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Human Responses to Propionic Acid. II. Quantification of Breathing Responses and their Relationship to Perception

James C. Walker[3], Martin Kendal-Reed[1,3], Sandra B. Hall[2], Walter T. Morgan, Vladimir V. Polyakov[1,4] and Richard W. Lutz[1]

Bowman Gray Technical Center, R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102, [1]School of Dentistry, University of North Carolina, Chapel Hill, NC 27599 and [2]Department of Statistics, Florida State University, Tallahassee, FL 32306, USA

[3]Present address: Sensory Research Institute, Florida State University, Tallahassee, FL 32306, USA

[4]Present address: Section on Pulmonary Medicine, Wake Forest University Baptist Medical Center, Medical Center Blvd, Winston-Salem, NC 27105, USA

Correspondence to be sent to: J.C. Walker, Sensory Research Institute, Florida State University, Tallahassee, FL 32306, USA. e-mail: jwalker@psy.fsu.edu

Abstract

In 20 normal and four anosmic participants, instantaneous inhalation and exhalation flow rates were recorded in response to 15 s stimulations with clean air or propionic acid concentrations (0.16, 1.14, 8.22 and 59.15 p.p.m., v/v) that ranged from peri-threshold for normals to clearly supra-threshold for anosmics. Each odorant/irritant delivery to the face-mask began with an exhalation. This allowed concentration to reach full value before stimulus onset, defined as the point where the participant began to bring the stimulus into the nose by inhalation. Two seconds after this stimulus onset, normals exhibited cumulative inhaled volume (CIV) declines of 39 and 14%, and latencies of 500 and 710 ms, with presentations of 59.15 and 8.22 p.p.m., respectively. With anosmics, 59.15 p.p.m. caused a 19% decline in CIV that began at 730 ms. Examination of the first inhalation after stimulus onset shows that the CIV declines in normals were achieved by a progressive decline in volume (InVol), beginning with a slight drop at 1.14 p.p.m., and a marked decline in duration (InDur) with only the highest concentration. Anosmics exhibited declines in InDur and InVol with only the 59.15 p.p.m. stimulus, and these declines were much more modest than the changes seen in normals. Comparison of these breathing results with perceptual responses from this same experiment demonstrates that: (i) in normals, odor perception rises slightly, but breathing does not change, with the lowest concentration; (ii) the higher breathing sensitivity (declines in InVol) of normals is paralleled by both the higher nasal irritation of these individuals and the presence of odor sensation; (iii) InDur declines in normals only with a stimulus concentration sufficient to cause marked nasal irritation in anosmics; and iv) in anosmics, modest but reliable declines in both InDur and InVol mirror the marked elevation in nasal irritation magnitude seen with only the highest concentration. In view of the failure of prior work to provide evidence that olfactory activation alone can cause any of the breathing changes we observed, we conclude that some breathing parameters are quite useful as rapid and sensitive measures of nasal irritation that arises from activation of nasal trigeminal afferents alone or in combination with the olfactory nerve.

Introduction

In everyday life, stimulation of the trigeminal and olfactory chemosensory afferents requires the movement, by breathing, of air into the nasal cavity. Nonetheless, it remains unclear what the exact relationships are among breathing behavior, degree of stimulation of the olfactory and trigeminal nerves, and the perception of odor and irritation.

Most of the work in this area, over the past four decades, can be grouped into two general categories. One approach has been to focus on the effect, for a given odorant concentration, of variations (either accidental or effected by the experimenter) in breathing pattern on the perceived magnitude of odor (von Gerhardt and Rauh, 1963; Gudziol and Gramowski, 1987). Generally, studies in this area employ weak to moderate odor stimuli that would be expected to elicit little or no nasal irritation, with the implicit assumption that there is no direct, reflexive effect of stimuli on breathing or sniffing parameters. Further, an idea underlying most such work is that, under constant experimental conditions, odor detectability or supra-threshold intensity is largely a function of the delivery rate of odorant molecules

to the olfactory receptor sheet as a result of either passive inhalation or active sniffing. Laing (Laing, 1982, 1983) summarized the key findings in this area and attempted to reconcile apparent discrepancies in prior reports as to whether perceptual ability could be enhanced by increasing inhalation rate. His work indicated that little or no enhancement in perception is achieved when individuals are instructed to employ sniff or inhalation parameters that deviate from their characteristic pattern. Thus, the confusion in prior research was attributed to the failure to take into account the characteristic way that a given individual normally inhaled or sniffed during odor perception.

The second line of research on the relationship between breathing and chemoreception takes the approach that, within a very short period of time after stimulus onset, breathing pattern is largely determined by the pattern of olfactory and/or trigeminal stimulation. This approach then seeks to find effects of this stimulation on breathing parameters. The majority of the focus in this area has concerned breathing changes with rather strong (and usually briefly presented) stimuli that cause moderate to severe nasal irritation. For example, Dunn *et al.* (Dunn *et al.*, 1982) and Shusterman and Balmes (Shusterman and Balmes, 1997) have shown that there is a reflex transient apnea that occurs in response to strongly irritating concentrations of carbon dioxide. These studies have provided some fundamental new information on the possible value of breathing changes as endpoints and they have collectively supported the notion that, at least for stimuli that cause clearly perceptible nasal irritation, breathing may be reliable as a non-verbal correlate of perception. It should be noted, however, that this view is based on an experimental approach in which very brief, and usually very intense, stimuli are abruptly presented. This contrasts with 'real-world' situations where stimulation occurs in the course of normal breathing. It seems reasonable to conclude that the breathing effects in such studies are largely mediated by the trigeminal system.

Recently, however, some evidence has accumulated to indicate that breathing responses might also be seen with much less intense stimulation. This research also has raised the possibility of an olfactory contribution to breathing changes. Normal individuals breathed slower and more deeply when exposed to environmental tobacco smoke (ETS) concentrations that elicited clear nasal and eye irritation (Walker *et al.*, 1993). This finding was replicated in later work, in which much lower ETS concentrations were included (Walker *et al.*, 1997). Surprisingly, the degree of change in breathing was the same for concentrations that elicited moderate irritation as for much lower concentrations that elicited no nasal irritation and very little eye irritation. Since these studies employed normal participants and 'whole body' exposures, the role of at least ocular trigeminal, nasal trigeminal and olfactory inputs must be considered in any attempt to understand the neural mediation of these breathing changes.

Somewhat more interpretable are a small number of olfactometer-based studies (Walker *et al.*, 1990b; Warren *et al.*, 1992, 1994) indicating that some nasally presented stimuli suppress inhalation volume. The findings thus far suggest that the degree of inhibition is related to the magnitude of nasal irritation, but is largely independent of odor magnitude. Based on this preliminary interpretation, a given amount of olfactory nerve activation may have little or no inhibitory effect on inhalation volume in the absence of trigeminal stimulation, but could contribute to this breathing change in the presence of trigeminal activation. In our view, studies provided thus far have not provided sufficiently detailed information to allow a thorough examination of the relationship between breathing changes and perception of odor and irritation. An insufficient number of trials were conducted for each stimulus condition, the limited amount of testing of anosmic individuals was not conducted in a manner identical to that applied to the normal individuals and a given individual was only tested for one session. As a result of such deficiencies, major questions remain as to the relationship between specific breathing parameters and the perceptual outcomes of separate and/or combined activation of olfactory and nasal trigeminal inputs.

The present study was part of an effort to answer some of these questions. A rather large set of normal individuals and a (necessarily) much smaller set of anosmic individuals each participated in four identical test sessions, in each of which the same range of propionic acid (PA) concentrations was presented. The selected concentration range was based on prior research (Walker *et al.*, 1989) and was expected to extend from sub- or peri-threshold for normal individuals to clearly supra-threshold for the anosmic participants. Ten trials of each of the four PA concentrations, in addition to ten control trials, were included in each of the four test sessions. Just before and during each stimulus presentation, instantaneous inhalation and exhalation flow rate was measured with 10 ms resolution. From these data, we examined the timing of an odorant effect on breathing by combining normalized raw data within and among participants. Based on the pattern of results from this approach, we also calculated parameters for the first inhalation after stimulus onset and examined the changes in these parameters with concentration and in relation to perceptual responses.

Materials and methods

Participants

Thirty-one normal and four anosmic individuals were recruited through newspaper advertisement and physician referral, respectively. Details of recruitment, pre-testing and the clinical status of the anosmics are provided in our report of perceptual responses (Kendal-Reed *et al.*, 1998). We selected a subset of the 35 participants who were tested

under identical conditions and for which we had sufficient raw breathing data. This filtering process eliminated 11 normals but none of the anosmics. The 20 normals that remained consisted of 15 females (28 ± 9.2 years old) and five males (23.6 ± 4.9 years old). Two anosmics were female (ages 27 and 46 years) and two were male (ages 33 and 58 years). Testing conditions and criteria used to decide whether sufficient data were obtained, per individual, are provided in the 'Procedure' section.

All participants were told that the purpose of the study was to examine sensory responses to odorous stimuli, and no indication was provided that breathing was being measured. Participants gave informed written consent and received financial compensation of $100 for participating in the study, which was approved by the Institutional Review Board of the UNC School of Dentistry.

Apparatus

Participants were tested in a 39 m^3 room where ventilation rate, mean temperature and mean relative humidity (RH) were 15 air changes per hour, 25ºC and 50%, respectively. Major components of the participant station are shown in Figure 1. Participants wore headphones through which recorded sounds of olfactometer operation mixed with 'white' noise were played. Clean air or one of four propionic acid concentrations (0.16, 1.14, 8.22 and 59.15 p.p.m., v/v) were generated by an air-dilution olfactometer (Walker *et al.*, 1990a; Warren *et al.*, 1992, 1994) in preparation of each trial.

Verification of olfactometer output was accomplished with the method of Maiolo *et al.* (Maiolo *et al.*, 1996). Whether clean air or an odor stimulus was being presented to the face-mask (Vital Signs Inc., Totowa, NJ), volume flow rate, RH and temperature of the air stream were 30 l/min, 35% and 25ºC, respectively. Pressure inside the inflated rim of the mask was continuously monitored to assess whether the participant's face was pressed snugly into the mask. Verification that this was the case ensured that the participant breathed only air from the olfactometer which, in turn, allowed quantification of inhalation and exhalation flow rates by the pneumotachograph mounted just downstream of the participant.

Procedure

Experimental design and trial sequence

Normal and anosmic participants completed four sessions, separated by at least 3 days, in which ten clean air trials and ten trials at each of the four PA concentrations were presented, in quasi-random order, over a period of ~2.25 h. Events during each trial are summarized in Figure 2. When instructed by the control computer, the participant entered the face-mask. Participants were simply asked to breathe normally and through only the nose when in place at the mask. (Intermittent monitoring of each participant, by looking through the transparent mask, revealed no instances of the lips being separated to allow any mouth breathing.) The PRE period, in which only clean air was presented, began with the first inhalation onset after the participant was fully in place at the face-mask and lasted 15 s. With the onset of the first exhalation after the PRE period, a flow valve operated to send clean or odorized air to the mask. This feature allowed the odorant/irritant concentration in the mask to rise to full value during the time the participant was exhaling. We defined stimulus onset as the beginning of the subsequent inhalation, since that is the point when airborne chemicals first entered the nasal cavity on a given trial. This point also marked the start of the 15 s DURING period.

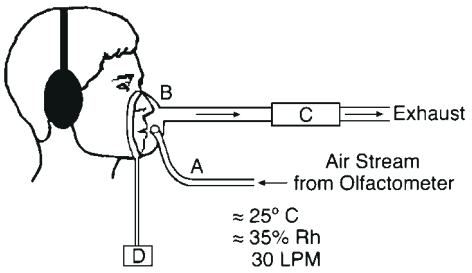

Figure 1 Key components used for odorant/irritant stimulation and measurement of breathing. An olfactometer-generated airstream **(A)** contained varying concentrations of PA or clean air, but was held at constant temperature, RH, and volume flow rate. The airstream was delivered to the participant's face-mask **(B)**. A pneumotachograph **(C)**, located downstream of the participant, was used to measure instantaneous flow rates of breathing. Verification that the participant was making a good seal was accomplished by a pressure transducer **(D)**, which measured the pressure in the inflated rim of the mask.

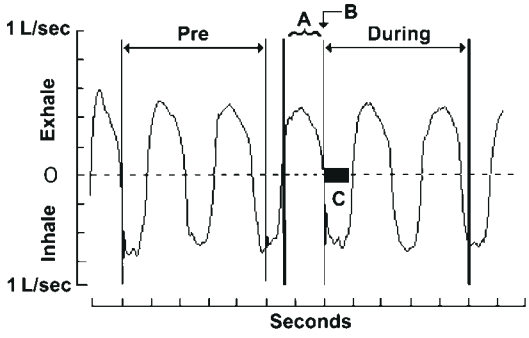

Figure 2 Timing of stimulus presentation in relation to breathing. Each mark on the time scale represents 3 s. The trace depicts instantaneous flow rates of inhalation (below '0' baseline indicated by dashed line) or exhalation (above baseline). Once the participant was in place at the face-mask, recording of breathing data commenced. The PRE period then began with the onset of the next inhalation and continued for 15 s. At the start of the subsequent exhalation a flow valve operated, delivering odorized (or clean air if a control trial) to the face-mask. Concentration rose to full value during time interval 'A', the remainder of this exhalation. The onset of the next inhalation ('B') began the 15 s DURING period. The initial 2 s of this period (the bar denoted by 'C') were selected as the basis for detailed analysis of the time course of breathing responses.

At the end of the DURING period, the participant was signaled by the computer to leave the mask and provide psychophysical responses. A detailed description of the measurement and interpretation of these responses is included in the report by Kendal-Reed *et al.* (Kendal-Reed *et al.*, 1998). Briefly, participants were presented with computerized line scales for reporting magnitudes of odor and nasal irritation, and were instructed to use a computer mouse to mark points that represented perceived intensity relative to two locations. The left end of the line scale denoted a complete absence of the sensation being reported and a location 68% of the way from the left to the right end represented the maximum intensity of that sensation experienced prior to the experiment. Participants were instructed to report odor magnitude independent of nasal irritation or the quality of any odor that might be present. Similarly, they were instructed to report the magnitude of nasal irritation, operationally defined as 'any stinging, scratching, burning or other irritating sensations from the nose', independent of odor magnitude. Advantages of this approach to measuring intensity of odor and nasal irritation have been discussed by Walker *et al.* (Walker *et al.*, 1997).

Data analysis

The experiment yielded a total of 9600 breathing traces: 24 participants × 4 sessions/participant × 50 trials/session × 2 periods/trial. Traces were eliminated if any of the following were observed: (i) decline in mask rim pressure and a downward shift in the breathing baseline, indicating an inadequate mask seal; or (ii) data collection began after inhalation onset; or (iii) the participant inhaled or exhaled so deeply that the dynamic range of the instrumentation was exceeded. Due to large variation in the rate at which flow rate declined at the end of exhalation, and the limitations of our hardware and software, triggering of data collection at the exact point that inhalation began was not possible. The controlling software was written with a bias to begin data collection early so that the 'trailing edge' of the preceding exhalation was included, thus minimizing the likelihood that true inhalation onset would be missed. This situation was observed in the small proportion of cases where the participant made a very rapid transition from exhaling to inhaling. In these cases the data were not used in our analysis. For the majority of traces, all values representing the end of the preceding exhalation were simply eliminated and the time base was adjusted accordingly. In order for data for a given participant to be included in further analyses, the above screening steps must have eliminated no more than 40 (of 200) total trials for a given participant and no more than 15 (of 50) trials for any given session. Analyses were based on 20 normal and four anosmic participants.

Two approaches were used to examine breathing responses to olfactory and/or trigeminal stimulation: cumulative inhaled volume (CIV) and breath-by-breath (BXB). The purpose of the CIV approach was to examine in detail the temporal pattern of breathing changes, separately for normals and anosmics, as a function of concentration. For each trial, inhalation flow rates were summated over the course of the DURING period of each trial. Since instantaneous volume flow rates are recorded at a sampling rate of 100 Hz, division by 100 yielded true volumes. The mean CIV for clean air trials for each session was calculated. Then all of the cumulative data for all concentrations for that session were expressed as a proportion of that volume. This meant that the mean CIV for the clean air exposures rose monotonically from 0 to 1 over the course of the PRE or DURING periods, while the CIVs for the four odorant concentrations rose, in most cases, to a value ≤1. Completion of this step for each session allowed data to be combined, within concentration, across sessions for a given participant and then across participants.

In addition to the use of CIV to examine changes in breathing as a function of concentration, we also used custom-designed software to calculate the volume and duration of each complete inhalation and exhalation. Based on the pattern of results seen with the CIV analysis, we focused on the proportional (percentage) change from the mean of the PRE period to the first breath of the DURING period. Using this approach, we examined inhalation volume and duration as a function of concentration, separately for the normal and anosmic participants. This was done first on a session-by-participant basis, then within participant across sessions and finally across participants. To allow data to be combined within and among participants, the mean percentage change for each concentration was expressed as an increment or decrement relative to the change seen on clean air trials.

The product of the analysis above was combined with perceptual data from this experiment in order to evaluate the value of inhalation volume (InVol) and duration (InDur) as correlates of perception. Odor and nasal irritation data for only those trials contributing to the breathing data set were expressed in terms of the percentage of the distance from the mean ratings on clean air trials (for that session) to the highest rating recorded (also for that session). Then the perceptual and breathing data were compared directly, separately for normal and anosmic participants.

Results

Time course of changes

Figure 3 shows the results of the CIV analysis, for normal participants, for the first 2 s after stimulus onset. Selection of this time period was based on two considerations. First, examination of the CIV data for the full 15 s stimulus period showed that all effects of concentration were evident by 2 s. A given concentration either had no effect or decreased the slope, within this 2 s time period, to a value maintained for the remaining 13 s. Second, since most inhalations lasted at least 2 s, almost all trials contributed to each of the 200 time

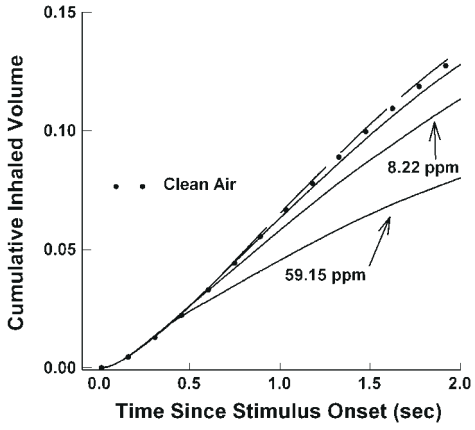

Figure 3 Normalized cumulative inhaled volume for the first 2 s of the stimulus period for normal participants (n = 20). At each time point, cumulative inhaled volume is shown relative to the mean volume of air inhaled in the complete DURING period of clean air trials. Lines for the lower two concentrations, which did not cause significant declines in cumulative inhaled volume, are unlabeled.

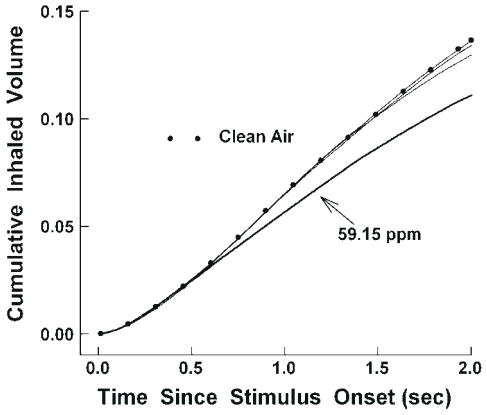

Figure 4 Normalized cumulative inhaled volume for the first 2 s of the stimulus period for anosmic participants (n = 4). Data are expressed in the same way as Figure 3. Only the highest concentration of 59.15 p.p.m. caused a significant decrease in cumulative inhaled volume.

points for which mean data are shown in this figure. Data are expressed, for each of the four concentrations, relative to that for clean air. Note that all stimulus conditions are identical for at least the initial 400 ms. This provides very strong evidence that the olfactometer maintained a constant flow rate across concentrations, sessions and participants. A pairwise t-test was performed at each time point, pairing each concentration against clean air, to determine the latency of any suppression of inhalation. A 0.05 significance level was employed. For the 59.15 and 8.22 p.p.m. concentrations, respectively, significant declines in CIV were first seen at 500 and 710 ms and inhaled volume was 39 and 19% lower. At no point were significant changes seen for the lower two concentrations

Comparable data for anosmics are shown in Figure 4. As with normals, all concentrations are the same for at least the first 400 ms. The degree of inhibition of breathing in response to 59.15 p.p.m. was much less than in normals and no effect on breathing was seen with the 8.22 p.p.m. stimulus. For the former, a significant decline was first observed at 730 ms and the reduction at 2000 ms was 19%. Comparisons between Figures 3 and 4 show that an intact olfactory system either increases the magnitude of the breathing change or causes one to be seen with stimuli that are ineffective in anosmics.

Based on the temporal aspects of the breathing changes we observed, we examined the percentage changes from the PRE period to the first complete inhalation after stimulus onset (see Figure 2). This approach also allowed us to investigate the relative importance of changes in inhalation volume and duration in causing inhibition of breathing in both normals and anosmics. Finally, these changes in breathing were compared to perceptual responses in order to

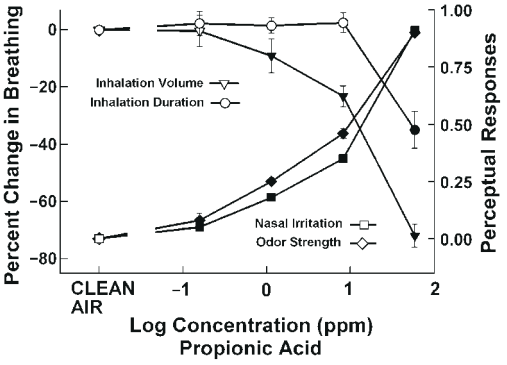

Figure 5 Comparison of breathing (left Y-axis) and perceptual (right Y-axis) responses for normal participants. For inhalation volume and duration, the percentage change from the PRE period to the first complete inhalation of the DURING period is plotted (mean ± SEM). For odor and nasal irritation, responses are expressed, as explained in 'Materials and methods', as a proportion of the within-session increment from the clean air mean to the highest rating recorded. Filled symbols denote cases where responses were significantly different at the 0.05 level.

evaluate the possible use of breathing patterns as sensitive and reliable correlates of perception.

Figure 5 depicts, for normals, changes in InVol and InDur for each concentration. These breathing responses are compared to the effect of each concentration on odor and nasal irritation. Since the effect of each of the five stimulus conditions is shown relative to that of clean air, all means for this control are zero. With both perceptual and breathing measures, each concentration was compared to clean air to determine which responses (denoted by filled symbols) are statistically significant at the 0.05 level. Odor and nasal irritation were reliably elevated with all four propionic acid concentrations, InVol declined with all but the lowest concentration, and InDur declined with only the highest concentration of 59.15 p.p.m. Comparable data for anosmics

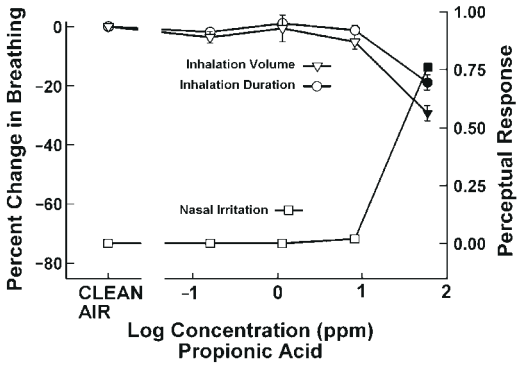

Figure 6 Comparison of breathing and perceptual responses for anosmic participants. Data are expressed, and statistical significance denoted, in the same way as Figure 5.

are summarized in Figure 6. Odor responses are omitted since all values were extremely low. Only the highest concentration caused significant increases in nasal irritation and declines in both breathing parameters.

Comparisons between Figures 5 and 6 support the following conclusions: (i) the presence of an intact olfactory nerve provides, in addition to odor perception, increased nasal irritation and breathing sensitivity; (ii) while InVol and InDur declines are both greater with an intact olfactory nerve, the former demonstrates much more clearly an olfactory contribution to breathing changes; (iii) InDur declines in normals only with a stimulus concentration sufficient to cause marked nasal irritation in anosmics, suggesting that this measure may be useful as a physiological marker of trigeminal sensitivity in normals; and (iv) in anosmics, modest but reliable declines in both InDur and InVol mirror the marked elevation in nasal irritation magnitude seen with only the highest concentration.

Discussion

The present results demonstrate that, in normal individuals with intact olfactory and trigeminal systems, brief nasal stimulation with propionic acid causes a concentration-dependent inhibition of breathing that may be seen as early as 500 ms. An olfactory contribution to this rapid response is demonstrated by the much weaker breathing response exhibited by anosmics. Examination of complete inhalations suggests that much of the enhanced breathing sensitivity that results from olfactory input is attributable to declines in volume in the absence of changes in duration. With two concentrations causing significant nasal irritation in normals, but no irritation or breathing changes in anosmics, volume declined while duration was preserved. Normals and anosmics were most similar with respect to changes in inhalation duration, which was significantly altered at only the highest concentration for both groups.

In evaluating whether the breathing changes we report are useful as correlates of perception or nerve activation, one must consider the fact that breathing is partly under conscious control. Several aspects of our experimental design and methodology argue against the likelihood that the changes we observed are voluntary in nature. Participants were unaware that their breathing patterns were being measured. Further, our system offers very low resistance to normal breathing through the nose, no measuring devices are visible to the participants and no mention of breathing beyond the instruction to 'breathe normally through your nose' was ever made. As illustrated in Figures 3 and 4, the temporal pattern of breathing changes that we observed does not seem consistent with the idea that these are voluntary changes in breathing. Latency and degree of suppression of breathing are orderly functions of odorant concentration, the changes begin as early as 500 ms and (though not seen in these figures) the reduced slopes established during the first 2 s are maintained for the duration of the stimulus. Since order of odorant concentrations is varied differently for each session and a wide range of stimulus intensities is presented each session, the likelihood seems remote that the concentration-related and rapid changes we observed could be attributed to demand characteristics or expectancy effects. Finally, we measure clear breathing changes before prior studies (Overbosch *et al.*, 1989) would indicate that perception is underway.

Our working hypothesis concerning the olfactory contribution to the breathing changes we observed with propionic acid is the same as that developed (Kendal-Reed *et al.*, 1998) to account for the olfactory role in nasal irritation. That is, the enhanced sensitivity is attributed to simultaneous weak activation of nasal trigeminal afferents and a generally higher level of olfactory stimulation. This scheme applies especially to stimuli causing weak to moderate nasal irritation, and declines in inhalation volume, in normals but no perceptual or breathing effects in anosmics. To illustrate, our model posits that the normal member of a pair of twins could honestly report nasal irritation in the same environments in which the anosmic member of the pair could honestly report a complete absence of this sensation. Exceptions would, of course, be encountered in those rare cases where all stimuli present were completely ineffective in activating trigeminal afferents or where the stimuli were just as potent for the trigeminal as for the olfactory system.

Our hypothesis provides an explicit framework for future experiments that could rule in, or rule out, the scheme that we propose whereby olfactory stimulation is an important component of the breathing changes and nasal irritation resulting from everyday chemical exposures. In addition, our model is consistent with the admittedly quite limited research on the neural mediation of breathing changes and nasal irritation. Three observations are worthy of discussion here. First, although it is often implicitly assumed that there is empirical support for the view that nasal irritation is solely the result of nasal trigeminal stimulation, this is not the case. Clearly, a sufficiently high level of trigeminal activation will

cause nasal irritation in the absence of an olfactory system; this requires stimulus concentrations far higher than are representative of indoor or outdoor environments. However, this does not speak to the possibility that, with much less intense stimuli, experiences of nasal irritation may be due in part to olfactory stimulation. Thus, observations showing higher perceptual or breathing sensitivity of normals to chemical stimuli are not in any way at variance with prior experimental data.

Second, the complete lack of empirical support for the notion that the trigeminal system alone accounts for nasal irritation may be one of several reasons to question strongly the idea that normals are unable to report this sensation accurately. Concern over the possibly confounding effect of the olfactory system on the reporting of nasal irritation appears to have led to the unwarranted belief that, when interpreting responses from normal individuals, only those stimuli that elicit nasal irritation due to trigeminal activation alone should be considered as 'true' irritation (Cometto-Muñiz and Cain, 1995) This view has, in turn, led to stated or implicit proposals to rely on endpoints that are solely a measure of trigeminal activation. This approach, if applied to the present work, would dismiss all data showing nasal irritation in response to stimuli that were undetected by anosmics or that failed to lower inhalation duration. This would have had the same effect as employing a nasal localization approach (Van Toller *et al*., 1987) as a measure of nasal irritation sensitivity in normals. All of these approaches ignore indirect evidence (Warren *et al.*, 1994; Walker *et al.*, 1997) that participants are able to report odor and nasal irritation as at least partially separable sensations. Perhaps most importantly, the 'trigeminal only' approach is of questionable ecological validity. In everyday life, individuals often experience odor and nasal irritation together. Thus any efforts to understand, and then minimize, severity of nasal irritation must contend experimentally with the frequent occurrence of this sensation along with odor.

Thirdly, the limited amount of work in which breathing has been measured along with odor and nasal irritation indicates that considerable odor sensation, and even some weak nasal irritation, may be present without any reduction in inhalation volume (Warren *et al.*, 1994). Phenethyl alcohol and *n*-amyl acetate were presented at concentrations thought to be well below the trigeminal threshold. Both elicited far more odor than nasal irritation, suggesting robust ability to separate the two sensations, but no breathing changes. Acetic acid, a compound for which relative trigeminal stimulatory effectiveness is much higher, elicited comparable levels of odor magnitude but much greater nasal irritation and a clear decline in inhalation volume. These observations are consistent with the ability to report odor and nasal irritation separately. In addition, they indicate that olfactory stimulation will have little or no effect on nasal irritation or inhalation volume in the complete absence of trigeminal stimulation. For this reason, it may be appropriate to view the decline in inhalation volume we observed as reflective of nasal irritation that is due, in most everyday instances, to low-level stimulation of nasal trigeminal afferents combined with much greater olfactory nerve activation.

The generality of our findings and the validity of our working hypothesis are best explored using additional stimuli. Propionic acid likely activates the trigeminal system via stimulation of polymodal nociceptors. Examination of data based on compounds that stimulate other classes of trigeminal afferents, as well as those that differ in terms of relative olfactory versus trigeminal stimulatory effectiveness, would shed light on this question. If the general pattern of results we observed is proven robust, we will attempt to extend the use of breathing parameters to the case of mixtures of varying complexity. A number of applied problems in occupational and environmental health might be attacked more effectively if a reliable and sensitive non-verbal correlate of irritation were validated.

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