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Human Responses to Propionic Acid. I. Quantification of Within- and Between-participant Variation in Perception by Normosmics and Anosmics

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Abstract

The objective of this study was to fully characterize normosmic perception of stimuli expected to cause widely varying degrees of olfactory and nasal trigeminal stimulation and to directly evaluate the possible role of olfactory nerve stimulation in nasal irritation sensitivity. During each of four identical test sessions, four anosmic and 31 normosmic participants were presented with a range of concentrations extending from peri-threshold for normosmics to supra-threshold for anosmics. For each session, odor (O) and nasal irritation (NI) sensitivities were summarized in terms of the concentrations required to produce four sensation levels ('iso-response' concentrations). Within-participant variation in these iso-response concentrations was <10-fold for 95% of normosmics, for both O and NI. For O but not NI, these apparent fluctuations in sensitivity were largely accounted for by the uncertainty surrounding the iso-response concentrations calculated for each session. Anosmics exhibited minimal within- and between-participant variation in NI and required, for all but the highest perceptual level, a higher concentration than almost all normosmics. Between-participant variation, expressed in terms of 90% confidence interval widths, was ~0.5 log units for both O and NI for the highest perceptual level, but increased to ~0.8 and 1.8 log units, respectively, for the lowest (peri-threshold) level. Our findings suggest that: (i) most apparent variation over time in O sensitivity is actually a reflection of the uncertainty surrounding estimates of sensitivity obtained for each session; (ii) within- and between-participant variation in O sensitivity is far less than is commonly reported; and (iii) low to moderate levels of NI in normosmics are the result of relatively weak trigeminal stimulation combined with much greater olfactory activation.

Introduction

There has been increasing demand, especially over the past decade or so, for quantitative data on the perception of odor and nasal irritation in human participants and for a greater understanding of the interactive effects of simultaneous olfactory and nasal trigeminal activation. At least three areas of application of such information can be noted.

First, a great deal has been learned recently concerning the initial transduction events in the olfactory epithelium (Buck and Axel, 1991), the pharmacology of the trigeminal periphery (Lundberg et al., 1988; Stjärne et al., 1989) and the topographic distribution of the afferents of both pathways in the nasal cavity (Keverne et al., 1986; Leopold et al., 1995). For a more complete understanding of the functional implications of this mechanistic information, it is necessary to have precise and well-understood measures of both the perceptual and physiological parameters of separate and combined olfactory and trigeminal stimulation.

Second, increased interest in the diagnosis and treatment of smell disorders has heightened the necessity for a more complete and quantitative description of normal odor perception and has increased the need for non-verbal correlates of perceived odor and nasal irritation. Current candidates for these non-verbal correlates include topographic electroencephalography (e.g. Kendal-Reed and Van Toller, 1992; Van Toller et al., 1993; Gori et al., 1995), chemosensory evoked response potentials (e.g. Lorig, 1994; Doty and Kobal, 1995), magnetic source imaging (e.g. Kobal et al., 1995) and respiratory responses (e.g. von Gerhardt and Rauh, 1963; Gudziol and Gramowski, 1987; Gudziol and Schubert, 1996; Kendal-Reed and Walker, 1996; Jalowayski et al., 1997; Walker et al., 1997). Interest in defining normal olfactory function has also underscored the need for a more complete understanding of the manner in which trigeminal input may influence responses to odorants and irritants (Laska et al., 1997).

Third, the importance of odor and nasal irritation in perceived well-being and comfort has been emphasized in recent years. This is of particular relevance as olfactory and trigeminal inputs likely play important roles in deter-

mining responses to indoor and outdoor environmental contaminants.

In our view, the current literature on human odor and irritation is somewhat limited in its ability to address these important areas. This is due in part to the relatively small number of compounds for which olfactory psychophysical parameters have been reported (Walker and Jennings, 1991). Equally important is the question of the confidence that can be placed in the available published data for use in, for example, diagnosing indoor air quality problems or quantifying degree of olfactory loss in a clinical setting. One reason for this lack of confidence is the considerable inter-laboratory variation in one of the simplest measures of performance: detection threshold (Devos *et al.*, 1990).

Also complicating the use of odor data is the generally held view that there is considerable variation among individuals and over time within a given individual (Burdach *et al.*, 1985; Stevens *et al.*, 1988; Lawless *et al.*, 1995). As has been suggested earlier, this view may actually reflect wide inter-laboratory variation in the rigor with which odorant stimuli are generated and odor perception is measured (Hyman, 1977; Stevens *et al.*, 1988; Walker and Jennings, 1991; Prah *et al.*, 1995; Reilly and Dalton, 1997).

With respect to nasal irritation, there are even fewer data that can be of use in understanding human reports of this sensation. Psychophysical studies have seldom included an instruction to participants to rate the presence or magnitude of this sensation, in part because participants often find this a difficult task (Cometto-Muñiz and Cain, 1994). A second issue with nasal irritation concerns the development of chemical (e.g. Abraham *et al.*, 1996) or animal (e.g. Alarie, 1981) models to predict human nasal irritation. Thus far the implicit assumption has been that this is mediated exclusively by the trigeminal nerve. To the degree that olfactory involvement in nasal irritation is evidenced (e.g. based on quantitative comparisons of nasal irritation in normosmic and anosmic participants), models of nasal irritation may need to be refined to account for olfactory involvement.

Our purpose in conducting the present research was to address some of the issues highlighted above with the aim of developing a paradigm that would optimize the value of the results of psychophysical studies of odor and nasal irritation. We combined precision air-dilution olfactometry and repeated testing of normosmic and anosmic participants to evaluate three nested sources of variation:

1. The uncertainty around an estimate of peri- and supra-threshold odor and nasal irritation sensitivity obtained for a given participant-by-session combination.
2. The variation in odor and nasal irritation sensitivity over time within a given participant tested repeatedly in an identical fashion.
3. The variation among participants in odor and irritation sensitivity.

We also sought to advance the understanding of the neural basis of nasal irritation by comparing, as suggested by Amoore *et al.* (1968), normosmic participants and participants that have normal nasal trigeminal input but lack olfactory input to the brain. This comparison allows an evaluation of the suggestion (Walker *et al.*, 1990b) that low to moderate levels of nasal irritation are the result of low levels of trigeminal activation combined with much higher levels of olfactory activation.

A third objective concerned the utility of respiratory measures as an integral part of human psychophysical studies of odor and nasal irritation. The results obtained which address this objective will be discussed in a forthcoming paper.

Propionic acid was selected as the stimulus based on prior work (Walker *et al.*, 1989, 1990b) which indicated that olfactory exceeded trigeminal sensitivity by a moderate (~1.2 log units) degree. These earlier results were used to select a set of four concentrations, one of which was expected to meet each of the following conditions: (i) just below reported odor thresholds for normosmic participants; (ii) above the odor but not the nasal irritation threshold for normosmic participants but still not detected by anosmics; (iii) above the odor and nasal irritation thresholds for normosmic participants but not detected (or peri-threshold) by anosmics; and (iv) above the nasal irritation thresholds of normosmic and anosmic participants.

Materials and methods

Participants

Two cohorts of normosmic participants were recruited by newspaper advertisement and a third cohort of anosmic participants was recruited by physician referral or advertisement. Cohort 1 comprised five males and five females, aged 18–35, who were assessed for any history of chemosensory dysfunction by filling out a brief health questionnaire, the responses on which indicated that none of these participants had a medical or occupational history associated with abnormally low odor sensitivity. Cohort 2 (six males, 15 females; age range 18–43) was also recruited by newspaper advertisement and assessed as described above. Cohort 3 comprised four anosmic participants (two males, two females; ages 27–59) recruited through referrals by oto-rhino-laryngologists of the University of North Carolina. Participant information for this group of anosmics is provided in Table 1.

After Cohort 1 was tested, Cohorts 2 and 3 were recruited and tested during the same time period. All participants in all three cohorts were tested using exactly the same experimental paradigm. Participants gave informed written consent and received financial compensation for taking part in the study, which was approved by the Institutional Review Board of the UNC School of Dentistry.

Table 1 A summary of anosmic participants

Subject number	Age	Gender	UPSIT score	Recruited by	Etiology
1	32	male	8	newspaper advertisement	unknown
2	59	male	11	newspaper advertisement	possible habitual over-use of nasal decongestants
3	46	female	17	physician referral	craniotomy
4	27	female	10	physician referral	Opitz–Frias syndrome (a genetic disorder affecting cranio-facial development)

Apparatus

Olfaction laboratory

Testing was carried out in a 39 m^3 laboratory in which the mean temperature and relative humidity (RH) were 25°C and 33% respectively. A high air exchange rate (15 changes/h) and an exhaust system that vented to the outside air minimized ambient odors.

Stimulus generation and presentation

Vapor phase stimuli were generated from liquid propionic acid (99% purity; from Sigma Chemical Co., St Louis, MO) placed in a glass saturator tube, maintained at 5°C (± 0.2°C), within an automated single channel air-dilution olfactometer. This device was previously described by Warren et al. (1992, 1994) and three key changes were made in the olfactometer for this study. First, the volume flow rate of clean or odorized air sent to the participant was increased from 10 to 30 l/min. This change, in combination with the ~250 ml volume of air downstream of the facemask, ensured that an excess volume of air was always available at the facemask, even with very vigorous inhalations.

Second, the olfactometer airflow was passed through a respiratory humidifier (Puritan-Bennett Inc., Carlsbad, CA), warmed to room temperature (~25°C) and humidified to a constantly monitored RH (mean of 34.6 ± 3.7%) before being delivered to the participant's facemask (Vital Signs, Inc., Totowa, NJ). This minimized nasal trigeminal nerve stimulation, which would be expected with the presentation of cool, dry air (Benignus and Prah, 1980). Small variations in airflow RH between sessions showed no correlation with ratings of odor strength or nasal irritation.

A third modification was effected to address observations made with Cohort 1. With a few participants there was a tendency to pull away slightly from the facemask when the highest concentration was presented. For a given participant, there was no evidence that this behavior influenced sensory ratings, but it did preclude the use of respiratory data for that trial. To minimize this problem for the second and third cohorts, an inflated rim was added to the facemask and a pressure transducer (Setra Systems, Acton, MA) was used to continuously monitor the pressure exerted by the participant's face on the facemask rim. Above
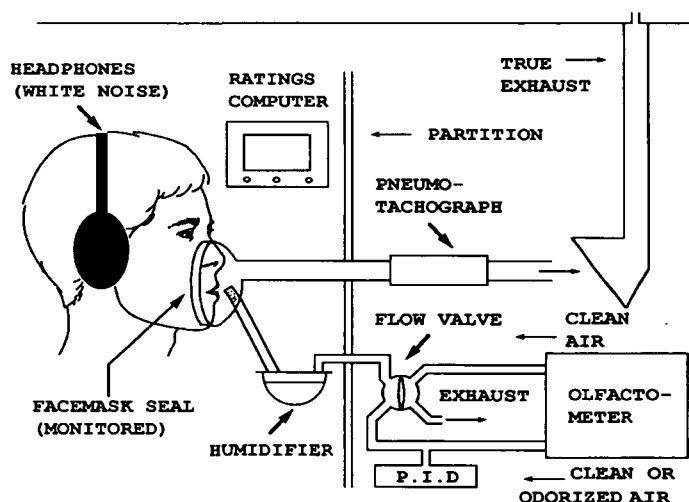

Figure 1 Schematic diagram of the test station for stimulus presentation, sensory and breathing measurements. Clean or odorized air from the olfactometer was delivered, at a constant volume flow rate of 30 l/min, to a snugly fitting facemask. A flow valve was used to control which of the two olfactometer outputs (clean air only, clean or odorized air) was directed to this mask. A heated water-bath respiratory humidifier was fitted between this flow valve and the facemask to warm and humidify the airstream. A pneumotachograph downstream of the facemask continuously measured the participant's breathing. Odorant concentrations were continually monitored by a photo-ionization detector (P.I.D.) to ensure that accidental overexposure would not occur.

a minimum pressure (3 cm water), leaks into or out of the facemask were eliminated.

The following fractions of propionic acid vapor saturation were used: $10^{-4.23}$, $10^{-3.23}$, $10^{-2.23}$ and $10^{-1.23}$. Gas chromatography (Maiolo et al., 1996) was used to calibrate the system once for the initial cohort of 10 normosmic participants (Cohort 1) and once again for the remaining participants (Cohorts 2 and 3). Actual airborne concentrations obtained from these two calibrations were expressed in units of log ppm (v/v) and were used in the analysis and presentation of results.

Relative odorant concentrations were also monitored by a photo-ionization detector (PID) (HNU Systems Inc., Newton, MA) during testing. The PID continuously sampled the odorant and was interfaced to the controlling software so that an immediate shut-down of the system was

programmed in the event that the concentration to be presented to the participant exceeded prescribed values. This prevented accidental odorant overexposure.

Procedure

Experimental design

All participants took part in four 2.25 h testing sessions, separated by at least 3 days. Each session consisted of 10 trials of each of the four propionic acid concentrations and 10 control trials during which only clean air was presented. All sessions for all participants were identical with the sole exception that the schedule of repeated presentation of the five different stimulus conditions (clean air control plus four propionic acid concentrations) was varied. Stimulus presentation order in every session was random except for the stipulation that no concentration was presented more than twice in a row.

Test station and pre-session instructions to participant

Participants were seated in front of the facemask used for odorant delivery, located at a test station depicted in Figure 1. Adjacent to this station was a computer screen and electronic mouse used for entering sensory ratings. Either clean or odorized air was delivered to the participant under the control of a computer-operated flow valve. The olfactometer was concealed from the participants' direct view by a partition to minimize distractions during testing. Prior to each session, the participant was asked to assess his or her nasal patency. Those who reported more than slight impairment in nasal breathing or an upper respiratory tract infection were rescheduled. Slightly less than 10% of the sessions were rescheduled for one of these reasons. Participants were instructed to go to the facemask when prompted and to ensure a good seal throughout each trial. They were asked to relax and breathe normally through only the nose. It was emphasized that a stimulus would not necessarily be perceptible on all trials.

Trial sequence

A trial (Figure 2) began when the participant was given auditory and visual cues to put on headphones through which a continuous sound (white noise intermixed with recorded olfactometer sounds) was played at a level sufficient to mask equipment and other laboratory sounds. Once the participant pressed his or her face into the facemask, the recording of respiratory data began. The pre-stimulus period, during which only clean air was presented, commenced with the onset of the next inhalation and continued for 15 s. The next exhalation onset then triggered the rapid (<0.7 s) operation of the flow valve which directed one olfactometer flow to the facemask and the other to exhaust. Synchronization of breathing and flow valve operation in this way ensured that the desired odorant concentration in the facemask reached asymptote prior to onset of the next inhalation (see Walker et al., 1990a).
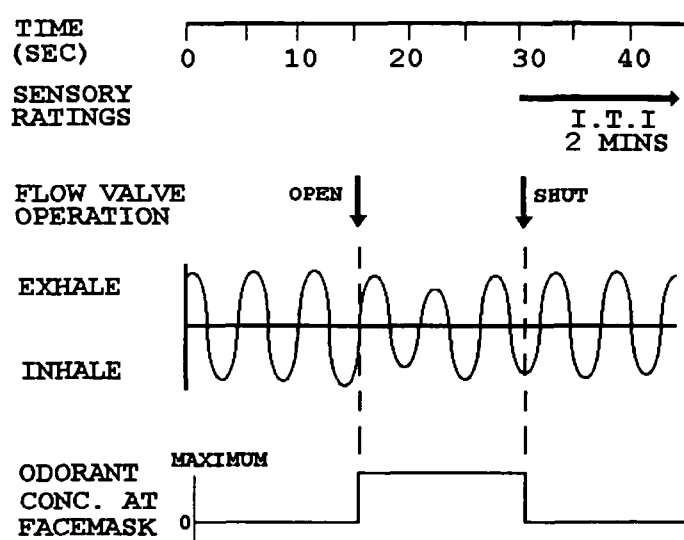

Figure 2 Sequence of events during each propionic acid trial. In the period before time zero, the participant was instructed to achieve a good seal with the facemask. During this time, only clean air was presented. After a minimum 15 s period, the flow valve was opened with the next exhalation onset. This redirected clean or odorized air from the exhaust to the facemask. Beginning with the onset of the next inhalation, respiratory data were recorded for a 15 s period, after which the flow valve was shut. The participant then immediately moved away from the mask to enter sensory ratings during the beginning of the 2 min inter-trial interval (I.T.I.).

During the 15 s stimulus period, a cue light was lit to alert the participant to attend to the perceptual qualities of the airstream. Then the flow valve was operated to divert clean or odorized air from the facemask and the participant was instructed to come away from the facemask and begin sensory ratings.

Immediately after moving from the facemask at the end of an odorant or clean air presentation participants provided ratings on the sensory aspects of the airstream using the adjacent computer screen. A computerized version of a visual analog scale, similar to that employed in earlier research (e.g. Walker et al., 1990a, 1993; Warren et al., 1992, 1994), was used to collect ratings of odor strength and nasal irritation magnitude. Participants entered responses on both scales after each trial by positioning the cursor at a point along a line (19 cm long on the computer monitor) and then pressing the mouse button. The visual analog scales always appeared on the computer screen in the same order at each trial. The scales for 'odor strength' and 'nasal irritation' had the anchor 'slight' positioned 3 cm from the left end of the scale and 'previous high' 6 cm from the right end of the scale. It was made clear to participants that a lack of sensation was to be reported by positioning the cursor at the leftmost end of the scale.

Definitions were posted in front of the participant. For odor strength, the instruction was: 'Please rate the magnitude or amount of odor sensation in the air you

breathe in at the facemask. This should be independent of the quality of the smell or any nasal irritation that may be present.' For nasal irritation, the instruction was: 'Please rate the magnitude or amount of any stinging, scratching, burning or other irritating sensations from the nose, independent of any odor that may be present in the air you breathe in at the facemask.'

The participant was instructed to make ratings relative to his or her sensory experience prior to the experiment. For example, if the participant positioned the cursor at a point midway between the left end of the scale and the point labeled 'previous high' on the nasal irritation scale, this indicated that the intensity of nasal irritation was half as intense as the strongest nasal irritation the participant had experienced prior to the experiment. Leaving the right end of the scales unanchored allowed for the possibility that sensation magnitudes could exceed the participant's previous maximum experience. The cues for the participant to start the next trial occurred 120 s after the end of the stimulus period.

Data analysis

For each of the 35 participants (31 normals, four anosmics) a complete data set consisted of four sets of 50 trials, on each of which were recorded ratings of the magnitude of odor and nasal irritation. The possible range of scores from the ratings task was 0–99. These data were transferred to a computer where statistical software (SAS Institute; Cary, NC) was used to determine, for each session-by-participant combination, the concentrations (in log ppm) corresponding to 10, 25, 50 and 75% of the 'distance' from the mean response on clean air trials up to the maximum rating recorded for that session. This procedure is illustrated in Figure 3 using data from a normosmic. The mean of odor strength ratings on clean air trials was 10 and the highest rating for this session was 95, yielding a 'distance' of 85. Propionic acid concentrations corresponding to the following four ratings were determined by simple interpolation: 18.5 (=10 + 10% of 85), 31.25 (=10 + 25% of 85), 52.5 (=10 + 50% of 85) and 73.75 (=10 + 75% of 85). These stimulus values were described as 'iso-response' concentrations since they approximated the stimulus intensities at which various sensation magnitudes are equated for different participants. This approach is analogous to the determination, for a set of sugars, of the concentration of each which corresponds to a given sweetness intensity.

This approach allowed us to estimate, in terms of the concentrations required to elicit various sensation magnitudes, the following: (i) for a given test session, the uncertainty around the concentration corresponding to each iso-response level; (ii) the session-to-session variation in these iso-response concentrations for a given participant; and (iii) the variation among participants in the mean concentrations corresponding to a given iso-response level.
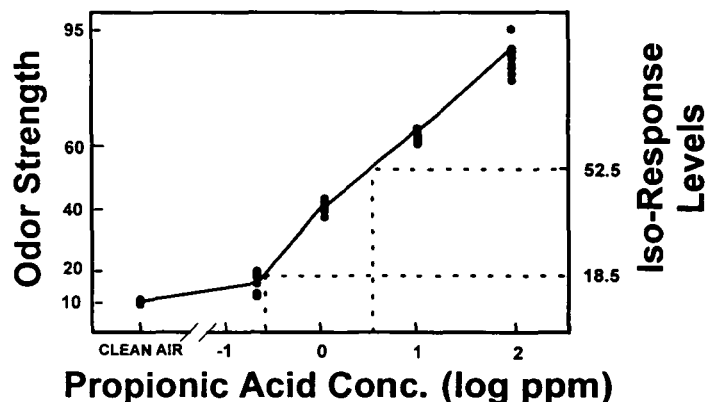
For estimating within-session variation, a bootstrap re-

Figure 3 Illustration of method used to summarize the results from each participant-by-session combination. The concentrations corresponding to various perceptual levels were determined by interpolation from a plot of the sensory ratings versus log propionic acid concentration. The possible range of sensory ratings was 0–99, with zero representing no odor or nasal irritation sensation. In this example from a typical normosmic participant, a concentration of $10^{-0.5}$ corresponds to the 10% isoresponse point and a concentration of $10^{0.5}$ corresponds to the 50% isoresponse point.

sampling technique was used (Efron, 1982). One repetition of bootstrap resampling consisted of drawing, separately for odor and nasal irritation, 10 samples (with replacement) from the 10 observed ratings for each of the four propionic acid concentrations and for the clean air trials. For each of the 100 times that this was repeated, for a given participant-by-session combination, the concentrations corresponding to the four iso-response levels were determined (see Figure 3) from the set of 50 ratings. One hundred resampling iterations was considered optimal, as it has been shown empirically that there is no statistical advantage to increasing this number (Efron, 1982, p. 28). This simulation technique has the interpretive advantage that, instead of making assumptions as to the underlying distribution from which the responses were drawn, sampling is done using only the actual data. Bootstrap resampling thus offered a straightforward means of extracting an estimate of the uncertainty around each of the four iso-response concentrations calculated from a set of 50 responses obtained during a single participant-by-session combination.

This procedure yielded, for each of the four sensation magnitudes, a distribution of the corresponding concentrations that could have arisen from the set of 50 ratings actually observed for a given session. In turn, each of these distributions was described in terms of the logarithmically expressed width of its 95% confidence interval. In this way, a quantitative estimate of intra-session precision was obtained for each combination of session and perceptual intensity.

To assess variation over time for a given participant, data for each participant-by-session combination were first reduced to a set of four iso-response concentrations.
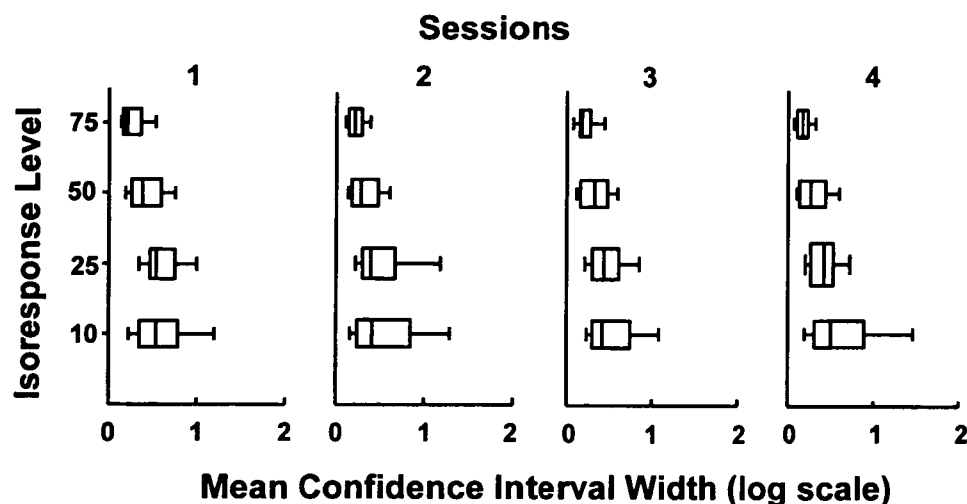
Sessions

Figure 4   Distribution of 31 normal participants in terms of intra-session uncertainty in iso-response concentrations (odor magnitude). Box-whisker plots show the 5th, 25th, 50th (median), 75th and 95th percentile values for the sizes of the intra-session confidence interval estimates, separately for each combination of perceptual magnitude and session.

Within-participant variation in sensitivity over the four sessions wasthen expressed in terms of the range, or span, of concentrations (expressed as logarithmic distances) associated with each iso-response level. To estimate between-participant variation, data for all four sessions, for a given participant, were reduced to a set of four iso-response concentrations. The distribution of participants, in terms of the concentrations required for the each of the four iso-response levels, was then examined.

Results

Within-session uncertainty

Figures 4, 5 and 6 depict the width of the confidence interval estimates as a function of session and perceptual intensity for odor and nasal irritation. Data for normosmic and anosmic participants are shown separately and no odor magnitude data are reported for the anosmic participants.

For the two plots dealing with the 31 normosmic participants, several points can be made. First, the bootstrap resampling simulation indicates that, with 10 trials at each of the four precisely controlled odorant concentrations, the average degree of uncertainty is generally constant over the four test sessions. Second, the width of the estimated within-session confidence intervals is rather similar for odor and nasal irritation. Third, the confidence intervals for both attributes are greatest with the peri-threshold level (10%) and decline markedly at higher perceptual magnitudes. For the lowest iso-response level the 90% confidence interval was ~0.6 log units (4-fold range) for odor and ~0.8 (6-fold range) for nasal irritation. At progressively higher perceptual magnitudes the widths of the confidence intervals for odor and nasal irritation declined steadily. The within-session

uncertainty in the nasal irritation results for the anosmic participants was comparable to that seen with normosmic participants at the 10% iso-response level; with the exception of session 2. For greater perceptual magnitudes, however, much smaller within-session variation was observed.

Within-participant variation

Figure 7 shows the distribution of within-participant concentration spans for each of the four iso-response levels. For each participant, variation at a given iso-response level was expressed in terms of the range of propionic acid concentrations required to produce each perceptual magnitude for the four test sessions. In this figure, odor and nasal irritation data are shown for the 31 normosmic participants and only nasal irritation data are shown for the four anosmic participants.

For normosmic participants the distribution of spans is rather similar for odor and nasal irritation at each of the four iso-response levels. Just as the within-session uncertainty is inversely related to the iso-response level (Figures 4, 5 and 6), participants are most widely distributed and within-participant variation is greatest at the lower two iso-response levels. For the anosmic participants, within-participant uncertainty appears to grow (stability declines) with increasing sensation magnitude.

The apparent association, for normosmic participants, between intra-session and intra-participant variation led us to evaluate the possibility that some of the apparent fluctuation in sensitivity, for a given individual, might instead be intra-session uncertainty. This question was addressed directly by examining the degree to which intra-session uncertainty predicted variation within a
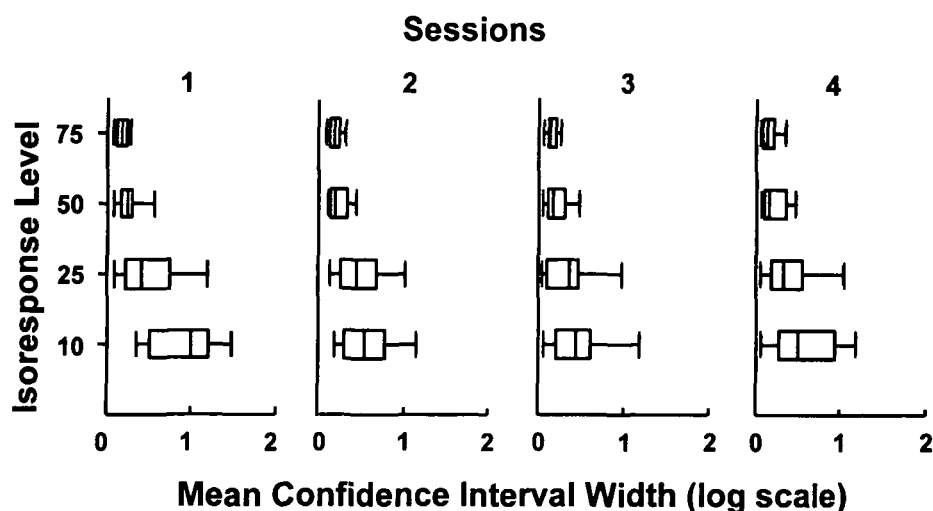
Sessions

Figure 5 Same as Figure 4, except that the distributions apply to nasal irritation instead of odor strength and show data from the same 31 normal participants.
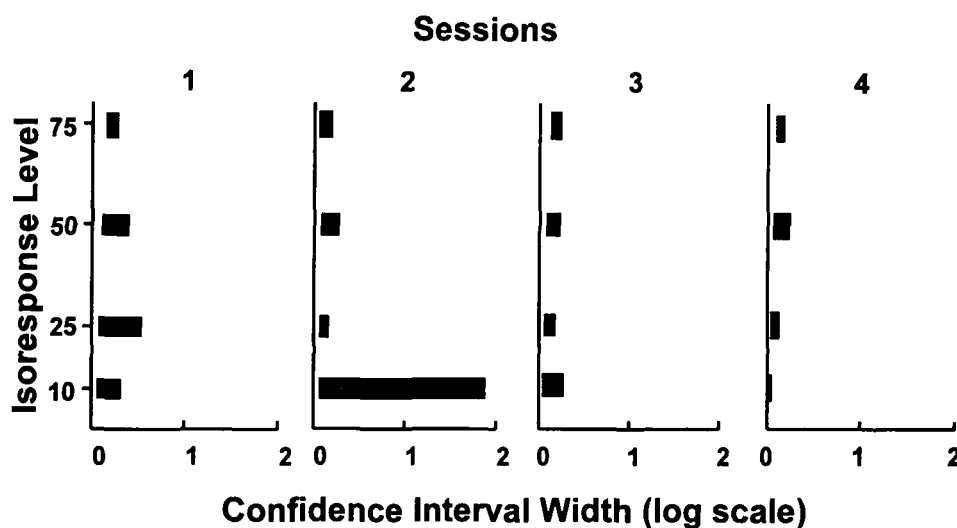
Sessions

Figure 6 Ranges, for the sample of four anosmic participants, in estimated confidence interval sizes used to gauge intra-session uncertainty in rating nasal irritation. As with Figures 4 and 5, all participants are represented for each combination of session and perceptual magnitude.

participant over time. For each combination of participant and perceptual level, simple regression analysis was used to examine the degree to which intra-session uncertainty (mean width of confidence interval for simulated iso-response concentrations) predicted apparent intra-individual variation (session-to-session span in iso-response concentrations). Regression results are shown in Table 2, separately for odor and nasal irritation.

For all four odor magnitudes, apparent session-to-session variation is largely attributable to uncertainty surrounding the iso-response concentrations obtained for each session. Thus, there may be little actual variation in odor sensitivity over time for a given individual, but significant inter-

individual differences in the number of trials required to measure iso-response concentrations to a given level of precision. This appears to be especially true for peri-threshold odor intensities. In contrast to these observations with odor strength, intra-individual variation in nasal irritation sensitivity is largely not accounted for by our measure of intra-session uncertainty. Weak though statistically significant relationships were observed for the two highest perceptual levels. However, for the two lower levels, the two components of variation were completely independent. We suggest therefore that there is considerable 'true' fluctuation over time in the stimulus concentration associated with a given intensity of nasal irritation. Given
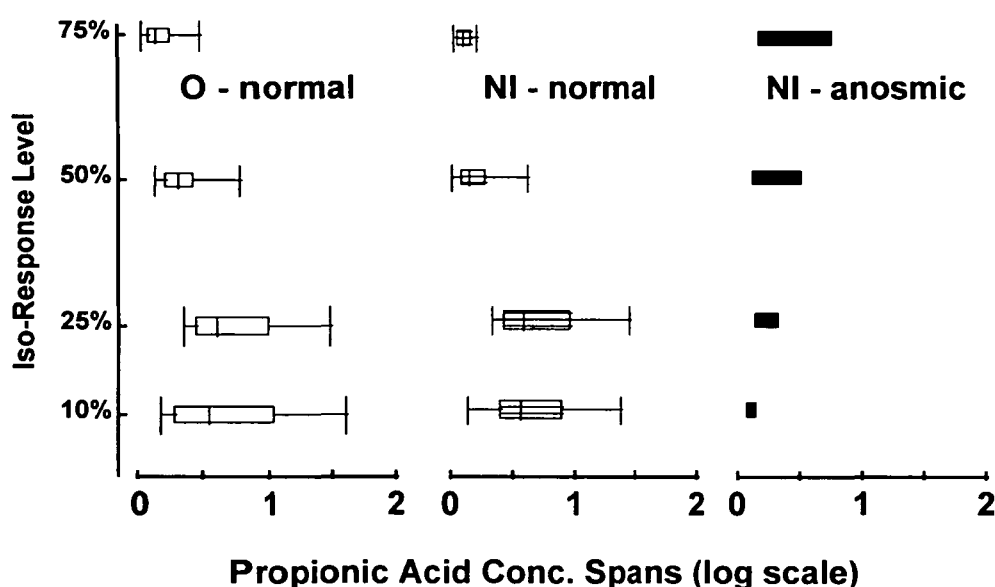
Figure 7   The distribution of intra-participant spans (session-to-session), expressed as logarithmic 'distances' in the concentrations required for each of the four iso-response levels are shown. The box-whisker plots show the 5th, 25th, 50th (median), 75th and 95th percentiles for the 31 normal subjects for both odor magnitude (O: clear boxes) and nasal irritation (NI: lined boxes). For the four anosmic participants (filled boxes) shown in the far right panel, only the range of spans is shown.

the small number of anosmics, and their low levels of both intra-session uncertainty and intra-individual variation, no formal comparison of these two sources of variation was made for these participants.

Between-participant variation

In order to examine between-participant variation in odor and nasal irritation we made the assumption that, even for those participants that exhibited the greatest variation, a total of 40 trials per concentration was sufficient to obtain reliable estimates of the concentrations required for the four perceptual magnitudes. Figure 8 depicts the distribution of these iso-response concentrations for both normosmic and anosmic participants. Each of the 31 normosmic participants is represented once at each iso-response level, for both odor and nasal irritation. The anosmic nasal irritation data were processed in the same manner, except that the four participants are represented by ranges only. For odor data, the concentration interval required to encompass 90% of the participants was <1 log unit at the 10% iso-response level but declined to ~0.5 log unit at the highest perceptual magnitude. The same pattern is seen with the nasal irritation data although the concentration interval is somewhat less at each perceptual level.

Comparison of the nasal irritation results for normosmic and anosmic participants leads to a number of conclusions. First, while caution is warranted given the small number of anosmic participants, these individuals appear to exhibit far less inter-individual variation than normosmics. This

Table 2   Results of regression analyses of the relationship between intra-participant variation and intra-session uncertainty in 31 normal participants

Perceptual (iso-response) level	Odor R^2/P value	Nasal irritation R^2/P value
10%	0.75/0.00	0.04/0.28
25%	0.54/0.00	0.01/0.56
50%	0.47/0.00	0.18/0.02
75%	0.34/0.00	0.15/0.03

variation increases with perceptual magnitude, in clear contrast to the pattern seen with the normosmics. The two groups are rather similar in terms of the concentrations required for the highest intensity of nasal irritation. At progressively lower perceptual levels, however, the concentrations required for normosmic participants cover a steadily expanding range and almost all normosmics require lower concentrations than anosmics. Some important implications of this higher nasal irritation sensitivity of normosmic participants are discussed below.

Discussion

We suggest that a reasonable estimate of threshold is provided by the concentration required for the 10% iso-response level. This approach yields values of 0.27 and 0.90 ppm for odor and nasal irritation, respectively, in normosmic participants and 10.59 ppm for nasal irritation
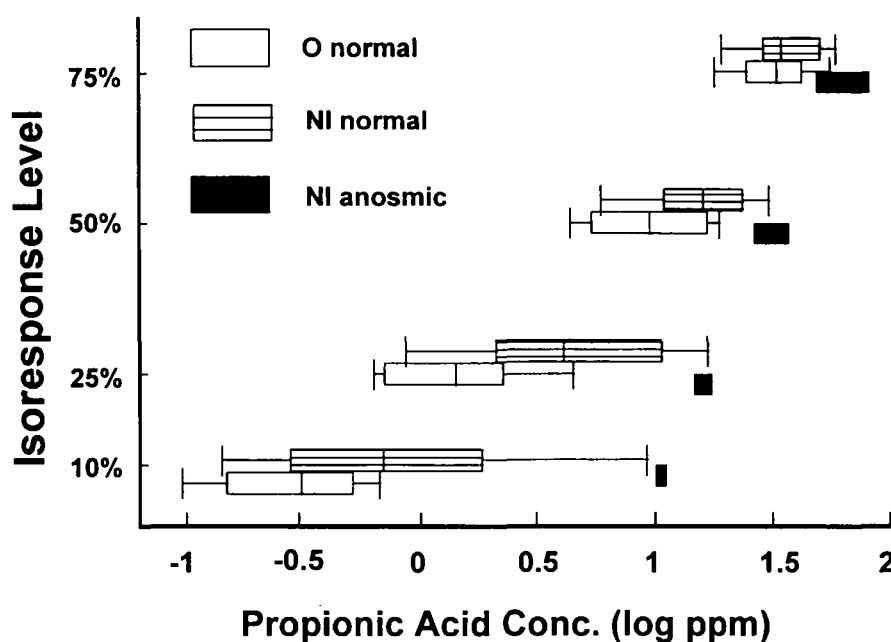
Figure 8 Inter-participant variation in the mean concentrations corresponding to each of the four perceptual magnitudes for odor and nasal irritation in normosmic participants, and for nasal irritation in anosmic participants. For the 31 normal participants, box-whisker plots depict the 5th, 25th, 50th (median), 75th and 95th percentile values. For the four anosmic participants, the ranges of concentrations at each iso-response level are shown. Small vertical offsets are used to aid visualization.

in anosmic participants. Our estimate of odor threshold for propionic acid is in good agreement with the range of values (0.003–4.57 ppm) provided in the compilation by Devos *et al.* (1990). Our results shed some light on prior work on intra- and inter-participant variation in odor sensitivity. Stevens *et al.* (1988) studied three participants' responses to three odorants over a 1 month period and reported that odor thresholds could vary 2000- to 10 000-fold over time for a given individual. This finding, in combination with the fact that there is considerable inter-laboratory variation in odor sensitivity (Devos *et al.*, 1990; Walker and Jennings, 1991), has led to a rather bleak assessment for the establishment of a database of human sensitivities to a chemically diverse set of odorants. The present findings may be cause for optimism in two ways.

First, variation in odor sensitivity over time for a given individual appears to be far less than that indicated by the Stevens *et al.* (1988) report. For the 10% iso-response level, which may be taken as a reasonable estimate of detection threshold, intra-individual concentration spans were only 1.3- to 110-fold (see Figure 7). Importantly, most of the apparent intra-individual variation in odor perception seen in this and other studies appears to be a reflection of various degrees of intra-session uncertainty exhibited by different participants. Intra-individual variation in odor sensitivity may be reduced to a negligible level with the combined use of air-dilution olfactometry and a sufficiently high number of trials per concentration per test session.

Secondly, our paradigm also appears to provide an estimate of inter-individual variation that is considerably less than that indicated by earlier work. Amoore *et al.* (1968) reported that the distribution of odor thresholds of normosmic participants to isobutyric acid exhibited a standard deviation of 0.42 log units. Similarly, Amoore and Steinle (1991) reported a standard deviation of ~0.6 log units based on tests with four odorants. Based on these two values, the concentration range required to encompass 90% of the responses from our participants should have been 1.58–1.97 log units. As seen in Figure 8, a concentration range of <1 log unit was needed, for all four perceptual magnitudes, to encompass 90% of the 31 normosmic participants. We suggest that conclusions regarding large intra- or inter-individual variation in responses to odorants be viewed with some skepticism until it can be determined that sufficient control over stimulus concentrations was obtained and sufficient numbers of trials per concentration were employed. If the observations we report for propionic acid are supported by future work, from other laboratories and with other stimuli, the expected value of a comprehensive database of human odor sensitivities would be considerably increased.

In an earlier quantitative comparison (Walker *et al.*, 1989, 1990b) of normosmic and anosmic nasal irritation sensitivity to several compounds, the former were ~1.2 log units more sensitive than the latter to propionic acid. This agrees well with the present finding that normosmic nasal irritation sensitivity exceeds that of anosmics by 1.07 log units. There is a paucity of work from other laboratories on the possible

olfactory contribution to nasal irritation. Allen (1929) and Elsberg *et al.* (1935) studied primarily physiological responses of anosmics to a variety of chemicals presented at unknown concentrations. Doty *et al.* (1978) obtained detailed ratings by both normosmics and anosmics, in response to 47 chemicals presented at 'high' but unspecified concentrations. Prah and Benignus (1984) reported the responses of four normosmics to a detection task that included the use of acetic acid and butanol, and found that trigeminal thresholds were greater than the olfactory thresholds for the same substances. Shams Esfandabad (1993) provided quantitative irritation data in a rigorous series of olfactometer studies using pyridine and formaldehyde. This work did not address anosmic participants, but demonstrated that normosmics were able to discriminate odor from irritation when asked to judge them jointly.

In a series of recent studies, Cain and colleagues (see Cain and Cometto-Muñiz, 1995) employed a mainly sniff-bottle-based stimulation approach to assess odor thresholds in normosmics and nasal irritation thresholds in anosmics. Due to a concern for the possible biasing effect on odor perception of nasal irritation or pungency, however, these investigations have not included nasal irritation sensitivity measurements in normosmics.

The systematic comparison of normosmic and anosmic participants is a practice that adds difficulty to such psychophysical experiments because there are relatively few suitable anosmic participants available for testing. However, there is a considerable advantage to including such participants and testing them in exactly the same way as normosmic participants. Comparison of the results of the two kinds of participants when both are treated identically provides important information on the neural mediation of nasal irritation. Consistent with our prior work (e.g. Walker *et al.*, 1990b), the presence of a functioning olfactory system appears to increase nasal irritation sensitivity. The most parsimonious explanation for this observation may be that low to moderate levels of nasal irritation in normosmic participants are a joint outcome of olfactory and trigeminal input. According to this interpretation, the greater variation among normosmic participants at the lower perceptual magnitudes reflects differences among normosmic participants in the integration of olfactory and trigeminal stimulation. Consistent with this view is the observation that, with low to moderate levels of nasal irritation, the between-participant variation exceeds that of either sensitivity to odor in normosmic participants (olfactory nerve activation) or nasal irritation in anosmic participants (trigeminal nerve activation).

Although it is unclear exactly what neural mechanisms underlie olfactory–trigeminal interactions in the perception of nasal irritation in normosmic participants, our findings do have immediate applications for efforts to develop physicochemical (e.g. Abraham *et al.*, 1996) or animal (e.g. Alarie, 1981; Abraham *et al.*, 1990) models to predict perceived nasal irritation. Specifically, models that implicitly assume that the trigeminal nerve is the sole basis of nasal irritation will not be predictive of the low to moderate levels of nasal irritation experienced in everyday life.

Clinical and experimental research may benefit from use of the paradigms we describe. For example, the relatively large normative dataset of 31 normosmics may be used for assessing the odor sensitivity of individuals whose self-reports suggest hyposmia. Those for which the isoresponse concentrations for odor are within the range of values for normosmic participants would be considered normal. However, individuals with higher isoresponse concentrations could be considered hyposmic.

Our paradigm might also be used to estimate, for one or more test sessions, the intra-session uncertainty using the bootstrap resampling procedure we employed. Participants exhibiting the lowest intra-session uncertainty could be selected for subsequent testing that could, for example, examine the effect of variables hypothesized to affect odor sensitivity. In this way, the interpretability of these results would be optimized since the uncertainty around each estimate of sensitivity is minimized.

In future work we plan to systematically extend the present paradigm to characterize responses of normosmic and anosmic participants to additional single odorants and to mixtures, using olfactometer techniques as well as controlled environment rooms (e.g. Walker *et al.*, 1997). These investigations will also include comparison of ocular-only, nasal-only and ocular-plus-nasal stimulation to help elucidate the interactions among chemosensory inputs in responses to airborne chemicals.

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