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Sensory Detection of Glutaraldehyde in Drinking Water—Emergence of Sensitivity and Specific Anosmia

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Abstract

A study of 30 young adults (15 males, 15 females), screened to have normal olfaction, measured detection of the flavor of glutaraldehyde, a biocide that could occur in disinfected potable water. Over the range of interest, up to 100 p.p.m., flavor derived from olfactory stimulation. Higher concentrations would cause oral irritation. Fourteen subjects failed to detect the glutaraldehyde in the first of four sessions of testing. Eight of the 14 (seven males, one female) continued to exhibit the anosmia throughout testing. The other six (one male, five females) began to detect the material in session two and exhibited increasing sensitivity over sessions two to four. Their average sensitivity never reached that of the 16 subjects who evinced no anosmia and who also improved their performance over sessions. The combined group of 22 could detect 17 p.p.m. Less thorough testing would have yielded much higher values. Specific anosmia for this dialdehyde has precedence in anosmia for various monoaldehydes, most notably isobutyraldehyde. The positive influence of experience with a material on detection has been found previously, most intriguingly by Wysocki and colleagues, who showed that experience could differentially induce sensitivity to the odorant androstenone and suggested that the phenomenon might occur for other compounds. Glutaraldehyde appears to be one, perhaps of many.

Introduction

The volatile organic compound (VOC) glutaraldehyde (1,5-pentanedial; CAS No. 111-30-8) has broad-spectrum biocidal activity. In addition to its widespread application in cold sterilization of dental and medical instruments, such as endoscopes, glutaraldehyde can serve *inter alia* to disinfect potable water. This motivates interest in a drinking water quality standard for the material.

Standards for drinking water

Standards for the presence of materials in drinking water often specify both primary maximum concentration levels, based principally upon criteria of public health, and secondary maximum concentration levels, based upon odor, taste and appearance. Primary standards normally derive from estimation of a no-observable-adverse-effect level (NOAEL) for the most sensitive indicator of toxicity of a material, calculation of total acceptable daily intake (ADI), apportionment of estimated exposure via the routes food, water and air, and computation of a maximum acceptable concentration per route from the ADI (Greim, 2000; van Leeuwen, 2000). Secondary standards or guidelines normally derive from psychophysical testing for the relevant perceptual attributes. Methodology for the testing varies, but normally entails estimation of a concentration that panelists can just detect or recognize. A secondary standard may indicate a maximum concentration at some fraction or multiple of this quantity. The threshold odor number (TON), with the value 1 set to the concentration of an operationally defined threshold, serves often as a means to express a secondary standard. In the USA, the Environmental Protection Agency (EPA) uses 3 TON as a secondary standard (US EPA, 1979). Since the EPA chooses not to enforce the secondary standard, it exists principally as a guideline.

Studies of animals exposed subchronically to concentrations of glutaraldehyde in drinking water, up to 250 p.p.m. in dogs and 1000 p.p.m. in rats and mice, uncovered no evidence of systemic toxicity to organs or tissue. For concentrations ≥50 p.p.m. in the dogs and 250 p.p.m. in the rodents, consumption of the water decreased, with compensatory and reversible renal physiological effects (Hermansky *et al*., 1995). Most likely, a direct sensory property of the material caused the decrease in consumption.

Chemosensory attributes of odor, chemesthesis and taste

Virtually all small VOCs (mol. wt < 200) have ability to evoke odor at low concentrations and chemesthetic sensations at higher concentrations (Cain and Cometto-Muñiz, 1995). [A terminological note: inhaled as vapors, VOCs

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evoke odor at low concentrations and what people often call pungency, but may legitimately call irritation, at higher concentrations. Sipped in aqueous solution, these VOCs evoke (olfactory-mediated) flavor, often called 'taste', at low concentrations and irritation at higher concentrations. Most small VOCs will stimulate the gustatory system only at very high concentrations.] In general, the more reactive a material, the smaller the gap between detection of its odor (or flavor) and its irritation. For glutaraldehyde vapor, the gap apparently lies somewhere below an order of magnitude (Ballantyne and Jordan, 2001). A similar gap seems likely to apply to aqueous solutions.

If the aversion of the dogs and rodents came from sensory stimulation, most likely it came from irritation. If defined as an adverse health effect, sensory irritation could form the basis for a primary maximum contaminant level in drinking water. Somewhere below this level would lie concentrations relevant to a secondary maximum contaminant level based upon flavor. This study concerned specification of the concentrations over which humans can detect the flavor of glutaraldehyde. As straightforward as this mission sounds, it happens to have uncovered a relative blind-spot in the methodology used in chemosensory studies.

Methodology for measurement of absolute sensitivity

The literature on chemosensory detection contains some lessons about how to obtain an accurate view of absolute sensitivity. In studies of olfactory detection, subjects have shown gains of a few-fold in measured sensitivity over repeated tests (Engen, 1960; Rabin and Cain, 1986; Cain and Gent, 1991). The results supported the interpretation that a gain may come from experience in the task rather than from an actual increment in sensitivity. That experience may sharpen detection, irrespective of modality, has long-standing precedent (Gibson, 1953). Nevertheless, not all increments in measured sensitivity need have the same origin. Some materials, most notably androstenone, may grow differentially in detectability from a person's first experience to subsequent experiences (Wysocki *et al*., 1989). In such cases, the number or availability of receptive sites may actually increase from stimulation (Wang *et al*., 1993). These instances could qualify as true induction of sensitivity rather than a mere uncovering of true sensitivity.

Irrespective of the origin of effects of experience in any instance, tests of chemosensory sensitivity rarely reckon with even their existence. Instead, most testing relies on measurements taken in a single session. Such measurements, quite common in the testing of contaminants in drinking water (Young *et al*., 1994), often assess detection of a few concentrations just a few times, or even just once. The American Society for Testing and Materials (ASTM) method E 679-79 for determination of odor threshold encourages presentation of just a single trial for each of a few concentrations in a small sample of subjects (ASTM, 1991). For comparisons of relative sensitivity across stimuli, single sets of measurements, though not necessarily single presentations, may suffice. For specification of actual absolute sensitivity, as for a potential contaminant, it seems at the very least advisable to discover whether performance changes as measurement proceeds. After all, people who drink tap water will do so regularly.

Just as single sets of measurements may underestimate sensitivity, they may also inflate apparent individual differences (Stevens and Dadarwala, 1993). Studies have reported individual differences as high as 100 000 to 1, with reports of 1000 to 1 being routine (Brown *et al*., 1968; Punter, 1983; Yoshida, 1984; Takagi, 1987, 1989). Superficial measurement with inevitably high random error may well play a part in the reported scatter, though some other factors, such as greater age in some subjects in a sample, may sometimes account for a fraction of the range (Cain and Gent, 1991; Stevens and Dadarwala, 1993). In order to determine whether apparent individual differences represent true differences, it is necessary to establish some stability in individual performance. Repeated tests serve this goal, just as they reveal whether experience with the stimulus counts.

Individual differences and specific anosmia

Individual differences of two kinds hold interest: those that lie within the principal distribution among subjects and those that form another distribution. A finding of two distributions can indicate existence of specific anosmia to a material (Amoore, 1969b, Brown *et al*., 1968; Stevens and O'Connell, 1991; Baydar *et al*., 1993). For androstenone, for instance, a third to a half of subjects, more males than females, fail to perceive it, with the basis for the defect apparently genetic (Wysocki and Beauchamp, 1984; Wysocki and Gilbert, 1989). A similar difference between males and females also exists for specific anosmia to the musk galaxolide (Wysocki and Gilbert, 1989).

Germane to the present study, Amoore *et al*. described a specific anosmia to isobutyraldehyde in 36% of persons tested (Amoore *et al*., 1976). The mode of the distribution for detection in the anosmics lay 260-fold above that in sensitive persons. The authors concluded, from tests with isomers, homologues and isosteric relatives of isobutyraldehyde, that the aldehyde functional group, particularly with linkage to an iso- or sec-form and a restriction to C_3–C_5 in molecular size, favored the anosmia.

Glutaraldehyde has received little attention with respect its properties as an odorant. Its reported odor threshold equals 40 p.p.b. (Ballantyne and Jordan, 2001), in the general vicinity of monoaldehydes of similar chain length (e.g. *n*-butyraldehyde = 37 p.p.b.; *n*-valeraldehyde = 61 p.p.b.; *n*-hexanal = 23 p.p.b.; Amoore *et al*., 1976). Its detectability in water has apparently not been investigated.

Materials and methods

Subjects

Fifteen males and 15 females, aged 18–40 years (average 25) participated.

Test facilities

Testing took place in a well-ventilated flavor-laboratory at normal temperatures (21–23°C) and humidity (40–50% RH).

Test material

The test material comprised an analytically confirmed sample of glutaraldehyde (2%) provided by Union Carbide Corp. It was diluted to concentrations of interest with commercial drinking water (Arrowhead).

Procedure

Screening

Pilot work implied that the flavor of glutaraldehyde came from olfactory stimulation at the concentrations of interest. Accordingly, screening of subjects for chemosensory function entailed a test of olfaction. Subjects took either the Connecticut Chemosensory Clinical Research Center (CCCRC) Test or the University of Pennsylvania Smell Identification Test (UPSIT) (Doty *et al*., 1984; Cain *et al*., 1988). Each has norms for normal and abnormal functioning. All subjects scored in the normal range.

Flavor testing

Pilot work had indicated that subjects could virtually never detect concentrations as low as 3 p.p.m. (v/v) and that most subjects, though not all, could detect concentrations as high as 100 p.p.m.. Because concentrations at or above 100 p.p.m. could cause oral irritation, either at the time of testing or thereafter, 100 p.p.m. was the highest concentration tested (see Appendix). The concentrations used in the main experiment ranged from 3.13 to 100 p.p.m. (3.4–110 mg/l), with six members that increased in concentration by 2-fold from one to the next.

Figure 1 shows the format of a data sheet for a test session and also illustrates the array laid out for a subject. A panel on the sheet corresponded to a tray with wells that contained 30 disposable plastic cups (30 ml capacity) of liquid and the numbers 1–10, as shown in the figure. For each of the 10 triads, one cup contained a concentration of the test material and two contained plain water (10 ml samples). An algorithm randomized the position of test material in a triad. The subject was blinded with respect to the position. A given tray held only one concentration of material, i. e. tray 6 held all concentrations of 3.13 p.p.m. (step 6 in Figure 1), tray 5 held all concentrations of 6.25 p.p.m. (step 5), tray 4 held all concentrations of 12.5 p.p.m. (step 4) and so on.

An experimental session began with tray 6. The subject sipped from the first cup of the first triad, expectorated the sample, rinsed with water and repeated the procedure with the second and third cups of the triad. During sampling, the subject needed to attend to which of the samples had the strongest flavor and at the end to choose one, guessing if necessary (three-alternative forced-choice). The subject also rated confidence in the choice, on the following scale: 1 = very low; 2 = low; 3 = moderate; 4 = high; 5 = very high. The subject then continued onto the second triad and repeated the same procedure.

When finished with the first two triads on tray 6, the subject moved on to tray 5 and sampled triads 1 and 2 in the same manner as for tray 6. This continued until the subject had sampled the first two triads of tray 1. By that time, the subject had scanned the entire range of concentrations, with two trials at each. After a 5 min break, the subject returned to tray 6 and sampled triads 3, 4, 5 and 6 in the manner described, moved on to tray 5 and so on. After another 5 min break, the subject returned to tray 6 again and sampled triads 7, 8, 9 and 10, moved on to tray 5 and so on.

A session lasted ~90 min and yielded ten trials per concentration step. Each subject participated in four such sessions. The median interval between successive sessions for an individual was 5 days.

Pinched-nose testing

At the end of the four sessions, the most sensitive male and most sensitive female served in four additional sessions that differed from the other sessions in just one way: the subjects wore nose clips over their noses to reduce olfactory stimulation. This served to check on whether non-olfactory as well as olfactory stimulation guided detection.

Results

Females

Figure 2 shows how percentage correct detection, cumulated over four sessions and corrected for chance, varied with log concentration in p.p.m. (v/v) for the 15 female subjects individually. Fourteen came under the influence of the stimulus, i.e. showed increasing detection with concentration, though two others (F02 and F04) failed to reach even 50% correct detection. The subject (F15), who failed to come under the influence of the stimulus, yielded virtually a horizontal function.

Males

Figure 3 shows the psychometric functions for the males. For those subjects who came under the influence of the stimulus, males and females exhibited similar results. Strikingly, however, seven males (M03, M04, M10, M12, M13, M14 and M15) failed to come under the influence of the stimulus.

Effect of experience

Collection of data over four sessions permitted inspection

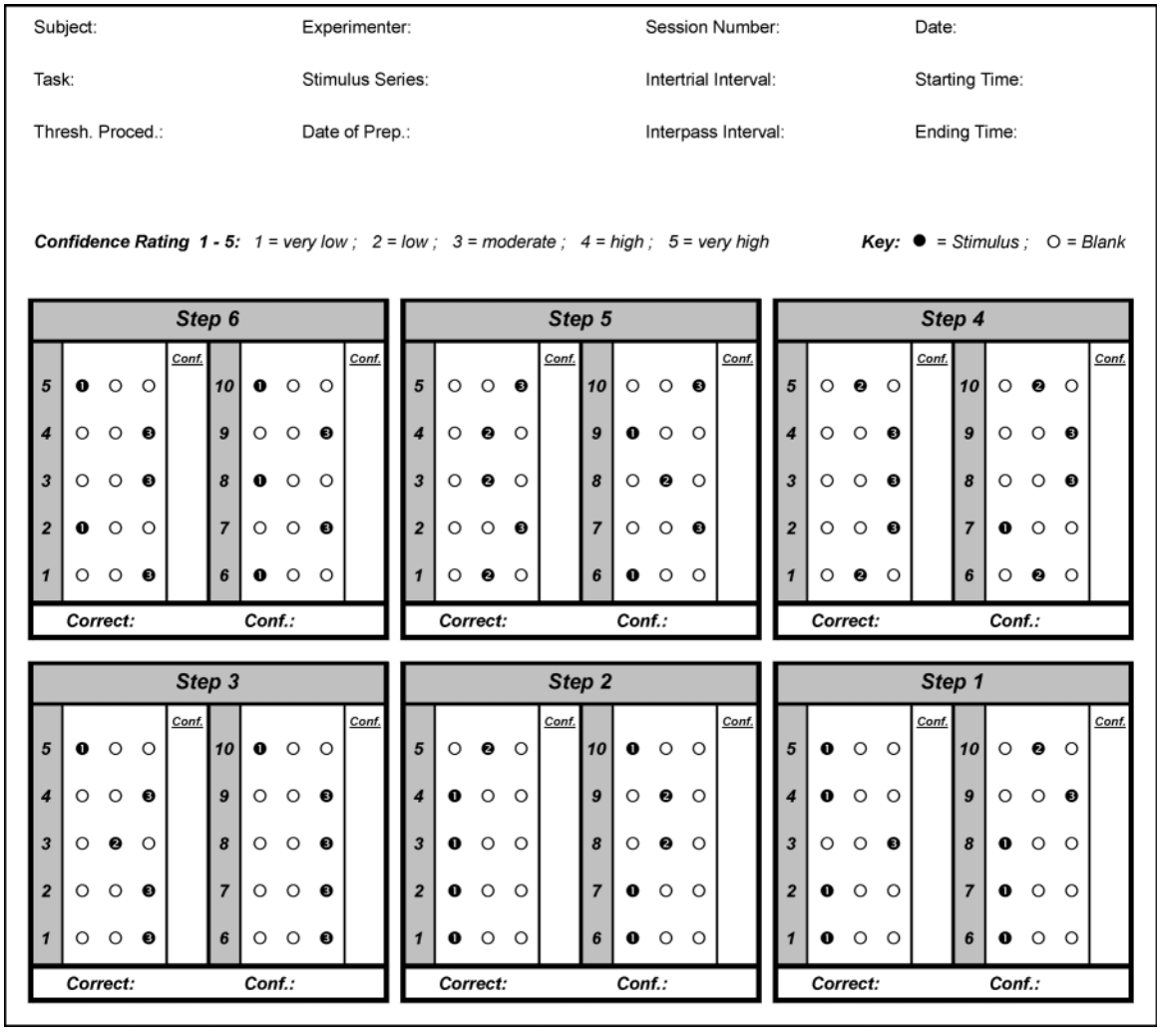

Figure 1 The subject received a data sheet of the format shown, but without the key for the correct answers, shown as darkened spots here. Each panel represents a tray of cups with the rows marked as shown. The columns to the right of the triads provided the space to write confidence ratings. The experimenter used the sheet with the key to set up the stimuli in the trays before the subject arrived. During a session, the experimenter merely monitored compliance with procedure.

of how experience influenced detection. Fourteen of the 30 subjects, eight males and six females, showed essentially no detection in session one (Figure 4). From session one to session two, however, six of these subjects (five females and one male) showed an abrupt increment in performance. For example, their detection of 100 p.p.m. increased more than 6-fold. For these subjects, the point of 50% detection went from indeterminacy in session one to 80 p.p.m. in session two and 48 p.p.m. by session four. The 16 subjects who exhibited detection in session one showed progressive improvement through their four sessions. The point of 50% detection progressed from 25 p.p.m. in session one to 13 p.p.m. in session four.

As Figure 5 illustrates, confidence tended to track performance. For example, in the crucial middle portions of the psychometric functions, between 25 and 75% correct, the gain in performance from the first to the fourth session for nonanosmics amounted to essentially one step in concentration (2:1) and differential confidence between correct and incorrect answers signaled the same gain.

Functions with the nose pinched

By showing how performance varied with the nose pinched and open in the most sensitive female and most sensitive male, Figure 6 demonstrates the olfactory-mediation of perception of the glutaraldehyde. With the nose pinched, performance fell to chance and rated differential confidence fell to zero.

Discussion

At what concentrations will persons with normal sensitivity to glutaraldehyde detect it in water? By session four, such subjects evinced detection significantly above chance at 6.25 p.p.m. [$t(15) = 2.33$, $P < 0.05$]. In session one, these

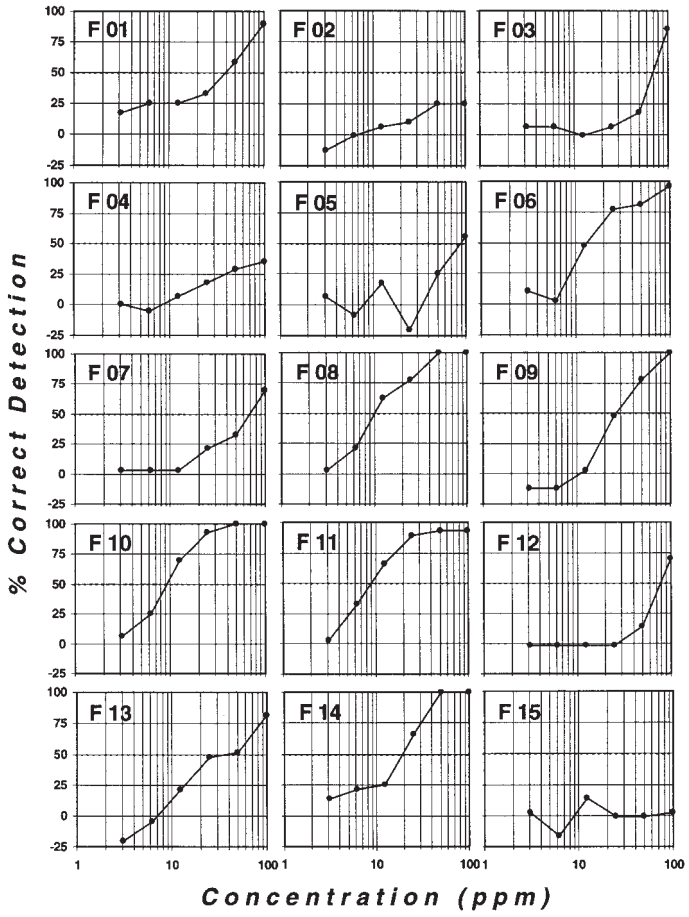

Figure 2 Psychometric functions show how the 15 females detected the flavor of glutaraldehyde over the concentrations 3.13–100 p.p.m. A function represents the results of four sessions, ten judgements per concentration per session. The data were corrected for chance by the customary formula (Gescheider, 1997).

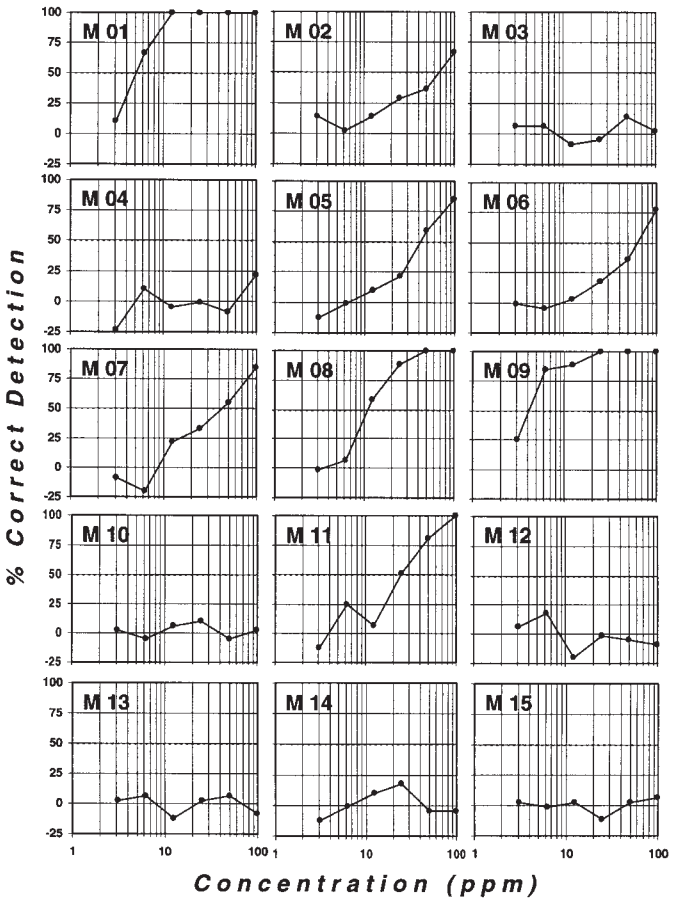

Figure 3 Psychometric functions show how the 15 males detected the flavor of glutaraldehyde over the concentrations 3.13–100 p.p.m. A function represents the results of four sessions, ten judgments per concentration per session. The data were corrected for chance.

subjects met the same criterion at 12.5 p.p.m. [$t(15) = 2.28$, $P < 0.05$]. For point of reference, had testing ceased after session one for the entire group of 30, the level would lie 12-fold higher, at 77 p.p.m. For all practical purposes, therefore, repeated testing, with its accompanying diagnostic possibilities, produced to an estimate of sensitivity an order of magnitude higher than single testing would have produced.

As indicated earlier, standards and guidelines may express maximum concentration of contaminants in water in terms of TON. A rule of TON = 3 applies relatively frequently, with threshold determined by ASTM method E679-79 or a variant. The ASTM method entails use of an ascending series and three-alternative forced-choice, with a single pass through the series per participant. If applied in this case, which we could do by scoring only the first trials at each concentration, 3 TON would equal 240 p.p.m. for the sample of 30 persons. The ASTM procedure would not have discerned the presence of anosmia, initial or otherwise. Subjects who fail to achieve detection of the highest concentration are assumed to have a detection point one-half concentration step above the highest.

Once aware that the sample does contain a reasonable number of anosmics, one can compute a threshold for the rest (number of subjects = 22). In the present tests, that equaled 17 p.p.m. in the fourth session and would give rise to a 3 TON of 51 p.p.m. (Figure 7). Irrespective of whether one would set the maximum allowable amount of glutaraldehyde in drinking water at a concentration as high as 50 p.p.m., the present comparison serves to illustrate that superficial testing will generally give the illusion that consumers will fail to detect levels they might readily detect.

If, as previously reported (Amoore *et al*., 1976), 36% of people have specific anosmia to isobutyraldehyde, then that defect has few rivals in its frequency. Most reported specific anosmias affect a much smaller percentage of persons (Amoore, 1969a). Subsequent to screening for the specific anosmia, presumably in a single session per subject, Amoore *et al*. empanelled 31 specific anosmics for further studies of ten aldehydes and four nonaldehydes. The investigators used the relative sizes of the anosmia among the aldehydes to

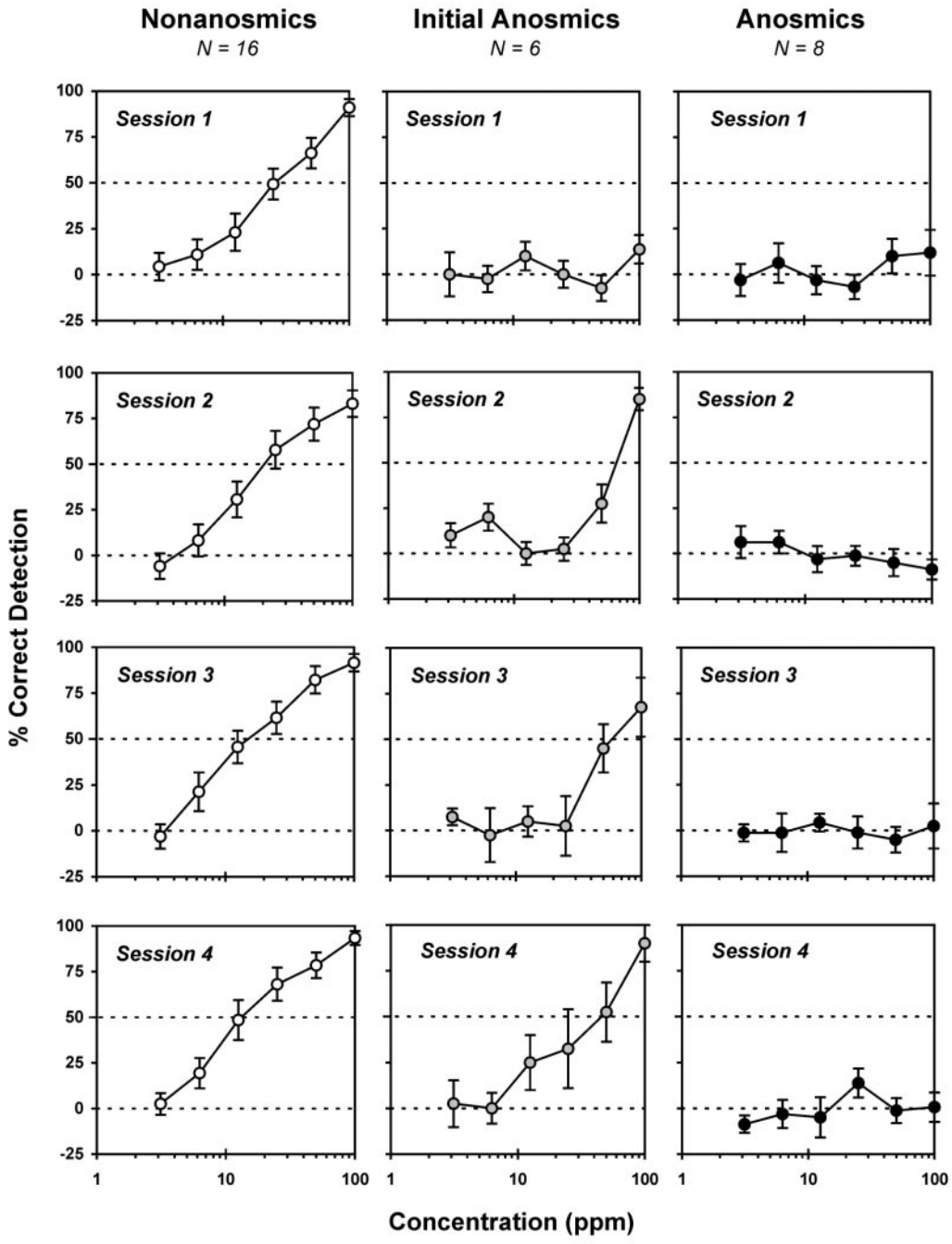

Figure 4 Psychometric functions for the groups of subjects nonanosmic to glutaraldehyde (n = 16), initially anosmic to it (n = 6) and anosmic to it (n = 8)—see Appendix. Bars show standard errors.

draw conclusions about structure and activity, as mentioned earlier. Although the present results by themselves indicate nothing in detail about structure–activity, they do suggest, along with the results of Amoore *et al.*, a rather high frequency of specific anosmia to aldehydes.

In a study of androstenone (Wysocki *et al.*, 1989), it was found that specific anosmics given daily exposure to the material had an induction of sensitivity, such that they became osmic to it by their second test, a week after their first. Although the gain occurred most dramatically from the first to the second test, it grew until approximately the fourth test. The induction favored neither sex. Anosmics given no experience between testing also showed progressive but less dramatic gain out to four sessions. Testing entailed less exposure to the material than in the present study, which makes it uncertain whether to compare the present

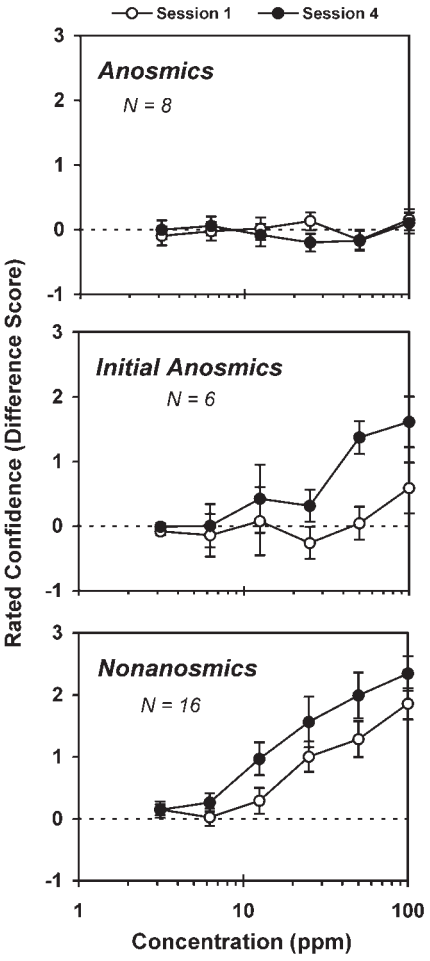

Figure 5 Functions for the ratings of confidence, expressed as the difference between ratings for correct minus ratings for incorrect answers, from sessions one and four in the three groups of subjects. Bars show standard errors.

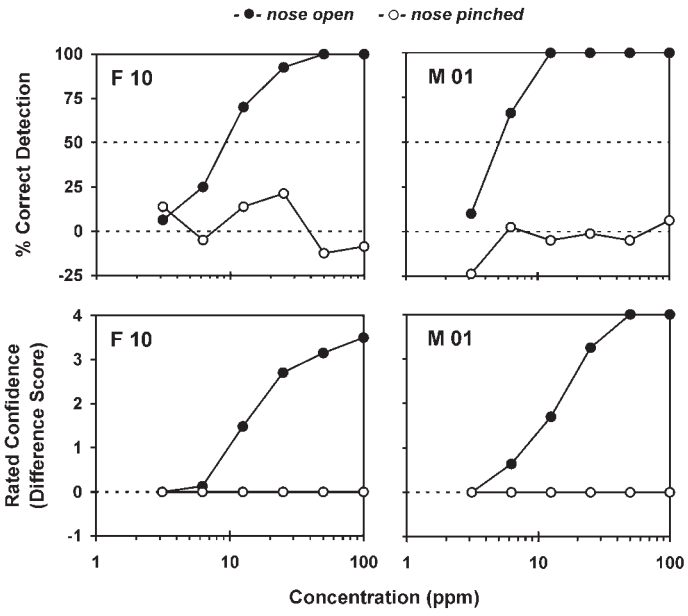

Figure 6 Upper: psychometric functions for subjects F 10 and M 01 with the nose pinched and open. Lower: ratings of confidence (difference score as in Figure 5) that corresponded with the performance shown in the upper part.

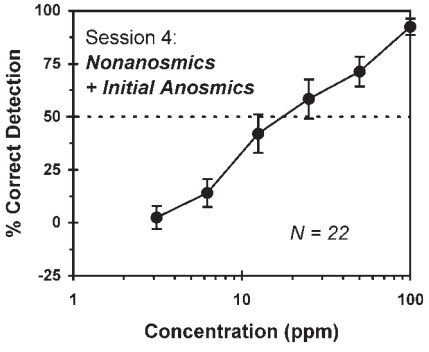

Figure 7 Psychometric function obtained in session four for the 22 subjects who could detect glutaraldehyde.

results to those given interpolated experience or those just tested repeatedly. Others have confirmed that exposure to androstenone can change anosmics to osmic status (Pause *et al*., 1999). Pause *et al*. gave just 1 min/day over 1 week.

Data from earlier work (Wysocki *et al*., 1989) imply that the induction of sensitivity to androstenone does not occur to all materials. Nevertheless, if the induction occurred only when the organism begins with a specific anosmia, then we can say that the question remains open. For most materials, specific anosmia occurs infrequently enough that the matter would generally escape notice. A study of whether induction would show up in physiological measures (Wang *et al*., 1993) found induction of sensitivity to androstenone in mice specifically anosmic to it and induction of sensitivity to isovaleric acid in mice specifically anosmic to it. This led the authors to state: 'Induction with two unrelated odorants implies that olfactory induction is a general phenomenon that may occur in a large fraction of the human population' (p. 998).

Subsequent behavioral experiments in mice and rats endorsed the claim of generality irrespective of specific anosmia (Voznessenskaya *et al*., 1994). This was also found to be the case in experiments in humans, but so far only in an unusual instance with the substance pemenone, somewhat of a simulant for androstenone. Exposure to it enhanced sensitivity to androstenone, irrespective of osmic status to the pemenone or androstenone (Stevens and O'Connell, 1995). The research of Wang *et al*. implicated the olfactory epithelium in induced changes in sensitivity (Wang *et al*., 1993). Studies on transection of the olfactory nerve have allowed the same interpretation (Yee and Wysocki, 2001).

More than a century ago, William James James, 1892) reminded us:

That 'practice makes perfect' is notorious in the field of

> motor accomplishments. But motor accomplishments depend in part on sensory discrimination . . . In the purely sensorial field we have the well-known virtuosity displayed by the professional buyers and testers of goods. One man will distinguish by taste between the upper and lower half of a bottle of old Madeira. Another will recognize, by feeling the flour in a barrel, whether the wheat was grown in Iowa or Tennessee. The blind deaf-mute, Laura Bridgman, so improved her touch as to recognize, after a year's interval, the hand of a person who had once shaken hers; and her sister in misfortune, Julia Brace, is said to have been employed in the Hartford Asylum to sort the linen of its multitudinous inmates after it came from the wash, by her wonderfully educated sense of smell. (p. 252)

We have known little about the bases for these feats, relegating them to practice, as if this constituted an explanation. In olfaction, 'practice' may actually have meaning in terms of changes in the peripheral nervous system. Experience or practice, in a more physiologically based sense, might account for some of the large individual differences reported in olfactory sensitivity. Some such differences might prove quite temporary. Only repeated measurements may reveal that aspect of individual differences.

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Appendix: on the definition of specific anosmia

Concerns about irritation dictated use of a maximum concentration of 100 p.p.m. in the investigation. This left indeterminate the concentrations where less sensitive subjects might detect glutaraldehyde. Although we have treated the results of these people as indicative of specific anosmia, we have offered no definition of the condition.

Two definitions have existed historically. One, principally graphic, relies upon the presence of a bimodal distribution of sensitivity, with an antimode. This definition, as usually expressed, makes only an intuitive appeal to statistics in that it relies merely upon how the distributions look. A factor that constrains the range of concentrations, as in the present case, can obscure the separation between distributions. How can one know, then, that the subjects in the present investigation would not detect glutaraldehyde at 101 p.p.m. and thereby show no antimode? The answer lies in the slope of the psychometric function for the 22 subjects who could detect glutaraldehyde. For detection to increase from chance level to 50% required an increment of concentration of a factor of six, with only a 5% coefficient of variation. In the most optimistic of circumstances, i.e. if all eight of our 'specific anosmics' began to detect glutaraldehyde just above 100 p.p.m., where average detection equaled 1.5%, their performance would not reach 50% until 600 p.p.m. Figure A1 shows the histogram that would result in that circumstance.

The other definition of specific anosmia relied upon statistics. When Amoore failed to find bimodal distributions with antimodes for chemicals that he thought should have revealed specific anosmia, he defined persons with thresholds two standard deviations above the mean of apparent normals as specific anosmics (Amoore, 1991). Without converging evidence, this rule has no more to recommend it than a rule based upon 1.5 or 2.5 SD, or any other number. Nevertheless, the data in Figure A1 would pass the rule of two standard deviations. Two standard deviations above the mean for normals equals 209 p.p.m., lower than the 600 p.p.m. estimated by the most optimistic trajectory.

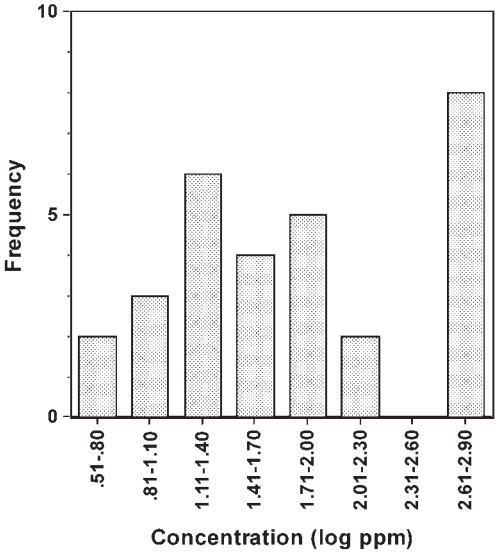

Figure A1 The distribution of concentrations for 50% detection among the 30 subjects. Fitted psychometric functions (normal-ogives) from individual subjects for sessions 2–4 served to calculate the concentrations. For the eight subjects (specific anosmics) who gave horizontal functions up to 100 p.p.m., we assumed their performance would increase above 100 p.p.m. at the same rate as that of the typical subject. Based upon such calculations and allowing for some variation from individual differences, the eight nondetectors would have reached 50% detection at concentrations in the highest bin, 404–794 p.p.m. What we show makes an excessively optimistic projection, but shows that an antimode would exist even under such a projection. Most likely, the eight persons would have needed even higher concentrations. Nominally, the size of the bins equaled 2:1, the step size used in the experiment and the typical size of bin in histograms used to illustrate specific anosmia (Amoore, 1991).

Finally, nothing requires that any analysis for specific anosmia use the point of 50% detection for comparisons. Equipped with psychometric functions for individual subjects, we could use any other criterion, 25% detection for instance. Two standard deviations above the mean for 25% detection equals 105 p.p.m., essentially at our highest test concentration. This would obviate extrapolation.

These various approaches to resolution of the difference between those with normal and those with poor sensitivity to glutaraldehyde depend upon gathering data for psychometric functions for individual subjects. Although time-consuming, the collection of the data necessary to allow consideration of stimulus–response relationships has evident benefits.