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Perception of pungent odorants alone and in binary Mixtures

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Abstract

The present investigation explores the stimulus-response (psychophysical) functions for total nasal perceived intensity for two pungent odorants, formaldehyde and ammonia, presented either alone or with different backgrounds of the other irritant. Stimuli were comprised of four formaldehyde concentrations (1.0, 3.5, 6.9 and 16.7 p.p.m.); four ammonia concentrations (210, 776, 1172 and 1716 p.p.m.); and their 16 binary mixtures. Ammonia functions showed a consistent upward concavity. At low, medium and high concentrations, the total perceived intensity of the mixtures showed hypoadditivity, simple additivity and hyperadditivity, respectively. That is, the intensity of the corresponding mixtures was significantly lower than, equal to, or greater than the sum of its components. The progressive involvement of pungency, aroused by common chemical sense stimulation, may be responsible for the increasing additivity observed. Simple additivity, and even hyperadditivity, may characterize mixtures involving common chemical stimulation.
Introduction

The nasal cavity of humans contains two major chemoreceptor systems: olfaction and the common chemical sense (CCS). The latter, so frequently neglected, has drawn more attention in recent decades since the pioneering work of Tucker (Beidler and Tucker, 1956; Tucker, 1961, 1963, 1971). This sensory modality gives rise, in humans, to sensations such as irritation, freshness, stinging, prickliness, burning and tingling which can be generically termed pungent sensations and are mediated principally by the trigeminal nerve.

It is difficult to study the functional properties of one of these two sensory channels without the influence of the other since they not only show an important interaction (Cain and Murphy, 1980), but also almost completely overlap in the chemical substances that can stimulate them. This means that almost all odorivectors show some capacity for stimulating the trigeminal nerve, even when no apparent pungency can be perceived (Cain, 1974a) and that almost all pungent substances also stimulate the olfactory nerve. In the case of CO₂, which has been employed in studies of the common chemical sense in the nasal and oral cavities (Cometto-Muñiz and Cain, 1982; Dunn et al., 1982; García-Medina and Cain, 1982; Cometto-Muñiz and Noriega, 1985; Cometto-Muñiz et al., 1987) the typical pungency is virtually devoid of odor (Cain and Murphy, 1980) or taste.

Interesting results were obtained studying subjects with unilateral destruction of the trigeminal nerve (Cain, 1974a) and anosmics lacking olfactory nerve function (Doty, 1975; Doty et al., 1978). The general conclusion from these studies is that there is an important trigeminal component in the total response of the nasal chemoreceptors to a great variety of inhaled chemicals.

In the present investigation, nasal perception was studied in terms of total perceived intensity of two pungent odorants [formaldehyde (H₂CO) and ammonia (NH₃)] when presented alone and in various binary mixtures. The aims of this study were to: (i) examine the psychophysical stimulus–response function for two odorants that also clearly stimulate the CCS; (ii) explore possible differences between genders regarding the perception of these pungent odorivectors (see Dunn et al., 1982; García-Medina and Cain, 1982; Cometto-Muñiz and Noriega, 1985); (iii) depict the influence of different backgrounds of each stimulus on the psychophysical function of the other stimulus, both in terms of rate of growth and relative position along the perceived intensity axis, and (iv) investigate the relationship between the perceived intensity of the various binary mixtures and the perceived intensities of their components presented alone at the same concentration.
Materials and methods

Stimuli

A two channel air-dilution olfactometer was employed to deliver the various concentrations of each of the two pungent odorants and their mixtures to the subject's nostril at a flow rate of 4 l/min, in 2.5-s bouts.

Formaldehyde (analytical grade purity) concentrations were 1.0, 3.5, 6.9 and 16.7 p.p.m., as measured by the chromotropic acid method (NIOSH, 1973). Ammonia (analytical grade purity) concentrations were 210, 776, 1172 and 1716 p.p.m., measured spectrophotometrically according to a standard technique (NIOSH, 1974). The total number of different stimuli was 24–four concentrations of formaldehyde, four of ammonia, plus 16 binary mixtures.

The concentration range of formaldehyde and ammonia was selected in order that the suprathreshold levels employed would reach a point where almost all subjects would agree that pungency was the completely dominating sensation. It was also necessary for the range to be compatible with the subjects safety and willingness to evaluate such pungent levels.

In a previous work with ammonia (Cometto-Muñiz and Cain, 1984) for inhalation periods (1.5 s) near those employed in this study (2.5 s) the threshold for a transitory reflex apnea provoked by ammonia was 800 p.p.m. on average, with a range of 400–1000 p.p.m. Based on this experience with ammonia, a range comprising two concentrations below and two above this threshold was selected.

With formaldehyde however, we had no previous experience about possible levels so concentrations rendering mild to intense pungency according to pre-tests were selected. Since it was not necessary to match concentration ranges of formaldehyde to corresponding ammonia, differences between the perceived intensities of the two ranges were not considered.

Since pungent sensations grow with inhalation time (Cometto-Muñiz and Cain, 1984), subjects were told that they could take out the nasal probe at any time before the 2.5-s bout if discomfort was intolerable, but they should mention this to the experimenter. During the testing sessions none of the subjects found that they were unable to tolerate any of the stimuli presented for 2.5 s.

At the beginning of the session, participants were asked to pause between stimulus presentations until all sensations from the previous stimulus had disappeared. Without exception all subjects eliminated any previous sensation with a few seconds of respiration. At the end of the session none of the participants complained or commented about any stimulus effect other than the
momentary odor or pungency evoked.

**Subjects**

Thirty subjects (10 men and 20 women) participated. Their average age was 21.2 (±4.8 SD) years. Men had an average age of 24.6 (±6.8 SD) years and women 19.4 (±2.1 SD) years.

All participants were nonsmokers. They were mainly university undergraduates or graduates and, at the time of participation in the test, were in good general health, lacking allergies, colds, or any respiratory tract diseases.

**Procedure**

Participants were instructed to use the method of magnitude estimation (S.S. Stevens, 1957, 1975) to judge the total nasal perceived intensity of each stimulus. Subjects assigned any number deemed appropriate to the first stimulus presented (called standard), and thereafter, they assigned to subsequent stimuli numbers reflecting total intensity relative to the standard.

Stimuli were presented in an irregular order (i.e., in no monotonic increasing or decreasing fashion, but not completely at random since the presentation of a very weak stimulus immediately after a very strong one or vice versa was avoided). By the end of the session, each subject made two estimates of each of the 24 stimuli.

At the beginning of the session, subjects chose one nostril (the more sensitive or, if both were equally sensitive, the more comfortable to work with) and used that nostril throughout. The other nostril remained free. In each trial, participants had to inhale for 2.5 s (paced by a metronome), and maintain the inhalation (or sniffing) effort as constant as possible throughout the different trials.

**Data analysis**

Since each subject was free to choose his or her own modulus, the variability around the mean value for each stimulus was artificially high. To eliminate the scatter due to differences in the choice of the initial judgement in the session, the data were normalized by bringing each subject's data to a common modulus (Lane et al., 1961; Cain and Moskowitz, 1974). Data were summarized in terms of the geometric mean of each subject's average response for each stimulus.
**Results**

Figure 1 depicts the stimulus-response (psychophysical) functions for total nasal perceived intensity for formaldehyde and ammonia when each irritant was evaluated alone or in the presence of various backgrounds of the other irritant. Ammonia functions show upward concavity in the linear coordinates of the figure. This concavity remains even if the data are plotted in logarithmic coordinates, confirming previous results (Cometto-Muñiz and Cain, 1984).

![Figure 1](image-url)

*Figure 1.* Total nasal perceived intensity as a function of concentration (p.p.m.) for formaldehyde and ammonia, alone and in the presence of various backgrounds of the other irritant. Each point represents the geometric mean of the average of two replicates made by each of 30 subjects.

A two-way ANOVA with interaction was performed over the group of formaldehyde functions (Figure 1, left side) with formaldehyde concentration
steps as one factor and ammonia backgrounds as the other. A similar test was run over the group of ammonia functions (Figure I, right side) with ammonia concentration steps as one factor and formaldehyde backgrounds as the other. The results for formaldehyde functions revealed significant effects for the different ammonia backgrounds ($F_{4580} = 152.27, P < 0.001$), for the formaldehyde concentration steps ($F_{3580} = 25.23, P < 0.001$), and for their interaction ($F_{12580} = 1.99, P = 0.023$). Similarly, the outcome for ammonia functions showed significant effects for the various formaldehyde backgrounds ($F_{4580} = 25.02, P < 0.001$), for the ammonia concentration steps ($F_{3580} = 164.91, P < 0.001$), and for their interaction ($F_{12580} = 2.14, P = 0.013$). The significance of the interaction term for both groups of functions indicates that functions within each group are not parallel.

Functions for formaldehyde and ammonia were analyzed by sex. Since each subject was free to assign any numerical modulus deemed appropriate to the first stimulus total nasal perceived intensity, it is not valid to compare the psychophysical functions obtained for males and females in terms of their absolute position along the perceived intensity axis. Nevertheless, we can compare the relative position of the various functions within the male group with their relative position within the female group. This comparison was made through a three-way ANOVA with gender, formaldehyde concentration and ammonia concentration as factors. The outcome showed significant effects for formaldehyde ($F_{4112} = 40.06, P < 0.001$), for ammonia ($F_{4112} = 61.97, P < 0.001$) and for the formaldehyde by ammonia interaction ($F_{16448} = 5.79, P < 0.001$), but not for the gender factor or any interaction involving it, including the interaction of gender by formaldehyde by ammonia ($F_{16448} = 1.17, \text{n.s.}$).

Figure 2 shows the relationship between the perceived intensity of the binary mixtures ($\Psi$ mixtures) and the sum of the perceived intensities of their components when presented alone at the same concentration as in the mixtures ($\Psi H_2CO + \Psi NH_3$). The dotted line represents the identity line of slope 1.00, around which the experimental points should have fallen if the total perceived intensity of the mixtures showed simple addition (i.e., if a mixture intensity was the sum of its components intensities). As seen, at low perceived intensities, mixtures are hypoadditive (i.e., lower than the sum of the component intensities); at intermediate intensities, the mixtures are additive; and at high intensities, the mixtures are hyperadditive (i.e., higher than the sum of the component intensities).
Figure 2. Total nasal perceived intensity for each of the 16 formaldehyde and ammonia binary mixtures as a function of the sum of their components perceived intensities, at the same concentration than in the mixtures, but presented alone. Straight line equation: $y = 1.50x - 18.63$, $r = 0.98$. The dotted line represents the identity line (slope = 1.00).

Figure 3 presents histograms for the type and degree of additivity for each binary mixture. Figure 3A depicts the total nasal perceived intensity of each of the four ammonia concentrations in the absence of formaldehyde and in the presence of four concentrations of formaldehyde. Figure 3B shows the same for the four formaldehyde concentrations in the absence and presence of ammonia. The bars that represent the perceived intensity of the various binary mixtures carry on their extreme right, a rectangle either empty or shaded. The empty rectangles indicate
hypoadditive mixtures. This means that the extreme left of the rectangle, where the segment representing the standard error begins, indicates the mixture intensity. The extreme right of the rectangle indicates the sum of that particular mixture components intensities when presented alone. The shaded rectangles indicate hyperadditive mixtures. The extreme left of the rectangle now indicates the sum of the mixture components intensities, while the extremer right (higher intensity) indicates the mixture intensity.

Figure 3. Histogram representing total nasal perceived intensity of various ammonia concentrations, alone and in the presence of growing formaldehyde backgrounds (A) or of various formaldehyde concentrations, alone and in the presence of growing ammonia backgrounds (B). Each bar represents the geometric mean (±SE) of the average of two replicates made by each of 30 subjects for that stimulus. Empty rectangles at the end of
the bars represent hypoadditivity degrees, while shaded rectangles represent hyperadditivity degrees (see text). Bars marked with an encircled number represent significative hypoadditivity (bars with empty rectangles) or significative hyperadditivity (bars with shaded rectangles) according to: 1, $P < 0.05$; 2, $P < 0.005$; and 3, $P < 0.001$; one mean $t$-test.

We compared each mixture intensity with the sum of its components intensities for each subject. The logarithm of the subjects' normalized magnitude estimations was used for the $t$-tests since such estimations show a log normal distribution (J.C. Stevens 1957; S.S. Stevens 1975). Results revealed that, from a total of 16 binary mixtures, seven showed significant hypoadditivity ($P < 0.005$), seven showed simple additivity (no significant differences between the mixture perceived intensity and the sum of its components perceived intensities), and two showed significant hyperadditivity ($P = 0.036$ and $P = 0.014$).

Thus, mixtures of low concentrations of the pungent odorants produce hypoadditivity of the evoked total nasal sensation, mixtures of intermediate concentrations produce simple additivity, and mixtures of the high concentrations produce hyperadditivity.

**Discussion and conclusions**

Figure 1 shows that ammonia functions exhibit a pronounced upward concavity while formaldehyde functions do not. This concavity remains even if the data are plotted in logarithmic coordinates. Previous investigations indicated that odor psychophysical functions are generally flatter than taste functions (see Cometto-Muñiz, 1981), but functions for pungency are characterized by higher growth rates than those for odor, independent of the scaling procedure employed—category scaling (Katz and Talbert, 1930) or magnitude estimation (Cain, 1976). Moreover, using CO$_2$ as the pungent stimulus, it was observed that nasal pungency growth rates can be higher than those of buccal pungency (Cometto-Muñiz and Noriega, 1985).

It has already been mentioned that almost all odorivectors have different degrees of a trigeminal or pungent component associated with them, depending on the particular odorant and the concentration at which the odorant is presented (Cain, 1974a; Doty, 1975; Doty et al., 1978). As suggested by Cain (1974b, 1978) for other odorivectors, the observed upward concavity might reflect a change from a predominant olfactory stimulation (odor) to a trigeminal one (pungency). This hypothesis deserved further investigation and we are currently studying the psychophysical function characteristics and the additive properties of perceived odor and pungency for various concentrations of these two pungent odorants presented alone and in binary mixtures.
Formaldehyde functions do not show upward concavity. In the linear coordinates of Figure 1, they show a small downward concavity which disappears when the data are plotted in logarithmic coordinates. Nevertheless, this stimulus evokes a definite pungent sensation which becomes salient at the highest concentration employed. Why is it then that upward concavity is not observed with formaldehyde? Perhaps higher concentrations are necessary for this concavity to appear. A more plausible explanation however is based on the relatively long time for formaldehyde’s pungent sensation to reach a maximum. Our current investigations indicate a significant difference between ammonia and formaldehyde regarding the latency for maximum pungency, with formaldehyde presenting a much longer latency than ammonia (data not shown). If both irritants were matched regarding perceived pungency, time could be as significant a factor as concentration. Some subjects commented that although they gave a number reflecting total perceived intensity at the established inhalation time (2.5 s), some stimuli—typically the formaldehyde dominating ones—kept growing in perceived intensity beyond that time. So it is possible that there was insufficient time for the higher concentrations of formaldehyde to build up to their maximum intensity level. The comparative study of the latency for maximum intensity of the pungent sensations evoked by different irritants is certainly worthwhile of future investigations.

Previous studies have found that females are more sensitive than males to nasal perceived pungency produced by CO₂ (Dunn et al., 1982; García-Medina and Cain, 1982 Cometto-Muñiz and Noriega, 1985). Female higher sensitivity was noted both physiologically, in the threshold for producing a reflex transitory apnea (Dunn et al., 1982; García-Medina and Cain, 1982), and psychophysically, in the evaluation of perceived pungency using sucrose sweetness as a reference modality (Cometto-Muñiz and Noriega, 1985). In our study, the three-way ANOVA showed no significant interaction of gender by formaldehyde by ammonia. Nevertheless, it could prove of interest to use these irritants in order to study possible gender differences regarding levels of perceived pungency by comparing the position of the psychophysical functions for each sex relative to a reference modality where no sex differences had been observed.

A review of the literature dealing with perceptual properties of odorant mixtures reveals hypoaddition to be most commonly found (Zwaardemaker, 1907; Foster, 1963; Jones and Woskow, 1964; Berglund et al., 1971; Berglund et al., 1973; Berglund, 1974; Cain and Drexler, 1974; Cain, 1975; Moskowitz and Barbe, 1977; Patte and Laffort, 1979; Laffort and Dravnieks, 1982; Laing and Willcox, 1983). Some investigations have found simple addition (Rosen et al., 1962; Baker, 1964; Koster, 1969), while hyperaddition seems to be an uncommon finding in odor mixtures (Koster, 1969).
In relation to perception of mixtures of pungent chemicals (stimulators of the CCS), there is an almost complete lack of data. This, again, is related to the strong interrelation between the olfactory and common chemical senses and the absence of chemical stimuli specifically tuned to stimulate just one of these chemoreceptor modalities. Nevertheless, some studies addressed the issue of functional comparison between the two modalities (Cain, 1976; Cain and Murphy, 1980; Cain, 1981; Cometto-Muñiz and Cain, 1982; García-Medina and Cain, 1982), while others focused on the study of the physicochemical basis for the ability to stimulate the CCS (Alarie, 1973; Doty, 1975; Doty et al., 1978). These investigations stressed the role of the CCS as a protective mechanism against the inhalation of dangerous substances. In view of this role, one could predict that pungent odorant mixtures should show a higher degree of additivity than benign odorant mixtures.

Results depicted in Figures 2 and 3 show that binary mixtures of formaldehyde and ammonia do not present a single type of additivity but rather vary, with hypoadditivity at low concentrations and hyperadditivity at high concentrations. Assuming that subjects use a response scale in which there is a linear relation between numerical response and underlying sensory magnitude, these results suggest that at low concentrations of the odorants employed, odor may predominate over pungency in total perceived intensity, so their binary mixtures behave perceptually as odor mixtures (i.e., hypoadditivity). At intermediate and high concentrations, pungency may predominate over odor, so their binary mixtures show, principally, simple additivity and two cases of hyperadditivity, suggesting that these types of additivity are associated with the progressively greater involvement of the CCS in the total perceived sensation. This possibility is currently being explored in our laboratory by asking subjects to evaluate the odorous and pungent component of the total nasal sensation and investigating the type of additivity of each component in the binary mixtures.

The hyperadditivity at high concentrations is not as general (two mixtures) or as statistically robust (P = 0.036 and P = 0.014) as the hypoadditivity at low concentrations (seven mixtures, P < 0.005). Nevertheless, as the concentration of the mixed irritants increases, there is a definite tendency for additivity to increase.

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