A Comparison of Two Methods for Determining Nasal Irritant Sensitivity

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ABSTRACT

Nasal irritation and irritant-induced reflexes (rhinorrhea and congestion) are prominent symptoms associated with indoor and ambient air pollution, and marked heterogeneity in individual sensitivity has been suggested. Nevertheless, there is currently no generally accepted functional index of nasal irritant sensitivity available for clinical use. To address this issue, we compared two objective measures of nasal irritant sensitivity: a CO_2 detection task, and CO_2-induced transient disruption of respiratory pattern (pulsed CO_2 acting as an odorless irritant). Using a respiratory flow thermocouple to produce a continuous recording of respiratory pattern, we challenged 20 normal adult volunteers (13 males and 7 females, average age 39.4 years) with brief (approximately 3 second) pulses of the odorless irritant carbon dioxide. Increasing levels of CO_2 (10–70%, vol/vol), paired with filtered air in random order, were presented unilaterally by nasal cannula of fixed geometry, synchronized with the inspiratory phase of the respiratory cycle. All subjects yielded CO_2 detection thresholds, whereas within the constraints of the testing method (subjective irritation rating ≤ "very strong"), only 13 of 20 subjects (65%) exhibited transient disruption of their breathing pattern. Further, although decreased respiratory volume (indirectly measured) appeared to be a common feature, several distinct patterns of respiratory alteration were observed, rendering objective scoring more difficult. Finally, some subjects showed CO_2-induced respiratory disruption intermittently from trial to trial, implying that rapid adaptation occurs. Determination of the CO_2 detection threshold therefore appears to be the more objective and consistently applicable endpoint for determining individual nasal irritant sensitivity. (American Journal of Rhinology 11, 379–386, 1997)

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Presented at the American Thoracic Society/American Lung Association Annual Meeting, May 10–15, 1996

Supported by United States Centers for Disease Control Agency for Toxic Substances and Disease Registry

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In a variety of indoor and ambient air pollution investigations, eye, nose and throat irritation have been among the symptoms most commonly reported by exposed individuals.[1–5] Accordingly, inter-individual differences in trigeminal irritant sensitivity are of considerable clinical, scientific, and regulatory interest. Respiratory pattern alterations were first proposed as an index of individual perceptual acuity to nasal irritants by Dunn, Cometto-Muñiz, and Cain.[6] Citing earlier work on respiratory reflexes,[7,8] the investigators used the odorless compound, carbon dioxide (CO_2), as a provocation stimulus, and recorded alterations in respiratory behavior by documenting temperature changes at the nares during breathing. Using a technique in which a subject's breathing pattern was regularized by a metronome (the CO_2 stimulus was controlled by the subject's insertion and removal of a cannula from one (or both) nostrils), the investigators documented a tendency of subjects to halt (or plateau) inspiration when an irritant stimulus of sufficient intensity was present; they termed this response "transient reflex apnea."

Further investigations applied this technique to studying variations in nasal irritant sensitivity as a function of age, gender, and smoking status, finding that younger subjects, females, and nonsmokers generally exhibited this response at a lower stimulus concentration, as well as giving higher subjective intensity ratings for a given stimulus concentration.[9–13] Since the original publication of the work of Dunn

et al. documenting "transient reflex apnea," two additional objective measures of nasal trigeminal response—both of which are electrophysiologic in nature—have been reported. These are 1) the "negative mucosal potential" that occurs transiently with nasal irritant stimuli;[14,15] and 2) stimulus-evoked potentials on electroencephalography (chemosomatosensory evoked potentials).[16,17]

In this experiment, we undertook to replicate portions of Dunn, Cometto-Muñiz, and Cain's work, as well as to evaluate aspects of the respiratory response to nasal irritants (i.e., changes in respiratory timing) that could not be examined using the original methodology. To achieve these ends, CO_2 was retained as the irritant of choice, but the method of stimulus presentation was modified. The criteria for evaluating these two methods included 1) objectivity of endpoint; 2) response elicitable dependably at (and above) a given stimulus concentration; and 3) response elicitable from the majority of experimental subjects.

METHODS

The study was cross-sectional, using volunteers over the age of 18 years who were, for the most part, either students or employees at a university-affiliated laboratory complex. Exclusion criteria included, among female subjects, current pregnancy (in order to avoid any temporal association with adverse pregnancy outcomes, which occur with a nontrivial background frequency). Although neither asthmatics nor individuals with self-identified nasal allergies were excluded, subjects with chronic bronchitis, emphysema, cystic fibrosis, or with prior episodes of angioedema or anaphylaxis were. Before testing, all subjects read and signed an informed consent document approved by the Institutional Review Boards of both the University of California, San Francisco and the University of California, Berkeley. Subjects then completed a questionnaire with items pertaining to demographics, prior otolaryngologic diagnoses, respiratory and other allergies, smoking history, and self-reported reactivity to common environmental stimuli. The differentiation between "rhinitic" and "nonrhinitic" subjects was based upon the response to the question: "Do you have regular episodes of sneezing, runny nose, nasal itching, or nasal congestion?"; additional questions regarding potential nasal allergies were administered to those answering "yes."

The exposure apparatus (Fig. 1) consisted of two banks of electronically controlled solenoid valves connected to rotameters (flow meters) with individual metering valves. Medical grade compressed air (Puritan-Bennett) was fed into one bank of valves, and CO_2 into the other. No attempt was made to humidify the gaseous stream, since CO_2 is readily soluble in water (as noted in the "Results" section, significant irritation from dry air alone was apparently not a problem). The rotameters were calibrated on a daily basis to deliver 0–70% CO_2 vol/vol at a total rate of 5 L per minute; the flow rate for air and CO_2 for each pair of flow meters varied reciprocally to deliver a constant total volume at each 10% concentration step. A solid-state timing device was used to precisely control stimulus duration, and CO_2 stimulus levels were selected—and CO_2 pulses triggered—manually. No attempt was made to mask the sound of the solenoid valves. Testing took place under room air conditions. Subjects were instructed not to eat, drink (other than water), or smoke (if applicable) for one-half hour before testing; they were also asked not to wear perfumes, colognes, or aftershaves on the day of testing.

The output of each bank of rotameters was conveyed to the subject via a separate 7-foot length of respiratory tubing (Model 2002; Salter Labs, Arvin, CA); the two outputs were then combined using a polyethylene "Y" connector, which rested on the subject's upper back. Mixed stimuli were delivered to the left nostril via a disposable, nonocclusive nasal cannula (Salter Model 1606). The right nostril of each cannula was occluded with paper tape, and was equipped with a standard respiratory flow thermocouple (Model 1221; Pro-Tech Services, Inc., Woodinville, WA). The thermocouple, in turn, was connected to a thermocouple-to-analog converter (Model TAC-386-TF; Omega Engineering, Inc., Stamford, CT), the output of which fed one channel ("airflow") of a strip chart recorder (Omniscribe B117-5; Houston Instruments, Austin, TX) run at 20 cm per minute. A second channel on the strip chart recorder ("event") registered a rectangular waveform corresponding to the electrical signal from the control unit to the solenoid valves (i.e., stimulus timing and duration). Nasal cannulae were replaced and the airflow thermocouple disinfected between research subjects.

On the day of testing the procedure was explained to the research subject, the nasal cannula fitted, and the subject was encouraged to breathe in a steady pattern while recording took place. Recording was stopped and the periodicity of breathing was determined from the strip chart record; the control unit was then adjusted to produce pulses whose duration corresponded to half of a breathing cycle plus 1 second (generally, 3.0 to 3.5 seconds). Recording was resumed and, while the subject was breathing steadily, a pulse of pure air (identified as such) was administered, beginning in late expiration. After initial recording, stimulus duration was further adjusted (as needed) to occupy the last second of expiration and the succeeding inspiratory phase. Any stimulus-related disruption of breathing pattern was noted, and up to three training trials were used to help subjects maintain a reasonably steady breathing pattern with "blank" (0% CO_2) stimuli (Fig. 2).

Sensory testing consisted of three elements: discriminatory testing, psychophysical rating, and documentation of respiratory pattern during testing. Subjects were told that the purpose of respiratory pattern monitoring was to control the timing of stimuli; however, they were not informed that their respiratory pattern itself was of interest. Trials consisted of pairs of stimuli—one air and the other CO_2 diluted in air—with the two presented in random order and separated by 12 to 15 seconds. The subject is alerted to impend-

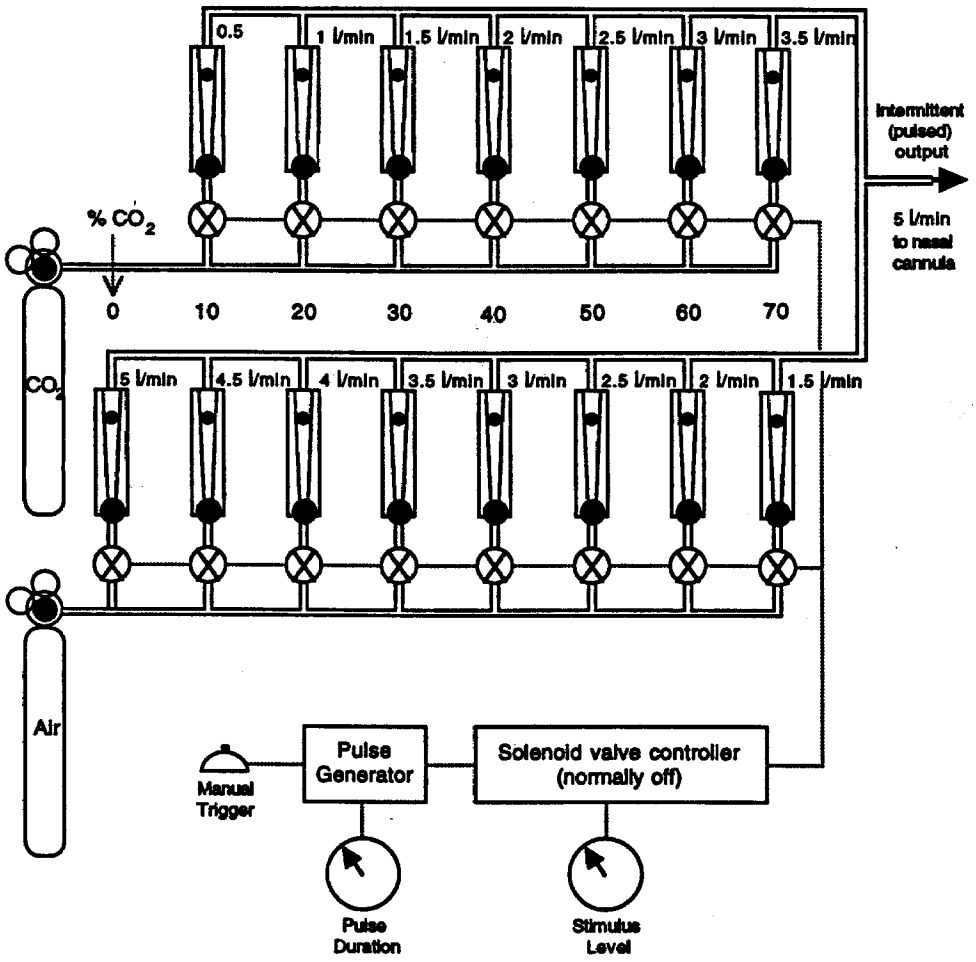

Figure 1. Schematic diagram of carbon dioxide dilution apparatus. Stimuli were triggered during last second of expiration in order to equilibrate CO_2 concentrations in delivery tubing before onset of inspiration.

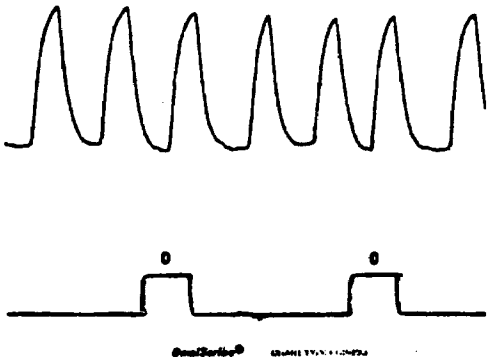

Figure 2. Respiratory tracing showing minimal response to inhaled stimuli. Lower tracing represent 0% CO_2 "blanks" with pulse duration = 3.5 seconds; upper tracing represents relative temperature at nares (inverted scale).

ing stimuli by the examiner ("This is A . . . and this is B"). After each stimulus pair, recording was suspended and the subject was asked to state which stimulus—"A" or "B"—was more irritating (a response of "no difference" was also permitted). If irritation was experienced, subjects adjusted the knob on a rotary potentiometer calibrated with the words "none," "slight," "moderate," "strong," "very strong," and "overpowering" (descriptors used by Clausen et al.[18]) to rate the more irritating stimulus. The potentiometer itself was calibrated to output 0–5 volts DC (continuously variable) to a digital voltmeter (0.01 volt resolution) that was read and recorded by the experimenter for each trial. The subject was asked to return the dial to "none" (0 volts) between each set of stimuli. Stimuli were presented in an ascending series, beginning at 0% CO_2, with three trials conducted at each level, and with approximately one minute allowed between trials. Subjects were blinded with regard to stimulus order and progression, and care was exercised not to provide auditory (or other) cues as to stimulus level. Testing stopped when subjects rated stimuli as "very strong," when the highest stimulus level was reached, or when subjects requested that testing be stopped. The "CO_2 detection threshold" was defined as the lowest concentration at which the subject correctly discriminated all three CO_2 stimuli from air, whereas the "respiratory disruption

threshold" was the lowest level at which a discernible change occurred in respiratory depth, waveform, or timing.

Demographic, stimulus discrimination, and psychophysical rating data were entered in an Apple Macintosh model 6115-CD computer using a standard spreadsheet program (Excel v. 4.0, Microsoft Corporation, Redmond, WA), which was also used to generate graphical output. Group means—geometric and arithmetic—were calculated using the StatView Student statistics package (Abacus Concepts, Berkeley, CA). Area-under-curve measurements were made using NIH Image, Version 1.60 (National Institutes of Health, Bethesda, MD).

RESULTS

Demographic characteristics for the 20 volunteers tested between June 22 and November 16, 1995 are summarized in Table I; a predominance of male, nonsmoking, and nonrhinitic subjects is apparent. The mean CO_2 detection threshold for the overall group was 29% (arithmetic) and 27% (geometric) (see histogram, Fig. 3). Irritation ratings for paired air stimuli ("blanks") averaged 0.06, and ranged from 0.00 to 0.43 (on the 0.00–5.00 scale); 12 of 20 subjects rated all blanks at 0.00. This is consistent with a lack of significant mucous membrane irritation due to a drying effect alone. Significant systemic absorption of CO_2—as manifest by a pharmacologic hyperpnea—was also not apparent, having been guarded against by the low flow rate, nonocclusive nasal cannulae, and high stimulus intermittency (low "duty cycle").

Thirteen of 20 subjects (65%) exhibited transient disruption of their respiratory pattern in relationship to pulsed CO_2 stimuli (Table II), a finding that sometimes occurred on an intermittent basis from trial to trial (see discussion). All CO_2 detection trials, on the other hand, were consistently correct at levels exceeding the threshold. In contrast to CO_2 detection thresholds, respiratory disruption thresholds were distributed nearly symmetrically, with an arithmetic mean of 33% (Fig. 4). Among the subjects who exhibited irritant-induced alterations in respiratory pattern, the (arithmetic) mean thresholds for CO_2 detection and for respiratory disruption were 28% and 34%, respectively. The mean age for

TABLE I

Demographic Characteristics of Experimental Subjects

Gender	Male	13 (65%)
	Female	7 (35%)
Age	Mean	39.4 years
	Range	22–67
Smoking status	Smokers	3 (15%)
	Nonsmokers	17 (85%)
Nasal allergies (Self-reported)	Present	9 (45%)
	Absent	11 (55%)

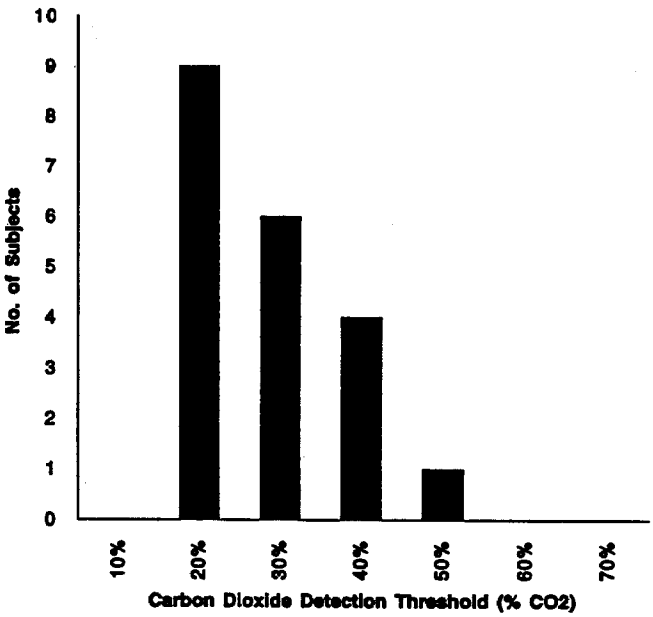

Figure 3. Distribution of CO_2 detection thresholds (n = 20).

the 13 subjects who exhibited respiratory disruption and for the 7 subjects who did not differed significantly (35 and 47 years, respectively; $p < 0.05$); gender, smoking status, and self-reported nasal allergies, on the other hand, did not distinguish the two groups. Individual subjects ranged from 0–3 (10%) concentration steps between the two responses (Fig. 5), with an overall correlation coefficient of 0.48. The mean (±s.e.) sensory rating for stimuli first eliciting respiratory disruption was 2.22 (±0.23), slightly greater than "moderate" on a 0–5 scale (range, 1.18–3.55).

Qualitatively, at least 5 distinct patterns of respiratory alteration were observed, including 1) diminished amplitude of respiration; 2) plateauing; 3) inspiratory pause; 4) exaggerated expiration; and 5) cough (Fig. 6, a–e). The potential significance of these various patterns is discussed below. In addition to the above descriptive categorization, selected respiratory tracings were scanned, and the area under the curve (AUC) measured. "Net" AUCs (area above the baseline minus area below the baseline) were calculated for the highlighted tracings appearing in Figure 6a–e, and were compared to the average of accompanying nonstimulus respiratory cycles. The ratio of the two, expressed as a percentage, were as follows: diminished amplitude, 24%; plateauing, 29%; inspiratory pause, −13%; exaggerated expiration, 21%; and cough, 16%. By comparison, the respective AUC for blank stimuli in Figure 6a–e (compared to nonstimulus respiratory cycles in the same tracing) were 78%, 138%, 138%, 109%, and 98%, respectively. Within the baseline tracing (Figure 2), the AUC for the respiratory cycles accompanying the two blank stimuli were 100 and 105% of the remaining (nonstimulus) cycles.

TABLE II

Occurrence of Transient Respiratory Disruption, by Subject, Stimulus Level, and Trial Number

CO_2 Level	20%			30%			40%			50%			60%			70%		
Trial No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Subject No.																		
1	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
2	−	−	−	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
3	−	−	−	−	−	−	+	+	+	0	0	0	0	0	0	0	0	0
4	−	−	−	−	+	−	−	−	−	+	−	−	−	+	−	+	+	+
9	−	−	+	+	+	−	+	0	0	0	0	0	0	0	0	0	0	0
10	−	−	−	−	−	−	−	+	+	+	−	−	+	+	+	0	0	0
14	−	−	−	+	−	−	+	−	−	+	+	+	0	0	0	0	0	0
15	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−	−	−
16	−	−	−	−	+	−	+	+	+	0	0	0	0	0	0	0	0	0
17	−	−	−	−	−	+	+	−	+	0	0	0	0	0	0	0	0	0
18	−	−	−	−	−	−	−	+	−	−	−	+	+	+	+	0	0	0
19	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0
20	−	−	−	−	−	−	−	−	−	+	−	−	0	0	0	0	0	0

Symbol Key: − = Response absent; + = Response present; 0 = Level not tested (≥ "very strong" rating).

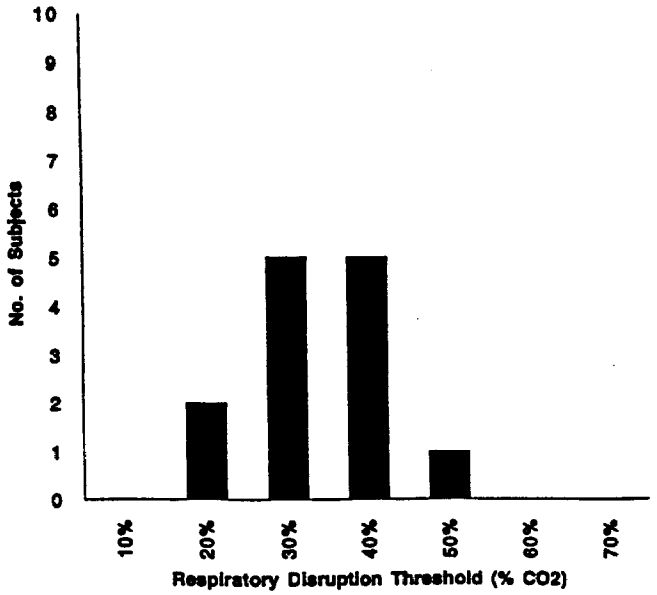

Figure 4. Distribution of respiratory disruption thresholds (n = 13).

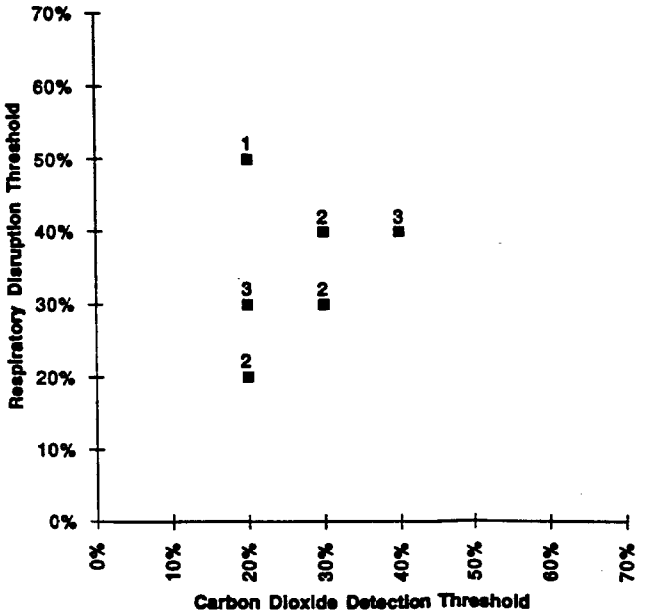

Figure 5. Distribution of respiratory disruption thresholds versus CO_2 detection thresholds; numbers of observations appear above symbols (total n = 13). The correlation coefficient (r) between the two parameters was 0.48.

DISCUSSION

The protocol we employed is a variant of the ascending series method of limits, using a "permissive choice" rather than a "forced choice" paradigm for the discrimination task (i.e., a response of "A," "B," or "no difference" was allowed in order to facilitate rating nonirritating stimuli as such).[19] We were also interested in evaluating the suitability of the respiratory disruption threshold as an index of nasal irritant perceptual acuity. An ideal "threshold" measure exhibits a number of characteristics, including: 1) objectivity; 2) stability; 3) response elicitable dependably at (and above) a given stimulus concentration; and 4) response elicitable from the majority of experimental subjects. The present data do not permit us to comment on the issue of test-retest (or longitudinal) stability, but do shed some light on the remaining issues. First, while the endpoint of tran-

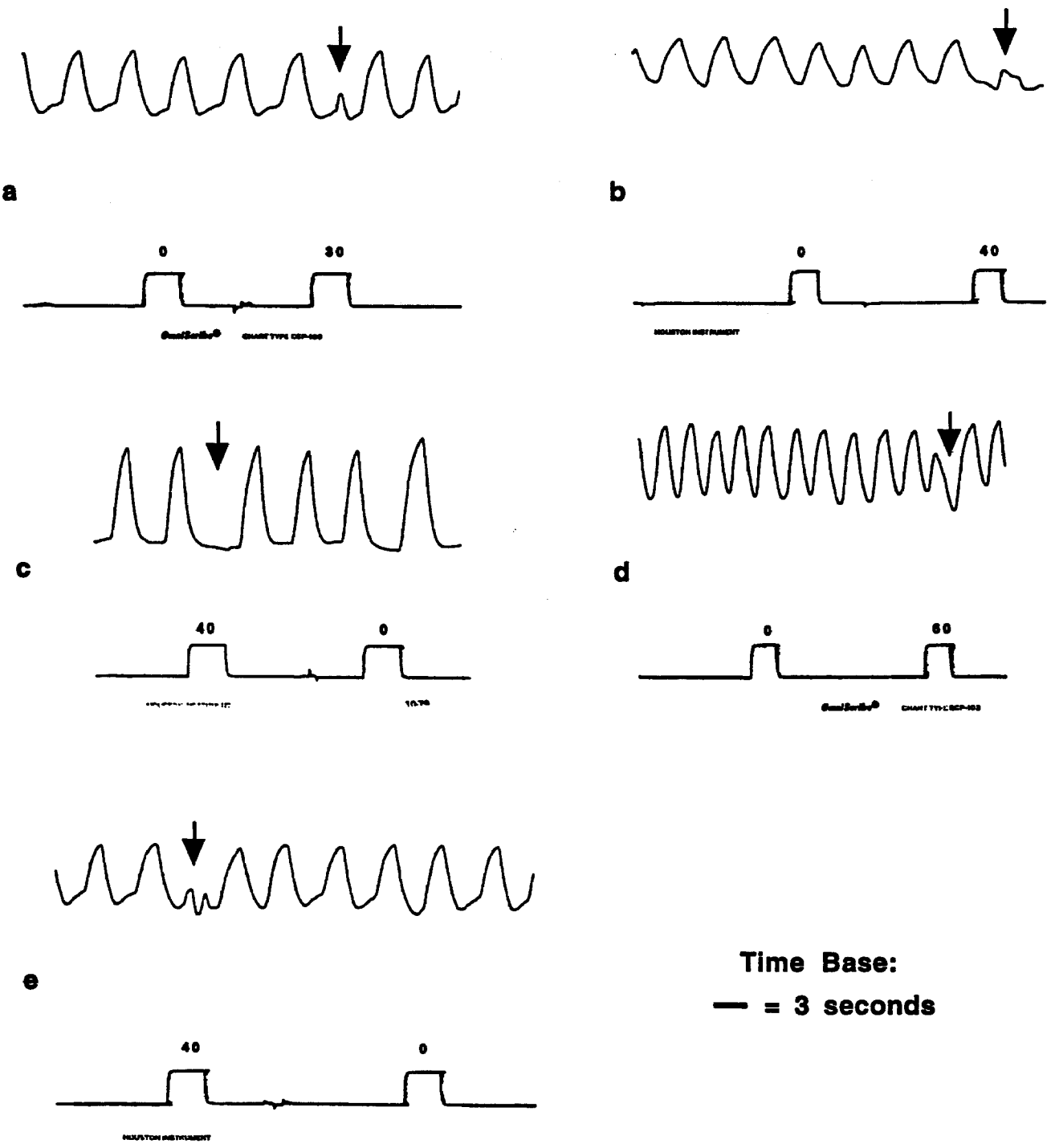

Figure 6. Representative respiratory tracings showing CO_2-induced respiratory disruption. (Numbers above rectangular waves indicate % CO_2.) Specific patterns observed included a) diminished amplitude of respiration; b) plateauing; c) inspiratory pause; d) exaggerated expiration; e) cough. Note in tracing "c" that there is also a minor degree of inspiratory pause evident with the "blank" stimulus.

sient respiratory disruption is objective, it is morphologically more diverse than was previously reported. (Previously published work has only documented two of these patterns: plateauing and diminished amplitude.[9,20])

Although diminished AUC appears to be a common denominator, some ambiguity may therefore arise in the functional equivalence of various respiratory disruption patterns, particularly when they occur in a subtle form. Second, within the limitations of our test method (70% maximum CO_2 level; termination of the testing sequence when stimuli rated as "very strong"), a substantial fraction (35%) of our subjects did not exhibit transient disruption of respiration.

In contrast to earlier published work, nonresponse in our study appeared to be more closely related to age than to smoking status.[6] Finally, only four subjects (Nos. 1, 2, 3, and 19) exhibited respiratory disruption consistently at—and above—the level at which the response first appeared in a given testing sequence; others exhibited the response intermittently. This gives rise to the possible interpretation that transient respiratory disruption may represent the equivalent of a "startle" response in some subjects, and that rapid adaptation (or desensitization) may occur at a given level of exposure.

In terms of the qualitative patterns of respiratory disruption documented, some observations are in order. First, although "inspiratory pause" could represent an extreme form of either reduced amplitude or plateauing, some subjects exhibited this pattern as their initial form of response, and it is therefore treated separately here. Second, both plateauing and cough suggest glottic closure, implying that significant laryngeal irritation has occurred (indeed, several subjects commented that they felt the stronger CO_2 stimuli "in the throat"). Thus, the relative role of trigeminal irritation—versus glossopharyngeal or vagus—is unclear when these response patterns are present. Finally, complex/combined respiratory disruption patterns are very common, and the occasional subject cannot regularize their breathing pattern, despite coaching.

Previous studies addressed a number of additional characteristics of this test, including 1) laterality (dirhinic stimuli elicited the response at a lower stimulus concentration than monorhinic), 2) duration (long stimuli were effective at lower concentrations than short), and 3) subjective equivalence (subjects exhibited the response at a relatively consistent rating of stimulus intensity, as measured on an auditory analog scale).[10] Of these variables, only the last was addressed in our work. In our study, all stimuli were administered monorhinically, and stimulus duration varied *slightly* from subject-to-subject (approximate range, 3.0–3.5 seconds), in order to present a constant interval relative to each subject's respiratory cycle. (Of note, no attempt has been made to correct for these relatively small differences in stimulus duration vis à vis temporal integration of irritation.) In apparent contrast with previous work, the irritancy rating at the threshold for respiratory disruption varied considerably in our subjects, centering at 2.22 (slightly greater than "moderate"), but ranging from 1.18 (barely over "slight") to 3.55 (between "strong" and "very strong") on a 0–5 visual analog scale. (A direct comparison of our rating data with earlier work is not possible, however, since different scaling systems were employed—visual analog scaling in this study and auditory analog scaling in Cometto-Muñiz and Cain.[10])

By contrast to the situation for respiratory disruption, our data indicate that the CO_2 detection task is technically straightforward, and has several methodologic advantages as an endpoint in measuring nasal irritant sensitivity. Principal among these are: 1) a clear-cut endpoint, and 2) the ability to test essentially all subjects, and to do so without reaching unacceptable levels of subjective irritation. As noted in the introduction, electrophysiologic methods have also been developed to document nasal irritation. Since respiratory behavior can be influenced by cognitive factors, electrophysiologic methods may circumvent a major drawback of the respiratory tracing technique—i.e., the need to either test naïve subjects, or to interpret results with attention to a subject's prior testing experience. In light of this and other considerations, electrophysiologic methods deserve systematic investigation as cross-sectional study tools. Notwithstanding these competing techniques, CO_2 detection thresholds provide potentially useful and relatively objective information on individual nasal irritant sensitivity. Issues to be addressed in future work include 1) test-retest stability of CO_2 detection thresholds; and 2) generalizability of CO_2 detection thresholds to other (more environmentally realistic) irritants.

ACKNOWLEDGMENT

Valuable advice was provided by William Cain, Ph.D., Enrique Cometto-Muñiz, Ph.D., Alfredo Jalowayski, Ph.D., and Theodore Witek, Dr.P.H.

REFERENCES

1. Centers for Disease Control. Discomfort from environmental tobacco smoke among employees at worksites with minimal smoking restrictions—United States, 1988. MMWR 41:351–354, 1992.
2. Fisk WJ, Mendell MJ, Daisey JM, et al. Phase 1 of the California healthy building study: A summary. Indoor Air 3:246–254, 1993.
3. Kharrazi M, Smith DF, Blake E, Vance WA, Goldman LR. Real-time surveillance of symptoms and odor complaints during the trial excavation of a toxic waste site. In: Hazardous Waste and Public Health: International Congress on the Health Effects of Hazardous Waste. Andrews JS, et al. (Eds.), Princeton Scientific Publishers, Princeton, NJ, 1994, pp. 806–814.
4. Kreiss K. The epidemiology of building-related complaints and illness. Occup Med 4:575–592, 1989.
5. Shusterman D, Lipscomb J, Neutra R, Satin K. Symptom prevalence and odor-worry interaction near hazardous waste sites. Environ Health Perspect 94:25–30, 1991.
6. Dunn JD, Cometto-Muñiz JE, Cain WS. Nasal reflexes: Reduced sensitivity to CO_2 irritation in cigarette smokers. J Appl Toxicol 2:176–178, 1982.
7. Angell-James JE, Daly MDB. Some aspects of upper respiratory tract reflexes. Acta Otolaryngol 79:242–252, 1975.
8. Ransom SW. Afferent paths for visceral reflexes. Physiol Rev 1:477–522, 1921.
9. Cain WS. A functional index of human sensory irritation. In: Indoor Air '87. Institute for Water, Soil and Air Hygiene, Berlin, 1987, pp. 661–665.
10. Cometto-Muñiz JE, Cain WS. Perception of nasal pungency in smokers and nonsmokers. Physiol Behav 29:727–731, 1982.
11. Cometto-Muñiz JE, Noriega G. Gender differences in the perception of pungency. Physiol Behav 34:385–389, 1985.
12. Stevens JC, Cain WS. Aging and the perception of nasal irritation. Physiol Behav 37:323–328, 1986.
13. Stevens JC. Plantinga A, Cain WS. Reduction of odor and nasal

pungency associated with aging. Neurobiol Aging 3:125–132, 1982.
14. Kobal G. Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. Pain 22:151–163, 1985.
15. Thurauf N, Hummel T, Kettenmann B, Kobal G. Nociceptive and reflexive responses recorded from the human nasal mucosa. Brain Res 629:293–299, 1993.
16. Kobal G, Hummel C. Cerebral chemosensory evoked potentials elicited by chemical stimulation of the human olfactory and respiratory nasal mucosa. Electroencephalogr Clin Neurophysiol 71:241–250, 1988.
17. Hummel T, Kobal G. Differences in human evoked potentials related to olfactory or trigeminal chemosensory activation. Electroencephalogr Clin Neurophysiol 84:84–89, 1992.
18. Clausen GH, Fanger PO, Cain WS, Leaderer BP. The influence of aging, particle filtration and humidity on tobacco smoke odor. In: Climate 200, Vol. 4, Indoor Climate. Fanger PO. (Ed.), VVS Kongres, Copenhagen, 1985, pp. 345–349.
19. Gescheider GA. Psychophysics: Method, Theory, and Application, 2nd ed. Hillsdale, N.J.: Lawrence Erlbaum Associates, 1985, pp. 46–49.
20. Warren DW, Walker JC, Drake AF, Lutz RW. Effects of odorants and irritants on respiratory behavior. Laryngoscope 104: 623–626, 1994. □