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Characteristic component odors emerge from mixtures after selective adaptation

Holly F. Goyert¹, Marion E. Frank^{2,*}, Janneane F. Gent³, and Thomas P. Hettinger²

¹ Department of Psychology, Cornell University, Ithaca, NY

² Center for Neurosciences, Department of Oral Health and Diagnostic Sciences, University of Connecticut Health Center, Farmington, CT

³ Center for Perinatal, Pediatric and Environmental Epidemiology, Yale University School of Medicine, New Haven, CT USA

Abstract

Humans cannot reliably identify the distinctive characteristic odors of components in mixtures containing more than three compounds. In the present study we demonstrate that selective adaptation can improve component identification. Characteristic component odors, lost in mixtures, were identifiable after presenting other mixture constituents for a few seconds. In mixtures of vanillin, isopropyl alcohol, *l*-menthol and phenethyl alcohol, this rapid selective adaptation unmasked each component. We suggest that these findings relate directly to how olfactory qualities are coded: olfactory receptors do not act as detectors of isolated molecular features, but likely recognize entire molecules closely associated with perceived olfactory qualities or “notes.” Rapid and focused activation of a few distinct receptor types may dominate most odor percepts, emphasizing the importance of many dynamic and specific neural signals. An interaction between two fundamental coding strategies, mixture suppression and selective adaptation, with hundreds of potential olfactory notes, explains humans experiencing the appearance and disappearance of identifiable odors against ambient mixture backgrounds.

Keywords

human olfaction; mixture suppression; odor identification; dynamic odor coding

1. Introduction

With a few sniffs [35,36,47], a human can presumably recognize “hundreds of thousands of different odors” [2], feats typically achieved over an adapted background mixture of ambient odors. However, humans have a quite limited ability to simultaneously identify odor qualities of individual compounds in multi-component mixtures. Component odors in mixtures containing as few as four moderately intense simultaneously presented components cannot be recognized above chance levels [44,45,46]. We hypothesized that rapid selective adaptation,

*To whom correspondence should be addressed; e-mail: mfrank@neuron.uchc.edu, telephone: 1-860-679-3354, fax: 1-860-679-4760, Dr. Marion E. Frank, Center for Neurosciences, Oral Health and Diagnostic Sciences, University of Connecticut Health Center, Farmington, CT 06030-1605, USA

Author Contributions: All four authors contributed equally.

Present Address: RR1 Box 453D, Edgartown, MA

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in effect, would subtract ambient odor components from a mixture. Odors of ambient stimuli would decrease, exposing characteristic odors of other compounds in a mixture for identification [38].

Our experiment first and foremost tested a single prediction reflecting dynamic human olfactory experience: identification of characteristic odors of component compounds in odor mixtures would be significantly improved after presenting the other components of the mixture. Odors of components of ambient mixtures would fade quickly into the background, allowing *dynamic odor coding* to identify newly introduced compounds by their characteristic odors.

There is a consensus that olfactory coding is based on combinatorial receptor activation [48]. One theory has stimulus molecules deconstructed into molecular features by non-specific olfactory receptors to be reassembled as a multiplicity of odor images in the olfactory cortex [7,67,72,73]. By studying identification of the odors of four compounds with overlapping molecular features in mixtures, we also address odor coding.

2. Materials and methods

2.1. Subjects

10 women and 4 men, non-smokers with normal olfactory function and mean age of 20.9 (SD 1.2) years, volunteered to participate. The research protocol was reviewed and approved the University Committee on Human Subjects of Cornell University.

2.2. Stimuli and labels

Component stimuli (*labels*) were: 5 mM vanillin (*vanilla*), 1 M isopropyl alcohol (*alcohol*), 0.5 mM phenethyl alcohol (*rose*), 1 mM *l*-menthol (*mint*) and water (*none*). Compounds were chosen because they are non-toxic, water soluble and used extensively in clinical testing [13, 16,32]. Vanillin, phenethyl alcohol and *l*-menthol were reagent grade (Sigma Chemical Co., St. Louis, MO) and isopropyl alcohol was pharmaceutical grade (Topco Associates, Skokie, IL). The compounds, Fig. 1, have overlapping and distinct molecular features, functional groups (“osmophores”) and molecular shapes (“molecular profiles”) that have been associated with odors [52,54]. The component stimuli, dissolved in water, have distinctive, recognizable, agreeable odors with moderate perceptual intensities. In addition to water and the 4 single compounds, complex stimuli, with the potential to elicit multiple distinct odors, were the 6 binary mixtures, 4 ternary mixtures and 1 quaternary mixture of the 4 compounds. Stimuli were diluted from stronger stock solutions so that concentrations of compounds were identical in component and mixture stimuli. For example, the alcohol-rose mixture contained 1 M isopropyl alcohol and 0.5 mM phenethyl alcohol. Stimulus solutions were prepared before the 16-day testing period and stored in capped bottles. Each of the 16 solutions was sniffed 4 times in a session.

2.3. Procedures

Adaptation and control test sessions were scheduled at least one day apart for each subject. Test sessions took place in an 18–21°C, odor-free room with non-recirculating air. Olfactory stimuli were dissolved in water and presented in 16, 250 mL polyethylene squeeze bottles fitted with caps having flip-up spouts. Clean, dry caps were used for each session. Each bottle contained 50 mL of solution, a sizable reservoir. The experimenter (HFG), who wore vinyl gloves throughout, demonstrated that the bottle should be squeezed 1 or 2 times with the flip cap up, then sniffed once or twice in order to obtain the full aroma. Average sniff duration is 1.6 seconds [35] and distinct characteristic odors can be correctly identified with one sniff [36].

Subjects were introduced to the labels used for the component odors in session 1 in order to engage odor recognition memory [59]. They were allowed to smell each single compound, including water, and were given time to think of meaningful associations to help remember the label. In sessions 1 and 2, before being presented with the stimulus pairs, subjects were required to correctly identify the 5 odors of the 4 single compounds and water, twice in a row. If mistakes were made, corrective feedback followed. Subjects could refer to the list of labels (*Vanilla, Alcohol, Rose, Mint, None*) at all times while sampling and identifying each randomized item. Six of the subjects met criterion without a mistake; 4 required a single repetition of the stimuli in one session, and 4 required a single repetition in both sessions. Correct identification of the single compounds averaged $97 \pm 1\%$, suggesting the labels were “veridical” matches for the odors [10,61]. Before testing of stimulus pairs began, subjects were told that test items might contain no odor, 1 odor, or a combination of up to 4 of the odors on the list and were instructed to identify every odor that they smelled.

2.4. Experimental design

The testing protocol consisted of presentation of stimulus pairs to sniff in opposite orders in adaptation (adapt then test) and control (test then adapt) sessions. First session presentation of adaptation or control sequences was alternated across subjects. About 5 seconds intervened between the 2 stimuli in a pair, the time needed to switch bottles. The test item of a pair contained all the compounds in the adapt item plus a single extra compound **X**. Adapt items were solutions of 0 (water), 1, 2 or 3 compounds; test items were the 1, 2, 3 or 4 compounds to be identified by their characteristic odors (Table 1). Each of the 4 compounds served as **X** in 8 pairs per session, yielding 32 pairs that were presented in a random sequence for each subject. The stimulus pairs were presented at least 1 minute apart, sufficient time to re-establish vapor concentration in the head space [57]. Table 1 shows the adapt-test pairs for adaptation sessions in which subjects sniffed adapt items and then immediately sniffed and identified the odors of test items. In control sessions test items were presented before adapt items; subjects sniffed and identified test odors, and then immediately afterwards sniffed adapt items. Thus, subjects sniffed 32 stimulus pairs and identified stimuli with adaptation or without adaptation in separate sessions that each lasted less than one hour.

2.5. Data Analysis

Identification of test items in adapt-test pairs in adaptation sessions evaluated the subjects’ abilities to identify the odor of each compound when it was an un-adapted compound **X** or a previously presented ambient compound. In control sessions, the test stimulus of the stimulus pair was presented first and identified. Identification of test items in control sessions established the subject’s ability to identify compounds as members of ambient plus **X** stimulus mixtures without selective adaptation. For example, in the A-AV pair listed for adaptation sessions in Table 1, subjects were asked to identify the components in the alcohol-vanillin mixture after adaptation to alcohol. However, when subjects were presented with the AV-A pair in control sessions, subjects were asked to identify the alcohol-vanillin components before presentation of alcohol. In this example, identification of vanillin was an extra stimulus control and identification of alcohol was an ambient stimulus control value. The control values were used to test for effects of selective adaptation on identification of characteristic odors of test stimuli.

Extra-stimulus and ambient-stimulus odor identification scores (1 point for each correct identification) were computed for each compound in 2, 3, or 4 component mixtures. Each compound appeared as **X** in 7 test-stimulus mixtures (3 binary, 3 ternary, 1 quaternary), and as an ambient stimulus in 12 test-stimulus mixtures (3 binary, 6 ternary and 3 quaternary), cf. vanillin example in Table 1. For analysis of variance (ANOVA), scores were expressed as proportions correct, with session (adaptation, control), stimulus status (extra, ambient), mixture

size (2, 3, 4 components) and compound (isopropyl alcohol, vanillin, *l*-menthol, phenethyl alcohol) as the 4 factors.

Stimulus pairs containing water tested the subjects' abilities to identify the odor of each compound as a single component (cf. "W" rows in Table 1). Single compound odor identification scores (1 point for each correct identification) were computed for each compound. Scores for ANOVA were expressed as proportions correct, with 2 factors, session and compound. Absent compound odor identification scores (1 point for each incorrect identification), "false positives," were also computed for each compound for the 12 times each was absent from test stimuli (cf. test mixtures without yellow shading, vanillin example, Table 1). Scores for ANOVA were expressed as proportions, with 2 factors: session and compound. All ANOVAs were repeated measures with the 0.05 α level adjusted with a Bonferroni correction for multiple post-hoc comparisons.

3. Results

3.1. Identifying odors of extra compounds added to ambient stimuli

Presenting a mixture of all compounds but one (**X**) to sniff for a few seconds before sniffing the mix with **X** added, improved identification of the extra compound's odor (Fig. 2). Compared to controls, correct identification of the characteristic odor of a mixture component was higher when it was freshly added to the ambient mix but lower when presented a second time as part of an adapted ambient mixture ($p < .0001$, Table 2). The few seconds of adaptation simultaneously increased correct identification of odors of newly added stimuli ($p < .0001$), upholding our primary hypothesis, and decreased correct identification of odors of already presented stimuli ($p < .001$), as would be expected with rapid adaptation.

This primary finding, formally a statistical interaction between adaptation (adaptation session vs. control session) and stimulus type (extra vs. ambient), is reiterated in Figs. 3 and 4. In the subsequent figures, data are configured to display other factors, mixture complexity and compound, that significantly affected the results.

Stimulus complexity (mixture size) influenced percent correct odor identification ($p < .0001$, Table 2). A 61% correct identification of component odors in 2-component mixtures exceeded the 54% for 3-component ($p < .001$) or 50% for 4-component ($p < .0001$) mixtures. However, compared to controls, odors of un-adapted extra stimuli were better identified and odors of adapted ambient stimuli less readily identified whether embedded in mixtures containing 2, 3 or 4 components (Fig. 3).

The 72 % correct identification of the odor of isopropyl alcohol in mixtures was greater than the 50 % identification of the odor of phenethyl alcohol ($p = .0001$), suggesting the alcohol odor may have been stronger in intensity than the rose odor (cf. Fig. 5 for correct identification of single components). However, although correct identification of the compounds' odors in mixtures varied ($p < .001$; Table 2), compared to controls, the odor of an un-adapted extra stimulus was more frequently identified and the odor of an adapted ambient stimulus was less frequently identified whether it was isopropyl alcohol, vanillin, *l*-menthol or phenethyl alcohol (Fig. 4).

In conclusion, the increase in salience of extra odor components, newly added to adapted ambient mixtures, occurred for three mixture complexities, four compounds, and, possibly, several odor intensities.

3.2. Identifying odors when presented alone and when absent

Subjects were taught to use specific labels for characteristic odors of test compounds before being presented with stimulus pairs. Included among stimulus pairs, each single compound appeared once as the test stimulus after water adaptation (Table 1). Identification of the odor of *l*-menthol as *mint* was perfect when menthol was presented before or after a sniff of water. However, characteristic odors of other stimuli were sometimes missed ($F(3,39) = 3.2$, $p = .03$) (Fig. 5, tall bars). Interestingly, the water sniff helped to identify phenethyl alcohol's *rose* odor ($p < .05$), the odor least often identified correctly in control sessions.

Percent incorrect identification of the odors of compounds that were not presented is also shown in Fig. 5 (short bars). Although these mistakes (false positives) were infrequent, identification of characteristic odors of absent compounds decreased with adaptation ($F(1,13) = 6.88$, $p = 0.02$).

Overall, subjects correctly identified single compounds 90 % of the time and chose incorrect odor labels for the compounds 10% of the time.

4. Discussion

4.1. Selective adaptation determines which odors in mixtures can be identified

Odors come and go. Hidden when stimulus compounds are presented at the same time in a mixture background, characteristic odors of compounds momentarily appear when newly added to the mix. To imitate this scenario, our subjects were presented with a stimulus to sniff before switching about 5 seconds later to sniff a stimulus containing one extra compound **X**. The brief selective adaptation greatly enhanced identification of characteristic odors of un-adapted **X** and reduced recognition of odors of previously presented compounds. We propose that odors of newly appearing compounds are often perceived for a few moments above the concealed odors of ambient compounds. The transient, ever-changing odor percepts are the consequence of two remarkably important and unexpectedly rapid physiological processes, mixture suppression and sensory adaptation.

Selective adaptation's impact on identification was large, about 50 % of the 80 % practical range of our subjects' ability to identify the odors. While testing stimulus pairs, correct identification of single compounds' odors averaged 90 % and false positives averaged 10 % (Fig. 5). When compounds defined as extra or ambient were presented with no adaptation in control sessions, identification of component odors averaged 55 % (the average of extra and ambient control bars in Fig. 2); however, after selective adaptation, identification of an extra compound's odor increased to 75 % and identification of odors of ambient compounds fell to 38 % (Fig. 2). The 20 % increment for extra compounds plus 17 % decrement for ambient compounds sums to 37 %, nearly 50 % of the subjects' 80 % identification range.

The strong influence of selective adaptation on odors suppressed in mixtures demonstrates *dynamic olfactory coding*, a rapid focusing of the olfactory system on newly introduced and stronger odors at the expense of veridical identification of the odors of all compounds that are present. To demonstrate this, we compared *veridical* identification (scoring 1 point for identifying odors of present compounds and 1 point for not identifying odors of absent compounds) with identification predicted by *dynamic odor coding* (scoring 1 point for identifying odors of newly added extra compounds and 1 point for not identifying odors of any other compounds, ambient or absent) (Fig. 6). *Veridical coding* fell far short of *dynamic odor coding* ($F(1,3) = 39$, $p < .0001$). Furthermore, veridical odor identification worsened going from 3 to 4 component mixtures ($p < .0001$) but identification focused on the extra compound did not ($F(1,13) = 16$, $p < .001$). Our subjects were equally good at identifying un-adapted extra odors in 3 component or 4 component mixtures (cf., Fig. 3). Humans may detect characteristic

odors of newly added compounds no matter how many ambient suppressed/adapted compounds are already present.

4. 2. Mixture component identification reveals analytic odor processing

We studied the processing of odor information in humans by asking subjects to identify an odor embedded in a mixture immediately after presenting other mixture components. Substantial effects of mixture suppression [45] and selective adaptation [41] on identification demonstrated the value of the method both technically and theoretically. By using 16 mixture permutations of 4 component compounds (Table 1), a robust statistical treatment based simply on correct identification was possible [24,27].

We used water soluble compounds with distinctive, recognizable and agreeable odors, compounds that had overlapping as well as distinct molecular features. The subjects easily learned to use odor labels for single components before testing sessions. The 4 compounds, with concentrations adjusted to moderate odor intensities consistent with mixture suppression [39], exhibited mixture suppression during testing. Control identification proved to be reduced in 3 and 4 component mixtures ($F(1,13) = 28.80$, $p < 0.0001$) even though odors of the 4 single compounds were not recognized with equal frequency ($F(3,39) = 4.27$, $p < 0.009$).

Studies of anosmic humans [12,17] have shown that phenethyl alcohol and vanillin mostly activate the olfactory nerve, CN I; but isopropyl alcohol [60] and *l*-menthol, like many olfactory stimuli, also activate the trigeminal nerve, CN V. Compared to extra controls (control bars at left of bars for extra stimuli in Fig. 4), correct identification of characteristic odors of extra compounds, with or without trigeminal epitope, increased by 18 % when added to the adapted mix. However, compared to ambient controls (control bars at left of bars for ambient stimuli, Fig. 4), the 5-sec adaptation reduced correct identification of vanilla and rose odors by 22 %, more than the alcohol and mint odors, which were reduced by 8 % ($F(1,13) = 5.33$, $p = 0.04$). We are uncertain whether this difference reflects fundamental differences in trigeminal and olfactory adaptation/identification [59] or slight mismatches in stimulus intensity, distinctiveness or familiarity. Nonetheless, identification of odors of the four component compounds all showed similar improvement after selective adaptation, providing evidence for analytical processing of odors not available from other experimental paradigms [25,26,56, 63].

We propose that by concealing ambient odor identity, mixture intensity suppression and adaptation allow for recognition of dominant characteristic odors. The odor of a very complex mixture without recognizable attributes would have little utility because mixtures typically lack repeatability and therefore identity, especially on variable backgrounds. Consistent with mixture suppression, odors of many common foods and fragrances appear to be dominated by notes of single compounds that impart characteristic, albeit crude, identities [20,25,26,61]. Because odor-component identification is suppressed in mixtures [44,45,46], we surmise that olfactory notes originate from very few components that dominate odors of mixtures due to their relative strength at a given point in time [39,56]. “Top note,” “middle note” and “bottom note” [62] are terms used to specify the timing of successive odors emerging from fragrant stimulus mixtures. Such identifiable odors are similar in principle to the odors that appear following selective adaptation that we report experimentally.

4.3. Characteristic odors rapidly wane after a sniff or two

In our experimental configuration, component odor recognition was already suppressed in mixtures. Yet, compared to controls, our subjects’ abilities to identify ambient odors declined an additional 31 % after 5 seconds of sampling, which changed relative odor saliency [38], making an extra odor easier to identify. In adaptation sessions we did not simply measure

declines due to waning odors, but tested ambient-odor identification after an extra compound had been introduced into the mix. Because identification in adaptation sessions was compared to control sessions when subjects received all *test* compounds without adaptation, we attribute declines in ability to identify ambient odors to a rapid sensory adaptation, not suppression. A few seconds of adaptation had quite significant consequences on odor identification, revealing an important odor coding strategy: a rapid reduction of ambient “noise”.

Although some human psychophysical experiments having subjects rate stimulus intensity suggest rapid adaptation/recovery and partial adaptation after a single sniff [53]; other rating experiments found odor adaptation may be incomplete even after several minutes or more, depending on the type of stimulus and its concentration [4,15,52]. Furthermore, cross-adaptation, effects of prior presentation of one compound on the odor intensity of another compound, may not consistently reflect molecular or odor similarity [53,68]. However, we demonstrated rapid selective adaptation with a clear loss in adapted-odor identification, an effect that may be associated with intensity reduction [41].

Animal studies also provide experimental data on the time course of olfactory adaptation and recovery from adaptation that are quite varied; loci and mechanisms of adaptation remain unclear [15,29,68]. Newt and salamander olfactory sensory neurons (OSN) adapt within 1 sec. and recover from adaptation within 10 sec. [31,34,74]. Rat and mouse OSN also partially adapt in a few seconds [5,50]; however, rapid OSN adaptation is not easily reconciled with olfactory bulb and olfactory cortex recordings showing adaptation taking a minute or more [29,66]. The mismatch may come, in part, from how sensory adaptation is defined. Some recordings from brain sites address decreased responding with repeated stimulus presentation (habituation), which likely involves different mechanisms than decreased responding with continuous stimulus exposure (adaptation). In fact, if OSN were to rapidly adapt and recover (as OSN may), responses to repeated stimulus pulses would not decline peripherally and the habituation observed would require central circuits. Also, inconstant odors due to sources that fluctuate or actions, such as varying head position and sniffing [47], may be experienced for long periods due to varying stimulus concentration. Nevertheless, under the controlled conditions of our experiment, the ability to correctly identify ambient compounds’ characteristic odors by associated labels [10] rapidly declined to a level sufficient to decisively promote recognition of single added compounds. Determination of how the adaptation we measured by identification quantitatively relates to decreases in intensity measured by ratings [41] requires further study.

4.4. Dynamic odor coding assures odor-quality constancy

Our finding that odor mixture components will reclaim their individual identities after selective adaptation raises important issues about mechanisms of odor quality coding. A general lack of a one-to-one correspondence between particular stimuli and individual receptors has led to a consensus that olfactory coding is based on combinatorial molecular feature detection [48]. One specific theory argues that OSN “deconstruct” an olfactory stimulus into its individual molecular features [7] and, according to this model, each individual molecular feature of the compounds smelled by our subjects would be separately coded by a distinct set of OSN. The compounds that we used shared some molecular features (Fig. 1). The olfactory responses to shared molecular features, theoretically, should have been adapted for extra as well as ambient compounds. Subtraction of responses to a key molecular feature would likely obscure odor identity; yet characteristic odors of extra compounds were more easily detected above the background. Subjects identified odors as the same as characteristic odors of the distinguishable individual compounds for which they had learned “veridical” labels [10]. Thus, the key constellation of molecular features of an individual odorous compound appears to remain irresolvable in mixtures.

There are studies that support a combinatorial model of odor stimulus recognition by overlapping and diverse receptor arrays [6,7,18,21,30]. Two common themes in these studies are that olfactory receptors are responsive to a wide range of chemical stimuli and most odorants activate multiple receptors. A given receptor type could be activated by a range of ligands, even those having different functional groups and presumably different odor qualities [7,30]. Octanal, a simple aliphatic aldehyde, was an effective stimulus for 55 different receptors, implying that octanal recognition might require activation of this large array [18]. Furthermore, some of the same receptors also responded to octanol, an alcohol analog with a different odor quality.

There are reasons why a broad spectrum of chemicals at high concentrations might activate receptors. For one, specificity of tuning may be greatest at the lowest concentrations [2] and the 10 to 100 micromolar stimuli used could result in non-specific binding [43]. Although it is hard to estimate what concentrations are “physiological,” many odorants are recognized in the gas phase at 1 part per million or less, corresponding to a vapor concentration of about 40 nanomolar, or roughly 1000 times weaker than that used in the liquid phase experiments noted above. Also, certain compounds, particularly aldehydes that can form Schiff bases with amino groups in proteins, might not be acting as true ligands, but rather as “reagents” at high concentrations. Another notable example is the use of high concentrations of dicarboxylic acids as olfactory ligands in mammals [48], when dicarboxylic acids as a group have negligible vapor pressure and no odor [52].

The olfactory receptors (OR) can display a high degree of specificity, as reflected by the ability of humans to discriminate optical isomers [40] and by the repeated ligand-driven isolation of the same OR in single-cell PCR experiments [64]. The original portrayal of rat OR I7 as an “octanal receptor” [71] further suggested specificity of ligand-receptor interactions but this concept may need reappraisal in view of subsequent identification of many receptors sensitive to octanal [18]. Specific anosmias in humans [1] are consistent with codes for particular odors representing restricted activation of some part of the olfactory neural circuit. The fact that the component stimuli we used did not cross-adapt implies that they did not elicit functionally significant overlap in receptor-activation patterns.

OSN display highly specific features. Each one of the hundreds of G-protein coupled ORs [8] is exclusively expressed in a subset of OSN that target a specific few glomeruli located in stereotyped positions in the olfactory bulb [51,58,65]. Furthermore, there is growing evidence that olfactory bulb output neurons, the mitral cells, are specific chemical feature detectors [43,54]. Each mitral cell obtains direct input exclusively from a single glomerulus to convey to the olfactory cortex. A hallmark of mitral cell responses is a sharpening of tuning attributable to inhibitory inputs from neighboring glomeruli, which receive direct input from different OSN and respond to different olfactory stimuli [69]. Overall, mixtures would suppress mitral cell activity while enhancing differential responses to a new stimulus. The masking of components in mixtures and their emergence following selective adaptation demonstrated by our study are logical perceptual consequences of olfactory bulb inhibitory circuits. Ambient odors fade quickly over time, allowing identification of the appearance of a new stimulus. However, the odor of the new stimulus will be identified only as long as it remains stronger than odors of other mixture components: its odor too quickly adapts. This changeability is the hallmark of *dynamic odor coding*.

An important corollary of our odor-identification study is that it demonstrates odor-quality “constancy” in the presence of different backgrounds, a necessary and sufficient condition for olfactory stimulus recognition. Taste-quality “constancy” in mixtures is also observed [3,22, 24,27] and the unmasking of a taste or olfactory mixture component following selective adaptation has been briefly noted previously [33,41]. A principal difference between taste and

smell lies in numbers of human taste and odor qualities. A total of 5 taste qualities [28,49] is sufficient to identify gustatory attributes of many different foods and drinks. Molecular receptors for the sweet and bitter tastes [23] are linked to heterodimeric GPCR complexes and multiple GPCRs, respectively, each expressed in single taste-bud receptor cells [55,70]. The hundreds of ORs devoted to olfaction, singly expressed in dedicated OSN, may likewise each be associated with one quality or odor note. If pure substances were to each have just two resolvable, distinguishable dominant odor notes of similar strength [20], an array of 350 different human olfactory receptors [19] could theoretically allow the recognition of more than 100,000 different chemicals. Predictably, because, unlike tastes, they are so numerous, odor qualities are difficult to bring to mind unless linked to actual objects in one's personal experience [10,11,37] and appear countless and difficult, if not impossible, to classify [9].

Although we ordinarily associate smells with objects, the idea that we recognize distinct coffees and wines by patterns generated by extremely complex "cocktails" of olfactory stimuli is implausible, given mixture suppression. Coffee is a highly variable commodity and only a few key odor notes will likely be common to all coffee preparations, allowing us to call them all "coffee." In a similar way, wine odor is likely dominated by a few chemicals, invariably including ethanol, with distinctive attributes of different wines recognizable [9], but a particular wine would not be expected to have a very large number of odor qualities [20,25,26]. Recognition, characterization and categorization of the complexes of odor qualities of variable objects may require some of the constellation of hierarchical-parallel circuits available in the central olfactory system for odor memory, cognition and affect [9,14,59,67].

In evolutionary terms, receptors are unlikely to have evolved for complex substances such as coffee and wine because both are variable and these popular examples are largely artificial as well. The receptors and possibly the associated neural circuits in the olfactory bulb [69], more likely evolved to detect specific constellations of chemical features, more or less representative of single compounds [42,43,54] —not complex and variable objects. It is likely for this reason that so many receptor classes are needed to characterize the multiple odor qualities.

4.5. Précis

Odor components, although masked in mixtures, rapidly reclaimed their identities after adaptation effectively subtracted other odors. Thus, odor-quality coding is both dynamic and specific. The odor code is likely a sensory language of molecular recognition using a very large receptor alphabet where letters are more informative than words.

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Fig 1. The four stimulus compounds

Component stimuli were: 5 mM vanillin, 1 M isopropyl alcohol, 0.5 mM phenethyl alcohol, and 1 mM *l*-menthol. The compounds have overlapping (hydroxyl, methyl, phenyl) and distinct (aldehyde, phenolic, terpene) molecular features that have been associated with odors. Complex stimuli included binary, ternary and quaternary mixtures of the 4 compounds.

Fig 2. Effect of selective adaptation on odor identification

Mean percent correct identification ($\pm$ se) with four compounds serving alternately as extra (X) or ambient stimuli in test mixtures is plotted. Compared to controls, adaptation increased correct identification of odors of the extra stimuli newly introduced in a mixture, $p < .0001$, but decreased correct identification of odors of the ambient stimuli, previously presented for a few seconds, $p < .001$.

Fig 3. Identification of odors of compounds in 2, 3 and 4 component mixtures

Mean percent correct identification ($\pm$ se) for test compounds embedded in 2 (Mix 2), 3 (Mix 3) or 4 (Mix 4) component mixtures is plotted. Characteristic odors of the 4 compounds were correctly identified more frequently in 2 component than 3 or 4 component mixtures ($p < .001$). Compared to controls, odors of unadapted extra stimuli were better identified and odors of adapted ambient stimuli

Fig 4. Identification of compounds as extra and ambient stimuli

Mean percent correct identification ($\pm$ se) of odors of four compounds in mixtures is plotted. The alcohol odor was more frequently identified than the rose odor ($p = .0001$). Yet, compared to controls, odors of each un-adapted extra stimulus were identified more frequently and odors of each adapted ambient stimulus were identified less frequently.

Fig 5. Identification of odors of single compounds and absent compounds

Mean percent correct identification of odors of single-components and incorrect identification of absent compounds ($\pm$ se) are plotted. Compared to controls, presenting water before single compounds improved correct identification of the rose odor ($p = .05$) and selective adaptation decreased the frequency of incorrect identification of odors of absent compounds (false positives) ($p = .02$).

Fig 6. Identification of odors in mixtures

Mean scores ($\pm$ se) are plotted for 3 and 4 component mixtures. *Veridical odor coding*, identifying characteristic odors of all compounds presented, *All Present*, including adapted ambient and un-adapted extra compounds, worsened with mixture size ($p < .0001$). However, *dynamic odor coding*, identifying only an extra compound added to the mix, *Extra Only*, was equally good for 3 and 4 component mixtures.

Table 1

32 adapt-test stimulus pairs* used in adaptation sessions.

Vanilla (V)		Alcohol (A)	
Adapt	Test	Adapt	Test
W	V	W	A
A	V ^A	V	VA
R	VR	R	AR
M	VM	M	AM
AR	VAR	VR	VAR
AM	VAM	VM	VAM
RM	VRM	RM	ARM
ARM	VARM	VRM	VARM
Rose (R)		Mint (M)	
Adapt	Test	Adapt	Test
W	R	W	M
V	VR	V	VM
A	AR	A	AM
M	RM	R	RM
VA	VAR	AR	ARM
AM	ARM	VA	VAM
VM	VRM	VR	VRM
VAM	VARM	VAR	VARM

* Vanilla (V), alcohol (A), rose (R) and mint (M) are odor labels; W = water. 4 sets of 8 stimuli are represented; each compound was extra compound **X** (bold type) in one set. Yellow shading identifies the 7 times vanillin was **X** (V set) and the 12 times vanillin was an ambient, adapted compound (**A**, **R** and **M** sets). Isopropyl alcohol, phenethyl alcohol and *l*-menthol were each also **X** 7 times and ambient 12 times.

Table 2
Mixture component identification: 4-factor ANOVA.

Source	df (factor, error)	F	p-value
Adaptation	(1,13)	0.22	0.64
Stimulus (extra, ambient)	(1,13)	58.64	0.0001
Mixture (2,3,4-component)	(2,26)	18.6	0.0001
Compound	(3,39)	6.38	0.001
Adaptation x Stimulus	(1,13)	35.68	0.0001
Adaptation x Mix	(2,26)	0.76	0.48
Adaptation x Compound	(3,39)	1.26	0.30
Stimulus x Mix	(2,26)	1.38	0.27
Stimulus x Compound	(3,39)	2.14	0.11
All other interactions			ns