarah E. Kemp

Givaudan-Roure Fragrances, Teaneck, NJ 07666, USA

[1]Present address: Synesthetics, Inc., Montclair, NJ 07043, USA

Correspondence to be sent to: Dr Sarah E. Kemp, Givaudan-Roure Fragrances, 1775 Windsor Road, Teaneck, NJ 07666, USA

Abstract

Olfactory detection thresholds for 11 structurally diverse musk odorants and one non-musk odorant were obtained from 32 subjects. Hierarchical cluster analysis produced four groups of subjects. One group (n = 12) was uniformly sensitive to all musks; another (n = 16) was uniformly insensitive. Two groups of subjects contained otherwise insensitive individuals who were exceptionally sensitive to cyclopentadecanone and musk xylol (n = 2) and to delta9-hexadecenolactone and tonalid (n = 2) respectively. We propose that the latter two groups are odor perception phenotypes (MSHM1 and MSHM2) that consist of multiple, specific hyperosmias to musk odorants.
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Introduction

The discovery of genes coding for presumptive odor receptors (Buck and Axel, 1991) throws olfactory psychophysics into a new light. It is now possible to conceive of a synthesis that links phenotypic description of individual differences in odor perception with genetic variation in receptor expression (Gilbert, 1992). The visual system provides a good model. Phenotypic differences in color vision, long defined by purely psychophysical methods, have now been linked to specific deletions and fusions in genes coding for middle-wavelength- and long-wavelength-sensitive visual pigments (Deeb *et al.*, 1992).

A genotype–phenotype synthesis in olfaction requires the psychophysical description of systematic, inter-individual differences in perception, e.g. the olfactory equivalent of protanomalous trichromacy. One experimental approach is to examine perceptual deficits known as specific anosmias. Specific anosmias have been used in studies of olfactory heritability (e.g. Wysocki and Beauchamp, 1984). However, they are rare in comparison to the number of odors that humans are able to detect. Further, odorants exhibiting specific anosmias and odors whose thresholds vary continuously across subjects may be served by different sensory pathways. Specific anosmias may not be the best model to study general olfactory perception.

An alternative approach is to obtain detection thresholds to multiple odorants and look for evidence of correlated sensitivities among a group of subjects. To date, studies of detection thresholds to multiple odorants have focused on grouping odorants whose thresholds covary, rather than

classifying on subjects according to their odor sensitivity profiles. Studies by Jones (1957), Brown *et al.* (1968) and Punter (1983) obtained thresholds to 20, 8 and 47 odorants, across 84, 60 and 16 subjects respectively. All three investigators analyzed their data with odor × odor correlation matrices; Jones (1957) and Brown *et al.* (1968) also used factor analysis. Other studies used a similar approach but pre-selected subjects on the basis of sensitivity to a particular odorant (O'Connell *et al.*, 1989; Stevens and O'Connell, 1991). While emphasizing the statistical grouping of odorants rather than subjects, all of these studies, along with that of Cain and Gent (1991), implicated a non-specific factor of general sensitivity in olfaction.

Our approach to the question of olfactory phenotype was to examine natural variation in olfactory thresholds among an unselected subject population. Our specific aim was to determine whether thresholds obtained across multiple odorants could be used to classify subjects according to odor sensitivity profiles. Casual observations in the course of our other research, as well as indications in the literature (Baydar *et al.*, 1993), suggested that acuity for the musks tends to co-vary from person to person. We therefore focused our search for an olfactory phenotype on a group of 11 perceptually similar but structurally diverse musks. We used hierarchical cluster analysis of rank order data to search for subjects with similar profiles of correlated sensitivity. Our goal was to quantitatively define an olfactory phenotype.

Materials and methods

Stimuli

Duplicate binary dilution series of 11 musks and a phenyl ethyl alcohol (PEA) control were prepared on a volume by volume basis in diethyl phthalate. The starting concentration for each dilution series was the highest achievable in practice: cyclopentadecanone (MCPD) 50% (w/w), civettone (CIV) 100%, DL-muscone (MUSC) 100% (w/w), omega-pentadecalactone (LAC) 87% (w/w), delta9-hexadecenolactone (AMB) 100%, ethylene brassylate (ETHB) 100%, musk xylol (MXYL) 10% (w/w), musk ketone (MKET) 10% (w/w), tonalid (FIX) 40% (w/w), celestolide (CEL) 9% (w/w), galaxolide (GAL) 100% and PEA 100%. For each dilution step, duplicate 10 ml samples were taken from the same stock solution.

Odorants were presented in 8 oz. cylindrical opaque white polypropylene bottles with snap-closure caps. Bottles and caps were deodorized prior to use by boiling in water for 2 h and air drying.

Three odorants were tested in each of four sessions, as follows: MUSC, AMB and PEA in session 1; ETHB, MKET and MCPD in session 2; CIV, LAC and FIX in session 3; and MXYL, CEL and GAL in session 4. The order of odorants in each session was balanced across subjects. Subjects with thresholds lower than the available dilutions were tested with further dilutions in an additional session.

Subjects

Thirty-two non-smoking individuals were recruited through newspaper advertisements. The data reported here are from 16 men and 16 women, ranging in age from 19 to 40 years (32.1 ± 5.9 years). Subjects were screened by self report for normal senses of smell, normal nasal breathing and absence of active head cold, sinus infection or allergy. Subjects provided their informed consent and were paid for participating.

Thresholds

Thresholds were measured using a two-alternative forced-choice (2-AFC) method of ascending and descending limits with seven reversals (Wetherill and Levitt, 1965). The detection criterion was two correct judgements in a row and the stability criterion was no more than three steps between reversals. Two alternating sets of each odorant series were used to ensure the same bottle was never used twice in a row. This allowed time for headspace in the bottles to recover between each 2-AFC test. The order of stimulus and blank for each 2-AFC test in a threshold measurement was randomized. Thirty-six random orders were prepared so that each subject received a different random order for a given odorant. These 36 orders were randomly allocated to subjects for each odorant. There was a minimum rest break of 10 min between odorants.

Odors were delivered by the administrator. Subjects took one continuous sniff as the administrator squeezed the bottle twice under their nose, and a clearing breath through the nose after every bottle smelled. At the start of each series of threshold measurements, subjects smelled the highest concentration in the series once to become orientated to the odor. Testing began in the middle of each dilution series at step 35. Subjects smelled a blank and an odorant and indicated which bottle contained an odor by raising one

finger for the first bottle and two fingers for the second bottle. Subjects were instructed to guess if they were unsure. Subjects were instructed not to eat or drink anything other than water for 1 h prior to testing and to refrain from wearing fragrance on the day of testing.

Initially, each dilution series was ascended in steps of two until two correct judgements in a row were made. From that point on, all movements up or down the series were made in steps of one, moving down when two correct judgements in a row were made and up when one incorrect judgement was made. Each change in direction up or down the series was counted as a reversal. Threshold measurement was terminated when seven reversals were completed with no more than three steps between the last six reversals. Individual thresholds were calculated by taking the arithmetic mean of the last six reversal steps.

Data analysis

There are drawbacks to the common practice of using dilution steps as raw data in odor × odor correlation matrices. First, threshold distributions are often not normal. This violates assumptions behind such statistics as Pearson's correlation coefficient. Second, the use of parametric statistics implicitly assumes that dilution steps within a distribution are perceptually equal distances apart. Yet dilution steps may not be equally spaced if the psychophysical curve is non-linear (Doty, 1975) or if volatilization rate varies with concentration. Third, the use of parametric statistics to compare multiple odorants assumes that dilution steps are of equal perceptual distances between, as well as within, odorants. This assumption is challenged by the use of different starting concentrations and dilution factors as well as by the existence of non-linear psychophysical curves. We therefore preferred to use a sensitivity measure based on ranks, rather than dilution steps, in further data analysis. Analysis based on rank order has the further advantage that it is less susceptible to distortion by instances where no threshold can be measured.

Individual threshold values were transformed to relative sensitivity rankings for further analysis. A subject's rank for a given odor ranged from 1 (least sensitive) to 32 (most sensitive). In the case of tied threshold values, the mean rank was assigned to each subject.

Hierarchical cluster analysis was used to identify homogeneous groups, or clusters, of subjects, based on the relative sensitivity rankings. Cluster analysis quantifies the degree of similarity between subjects by calculating a distance measure between all possible pairs of subjects. The two most similar subjects are then grouped together and the distance measure recalculated. This iterative process continues until all subjects are members of a single cluster. The resulting hierarchical clustering solution can be visually displayed as a dendrogram, in which the distance between successive clustering steps is rescaled to a standard numerical range.

Results

A hierarchical cluster analysis was performed on the rank data for the 11 musks. It was based on a squared Euclidean distance measure, and an average between-groups linkage algorithm. The analysis arranged subjects into two large clusters (labeled 1 and 2 in Figure 1) and two small clusters (labeled 3 and 4). Each subject's overall sensitivity rank (from 1 to 32) was calculated as the mean of ranks across the 11 musks, and is included in Figure 1. Cluster 1 consisted of 12 relatively sensitive subjects, and cluster 2 of 16 relatively insensitive subjects. The four subjects in clusters 3 and 4 had mixed sensitivities, as evidenced by large standard deviations in overall sensitivity rank. This initial cluster analysis suggested that 87.5% of subjects (those in clusters 1 and 2) were grouped according to overall sensitivity ranks.

To interpret the dendrogram further, we calculated for subjects in each cluster a mean rank for each odorant (Table 1). The grand mean for all 32 subjects was 16.5. Subjects in cluster 1 had a high overall rank and their mean rank for each musk was consistently larger than 16.5. Accordingly, these subjects can be classified as relatively sensitive. Cluster 2 subjects showed the reverse pattern: their overall rank was lower than 16.5 and their mean rank on each odorant was uniformly lower than that of cluster 1 subjects. Therefore, cluster 2 subjects can be classified as relatively insensitive. Subjects in clusters 3 and 4 were insensitive in varying degree to most musks. However, they had high mean ranks for certain musks: MCPD and MXYL in cluster 3, AMB and FIX in cluster 4. Subjects in these clusters can be classified as having mixed sensitivity. In particular, they displayed high sensitivity to two musks but were broadly insensitive to the rest.

We next compared the dendrogram results to sensitivity rankings for the non-musk odorant PEA. If PEA rank were unrelated to overall musk rank, clusters 1 and 2 could be

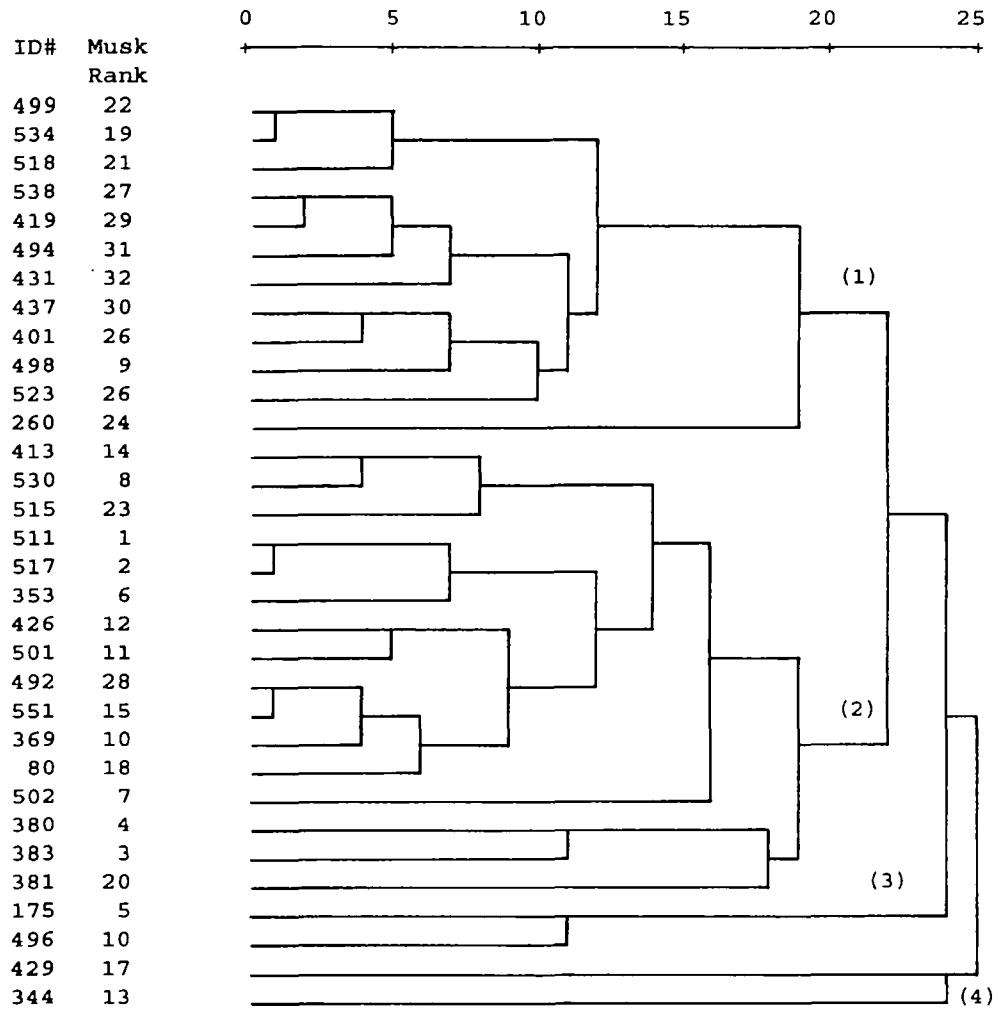

Figure 1 Dendrogram of the hierarchical cluster analysis using rank-transformed threshold data for the 11 musks tested; the clusters correspond to sensitive (1), insensitive (2), and mixed sensitivity subjects (3 and 4).

interpreted as reflecting differences in sensitivity to specifically musky odorants. However, PEA rank was positively correlated with overall sensitivity rank ($r = 0.41$, $P < 0.001$, $n = 32$). This is consistent with a trait for general olfactory sensitivity, rather than a selective sensitivity to musky odorants.

From 352 determinations of musk thresholds, there were 18 instances where no threshold could be obtained, due to a subject's complete insensitivity to a given odorant. These instances were distributed across eight musks [MCPD (5), MXYL (4), FIX (3), CEL (2), ETHB, GAL, MKET and MUSC] and across 11 subjects [nos 383 (4), 511 (3), 175 (2), 429 (2), 534, 426, 517, 502, 381, 530 and 344]. Instances of complete insensitivity were less prevalent in cluster 1, than in clusters 2, 3 and 4 (0.8, 6.8, 9.1 and 13.6% respectively), as were the proportions of subjects exhibiting complete insensitivity to one or more odors (8.3, 43.8, 50.0 and 100% respectively). Thus, complete insensitivity was less frequent among subjects in the sensitive cluster and more frequent among subjects in the insensitive and mixed sensitivity clusters.

Discussion

We found that 87.5% of the sample population could be classified into two clusters: broadly sensitive subjects and broadly insensitive subjects. Because threshold for the non-musk control odor covaried with overall musk sensitivity, these two clusters may reflect differences in sensitivity to a wide array of odorants, a result consistent with previous work implicating a trait of general olfactory

Table 1 Mean ranks on test odorants for subjects in the clusters in Figure 1

Cluster	Musk													
	AMB	CEL	CIV	ETHB	FIX	GAL	LAC	MCPD	MKET	MUSC	MXYL	Mean	SD	PEA
1 (n = 12)	22.7	24.0	24.5	22.5	19.2	19.9	22.3	19.7	20.3	19.3	23.2	21.6	2.0	21.9
2 (n = 16)	12.0	12.5	11.3	15.0	13.1	14.3	10.7	13.1	15.5	17.1	10.8	13.2	2.1	15.4
3 (n = 2)	2.5	4.3	14.0	7.5	14.5	16.0	22.0	29.5	4.5	8.0	29.5	13.8	9.7	3.0
4 (n = 2)	29.0	15.8	13.0	1.5	29.5	14.8	22.8	12.0	14.3	3.5	9.5	15.1	9.0	6.0

sensitivity (Jones, 1957; Brown *et al.*, 1968; Punter, 1983; Cain and Gent, 1991), as well as with results suggesting that measures of acuity may share a common source of variance with tests of identification, discrimination, etc. (Doty, 1994). General sensitivity could be produced by peripheral features that increase detection threshold, such as a reduced olfactory cleft or fewer sensory cells in the olfactory neuroepithelium. Alternatively, general sensitivity could result from different levels of olfactory receptor (OR) gene expression. Sensitive subjects might produce more ORs and have a correspondingly higher sensitivity to odorants. In either case, the existence of a general sensitivity will obscure individual differences in specific sensitivity and may hinder the analysis of genotype–phenotype linkage.

We further identified two clusters of subjects who were broadly insensitive, but very sensitive to specific musks. One group was differentially sensitive to MCPD and MXYL and the other to AMB and FIX. We hypothesize that these clusters consist of persons with distinctive olfactory phenotypes, namely multiple, specific hyperosmias to musks. Accordingly, we have provisionally given these clusters the phenotypic designations MSHM1 and MSHM2 (for the MCPD/MXYL and AMB/FIX patterns respectively). Based on our study population, the MSHM1 and MSHM2 phenotypes each have a prevalence of 6.25%. These phenotypes are defined statistically and there is a possibility that the observed patterns of sensitivity occurred by chance. However, the stringency of our threshold determination protocol makes it unlikely that a given subject would be a random outlier on two odorants, especially as the odorants in each phenotypic pair were tested in different sessions. In any case, two subjects showed each pattern of selective sensitivity, an outcome that is even more unlikely to be random.

The odor sensitivity profiles of MSHM1 and MSHM2 individuals (i.e. multiple specific hyperosmias to musks against a background of relative insensitivity) cannot be accounted for by the factors (described above) that explain reduced general sensitivity in cluster 1 individuals. A different account appears to be required. We propose that these phenotypes may be due to polymorphism in OR genes or gene expression. MSHM1, for example, could be associated with duplications in the genes coding for ORs that selectively bind MCPD and MXYL. Normal gene expression would then lead to relatively higher numbers of these ORs in MSHM1 individuals. An analogous situation exists in color vision pigments (Neitz and Neitz, 1995). An alternative scenario is that most individuals in the population have a similar OR gene repertoire, and that MSHM1 and MSHM2 individuals display atypical control of gene expression. For example, they may selectively overexpress OR genes for receptors that have high affinity for the musks in question. A goal for further research is to examine these and other possibilities at the level of molecular biology, relate them to quantitative sensory traits at the level of psychophysical analysis and thereby establish an olfactory genotype–phenotype linkage, as has been done for color vision anomalies and visual pigment genes by Deeb *et al.* (1992).

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