Chem. Senses 31: 227–235, 2006

doi:10.1093/chemse/bjj023
Advance Access publication December 29, 2005

Temporal Integration in Nasal Lateralization of Ethanol

Paul M. Wise, Thomas M. Canty and Charles J. Wysocki

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA

Correspondence to be sent to: Paul M. Wise, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA. e-mail: pwise@monell.org

Abstract

Two experiments examined the trade-off between concentration and stimulus duration in nasal lateralization of *n*-ethyl alcohol. In nasal lateralization, a common measure of irritation threshold, subjects receive chemical vapor in one nostril and clean air in the other. Subjects try to determine which nostril received the chemical. Within experimental runs, subjects received fixed concentrations (1650–5000 ppm) of ethanol, and duration was varied to find the shortest, lateralizable stimulus. In Experiment 1, a small group of subjects was tested intensively to obtain stable individual data. In Experiment 2, a larger group was studied using more rapid methods. In both cases, subjects could lateralize increasingly weaker concentrations with longer stimulus presentations. Hence integration occurred. However, more than a twofold increase in duration was required to compensate for a twofold decrease in concentration to maintain threshold lateralization. These results suggest that an imperfect, mass-integrator model can describe short-term integration of nasal lateralization of ethanol.

Key words: chemesthesis, dynamics, irritation, trigeminal

Introduction

When airborne chemicals interact with endings of somatosensory nerves, resulting sensations include burning, warming, cooling, pungency, irritation, and stinging (Bryant and Silver, 2000; Doty and Cometto-Muñiz, 2003). Under some circumstances, chemical somesthesis, or chemesthesis (Green *et al.*, 1990), can cause problems. Government regulators view chemical irritation as a material impairment of health and set many occupational exposure limits based on irritation (NIOSH, 1994; Cain, 1996). Researchers have made considerable progress toward understanding chemesthesis (Bryant and Silver, 2000; Doty and Cometto-Muñiz, 2003; Doty *et al.*, 2004). However, basic data that relate stimulus to sensation remain limited in some areas, especially among humans.

Data concerning temporal integration in detection of nasal irritation are particularly limited. Through temporal integration, sensory systems sum stimulus energy over time to detect weaker signals than they otherwise could (Garner and Miller, 1947; Baumgardt, 1972). Sensory scientists also use the term ''time–concentration trading'' to indicate that a given sensory impact may come from either a long presentation of a weak stimulus or a short presentation of a stronger stimulus. Because integration occurs, researchers must explore the domain of time to fully understand any sensory system.

Studies have shown that suprathreshold irritation grows as stimulus duration increases (see Hummel, 2000; Frasnelli *et al.*, 2003; Hummel *et al.*, 2003). For a fixed concentration, perceived irritation may grow over the course of seconds (Cometto-Muñiz and Cain, 1984; Anton *et al.*, 1992; Wise *et al.*, 2003) or even over many minutes (Cain *et al.*, 1986; Hempel-Jorgensen *et al.*, 1999). Fewer studies have investigated integration at threshold level. However, some studies have examined short-term integration in nasal lateralization of carbon dioxide (CO_2) and ammonia (NH_3) (Wise *et al.*, 2004, 2005).

In a nasal lateralization paradigm, subjects simultaneously receive clean air in one nostril and chemical vapor in the other. Subjects must identify the nostril that received chemical vapor. Humans have little or no ability to lateralize odors (Kobal *et al.*, 1989; Doty and Cometto-Muñiz, 2003; Porter *et al.*, 2005). As mentioned above, chemesthesis is mediated by the somatosensory system, which does register location. Thus, subjects can lateralize chemicals they can feel. Investigators now commonly use lateralization rather than detection to measure chemesthetic thresholds in the nose since subjects usually smell most chemicals at concentrations too low to feel (Doty and Cometto-Muñiz, 2003; Wysocki and Wise, 2003). That lateralization thresholds agree well with detection thresholds measured in subjects who lack a sense of smell supports the validity of the method (Cometto-Muñiz and Cain, 1998; Cometto-Muñiz *et al.*, 1998a).

Wise *et al.* (2004, 2005) measured the briefest stimulus durations that allowed reliable lateralization for a range

of concentrations of CO_2 (~10,000–65,000 ppm) and NH_3 (37–721 ppm). For both compounds, fixed-ratio decreases in concentration could be compensated for by fixed-ratio increases in stimulus duration to maintain a constant level of lateralization performance, at least for durations up to ~3 s. These findings suggest that detection depends in some simple way on total mass delivered to the nose. In a model of integration, detection would depend on the product of time (T) and concentration (C). A model of this type is commonly used in toxicology. Haber's rule states that T multiplied by C equals a constant for a fixed outcome (see Miller *et al.*, 2000).

For lateralization of both CO_2 and NH_3, however, it required more than a twofold increase in stimulus duration to compensate for a twofold decrease in concentration. For CO_2, it required a 3.4-fold increase in duration. Integration was closer to perfect for NH_3 but still fell short: a 2.5-fold increase in duration was required to compensate for a twofold decrease in concentration. These findings support an imperfect mass integrator. A more general form of Haber's rule can model imperfect integration: $C^nT = k$ (Miller *et al.*, 2000). Solving for T yields $T = kC^{-n}$. Since the equation is a power function, plotting threshold stimulus duration versus concentration in log–log coordinates allows data to be fit with a simple linear equation:

$$\log(T) = -n[\log(C)]+k.$$

The term "T" represents the minimum (threshold) stimulus duration required for reliable lateralization. The term "C" represents concentration, and k is a constant. The slope, n, indicates degree of integration. A slope of -1 indicates complete integration or perfect time–concentration trading. A slope less than -1, that is, a steeper slope, indicates imperfect integration. This model described lateralization of CO_2 and NH_3 quite well over the range of concentrations studied (Wise *et al.*, 2004, 2005).

CO_2 stimulates by acidifying tissue (Hummel, 2000; Shusterman and Avila, 2003). NH_3 is a base. The current experiments extend these results to ethanol, a nonreactive, volatile organic compound (VOC). In Experiment 1, a small number of subjects were tested intensively. In Experiment 2, a larger group was tested by more rapid methods.

Experiment 1

Experiment 1 examined the trade-off between stimulus duration and concentration by measuring the duration thresholds for lateralization of six concentrations of ethanol. A small group of subjects was tested intensively to obtain stable individual data.

Materials and methods

Subjects

Three male (aged 25–35) and three female (aged 23–29) healthy nonsmokers participated. Subjects provided informed consent on forms approved by the Institutional Review Board of the University of Pennsylvania. Authors P.M. Wise and T.M. Canty were subjects 4 and 5, respectively. Other subjects were paid. The authors were blind to any conditions that could cue responses trial by trial. Data from the authors resembled data from the other subjects.

Apparatus

An olfactometer presented stimuli to the nose (Figure 1). The design had two key features. First, it allowed stimulus presentation with minimal changes in humidity, pressure, and temperature. Flow from all channels entered the nose at 5 (±0.05) l/min, 35 (±0.7)°C, and 85 (±3)% relative humidity. Second, the device allowed good control of stimulus timing. Fast-response solenoid valves switched from clean air to VOC-laden air very close to the nostril (Wise *et al.*, 2004, 2005).

Three channels for each nostril. Flow from an air pump was first dried and filtered. Next, air was warmed and humidified. The treated air fed three channels for each nostril. One channel (background) continued to the nose with no further treatment. The background channel constituted a steady flow that entered the nostril before and after stimulus presentations. Two other channels passed to a Teflon manifold. One channel (blank) consisted of clean air. The other channel (ethanol) was split into two subchannels. One subchannel flowed through a chamber containing pure ethanol. The other subchannel bypassed the ethanol chamber. The two subchannels rejoined at a mixing chamber before passing to the manifold. The ratio of the flows through the two subchannels, controlled via rotameters, determined the vapor phase concentration of ethanol. Three-way, Teflon solenoid valves determined whether the blank or ethanol passed through

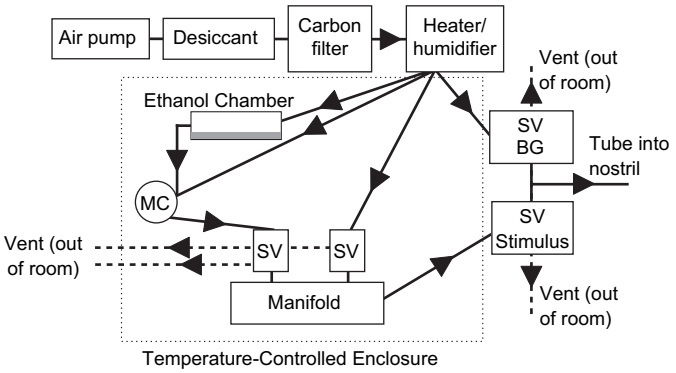

Figure 1 Simplified schematic of the olfactometer. Note that, for the sake of simplicity, the diagram illustrates channels for one nostril only. The full olfactometer has duplicates of the parts pictured. MC = glass mixing chamber, SV = three-way Teflon solenoid valve, and BG = background. Arrow heads on lines indicate direction of airflow. Solid lines indicate tubing that carries flow toward the subject. Dashed lines indicate tubing that carries flow out of the room (vent). Flow rate through all channels is controlled by rotameters (not shown). The text provides more detail.

the manifold, that is, whether the nostril in question received ethanol or a blank. When not directed into the manifold, flows were vented from the room. All flows were constant.

Switching at the nose. The background channel passed to a three-way Teflon solenoid valve close to the nose. The valve normally gated flow into a tube that entered the nostril. Flow from the manifold (either ethanol or blank) passed to another valve. The second valve normally gated flow out of the room. To present a stimulus, a computer energized the background solenoid to vent the background flow from the room. After a delay of 10 ms, the computer activated the stimulus solenoid to gate ethanol (or blank) to the nose. The 10-ms delay reduced pressure transients from switching. Timing of switching was controlled by an analog DAQ card (PCI-6023E, National Instruments, Austin, TX). Needle valves (not shown) equalized back pressure between vent lines and the tube that entered the nostril to help reduce pressure transients.

Calibration. All measurements were made at the output of the olfactometer. Experimenters checked flow rate (Gillibrator 2 flow-meter, Gillian Instrument Corp., Wayne, NJ), humidity (Digitron 2020R hygrometer, Topac Instruments, Hingham, MA), and temperature (BAT-12 thermocouple reader, Physiotemp Instruments, Clifton, NJ) for all channels daily, after the device warmed up. A fast-response pressure transducer (CyQ line, custom made, Cybersense, Nicholasville, KY) verified that minimal changes in flow occurred with switching between background and stimulus. Vapor phase concentrations of ethanol were set for both nostrils using a photoionization detector (MiniRAE 2000, RAE Systems, Sunnyvale, CA). Experimenters calibrated the detector daily using a standard gas. Experimenters rechecked flows and concentrations periodically throughout the day, making small adjustments when needed.

Stimulus presentation. Subjects practiced velopharyngeal closure during stimulation. In velopharyngeal closure, subjects use the soft palate to isolate the nasal cavity from the rest of the airways (Kobal and Hummel, 1991). This breathing technique helps prevent fluctuations in pressure and flow in the nasal cavity from respiration. Stimuli were injected through flexible Tygon tubes (4-mm outer diameter) that extended about 0.75 cm into the nostrils. Flow exited the nostrils around the tubing.

Stimuli

All subjects received ethanol at 1650, 2050, 2560, 3200, 4000, and 5000 ppm. Pilot work determined the highest concentration: The apparatus failed to reliably produce concentrations higher than 5000 ppm. Pilot work also determined the lowest concentration: Subjects failed to reliably lateralize concentrations lower than 1650 ppm at durations of 10 s or less. The six concentrations selected divided the resulting range into equal log steps (successive concentration steps increased by a factor of 1.25). In addition, subjects who lateralized 1650 ppm received 1330 ppm.

Procedure

To begin a trial, subjects placed the tubes in their nostrils, established velopharyngeal closure, and clicked a mouse. The mouse click triggered a countdown of 10 s. Beeps accompanied the last 3 s. After the last beep, the computer presented a stimulus of variable duration. The nostril that received ethanol varied randomly from trial to trial. Subjects remained in position for 3 s after the stimulus ended, then recorded which nostril received ethanol. After a 45-s intertrial interval, the computer prompted the subject to begin the next trial. Including the countdown, more than 55 s elapsed between stimulus presentations.

Concentration of ethanol was fixed within threshold runs. The computer varied the stimulus duration according to a 2-up, 1-down staircase procedure (Wetherill and Levitt, 1965) to find the shortest pulse subjects could reliably lateralize. To reduce the chances of spurious thresholds, the protocol required four consecutive correct responses before the first reversal was counted (Wise *et al.*, 2004, 2005). Thereafter, the computer collected six reversals. Consecutive steps changed by 0.10 $\log_{10}$ units. For example, a 1000-ms pulse would increase to 1256 ms (or decrease to 794 ms). Pilot runs suggested this step size for a balance between reasonable speed in data collection and accurate estimation of thresholds. Runs started with stimuli about 20–30% longer than the best estimate of duration threshold. Initial estimates for each subject were based on five to six practice runs at various concentrations. Long starting durations provided a relatively clear sample of the target sensation and helped avoid spurious thresholds. Most runs required about 25–35 trials. Subjects rarely completed more than a single run in a day. If they did complete more than one run in a given day, 15–20 min separated successive runs. Subjects completed at least three runs for each of the six concentrations from 1650 to 5000 ppm. Runs occurred in irregular order (due to scheduling issues, plans for blocked, and random orders failed for some subjects). After this testing was complete, subjects who lateralized 1650 ppm also completed one to two runs at 1330 ppm.

Data analysis

Thresholds at or below the duration where subjects first achieved four consecutive correct responses counted. This criterion was applied to reduce the risks of spurious thresholds. Subjects repeated runs that failed to meet this criterion. For each experimental session, threshold was estimated by averaging across reversals. For each concentration, threshold was estimated by averaging across experimental sessions. For each subject, experimenters plotted threshold duration versus concentration in log–log coordinates. Mass-integrator

models (linear functions) were fit to the resulting curves via least squares regression (see Introduction).

Results and discussion

With decreasing concentration, subjects could successfully lateralize by increasing stimulus duration, at least to some perithreshold concentration which differed across subjects (Figure 2). Reliable lateralization failed below 2050 ppm for subject 5 and below 2560 ppm for subject 6. The other four subjects were unable to reliably lateralize at 1330 ppm, even for pulses as long as 10 s. Threshold pulse duration for the lowest detectable concentration ranged from 2444 to 4911 ms (average = 3403 ms). Linear functions, in log–log coordinates, accounted for 87–96% (mean = 93%) of the variance in thresholds. This result suggests simple integration, that is, that detection depended in some simple way on total mass delivered to the nose (see General Discussion). Geometric

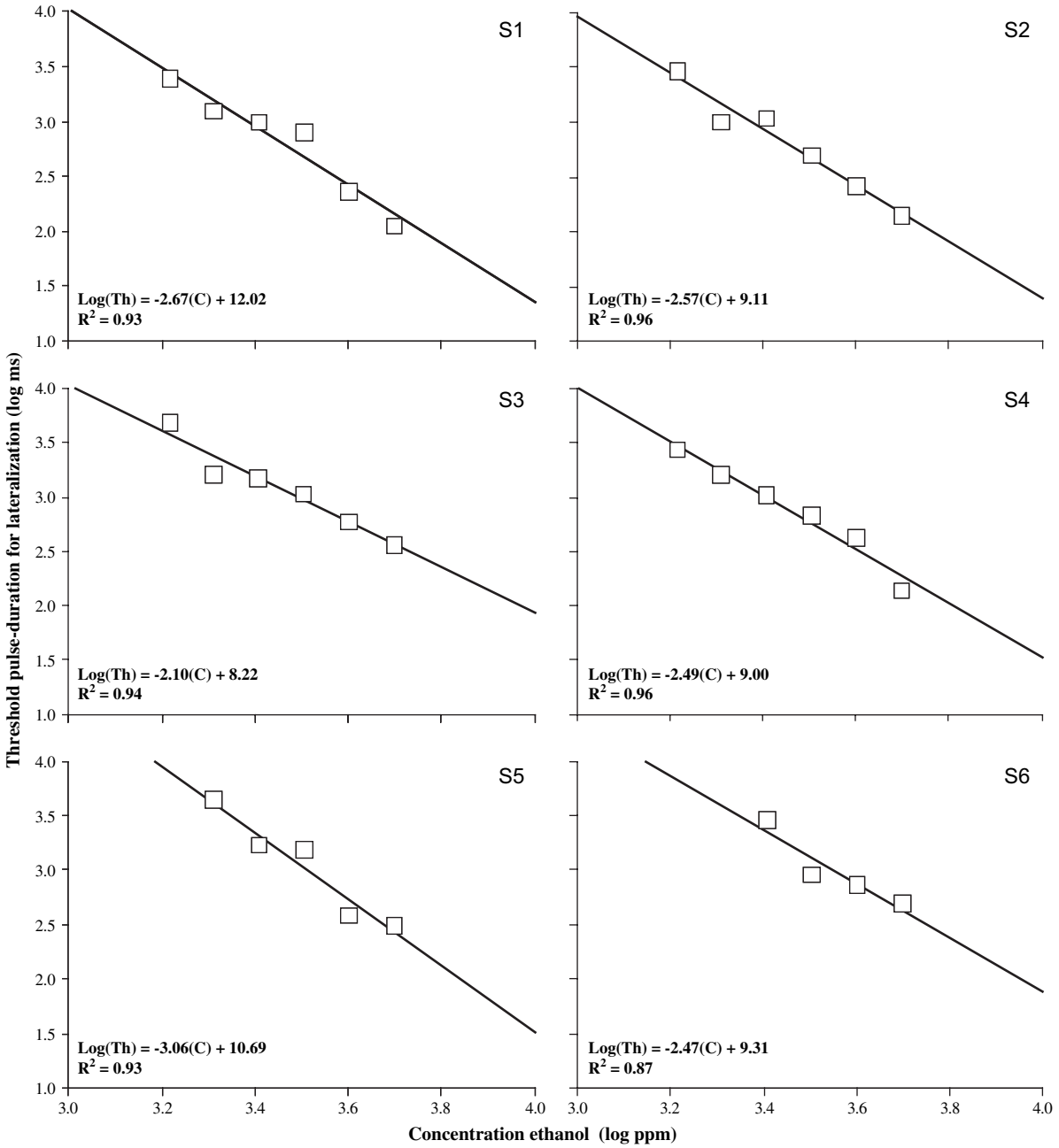

Figure 2 Threshold stimulus duration (log ms) versus concentration of ethanol (log ppm) for six subjects (1–6). Trend lines represent linear functions, fit through least squares regression. Equations for the best-fit functions appear above.

mean slope, calculated across subjects using absolute values, equaled −2.54 (95% confidence interval from −2.24 to −2.89). On average, an increase in duration of ~5.8-fold was needed to compensate for a twofold decrease in concentration to maintain threshold level lateralization. This result suggests imperfect integration.

Experiment 2

Obtaining stable integration functions from individual subjects (as described above) required many sessions spaced over months. In these early stages of model development, such data are desirable. However, such methods might prove impractical for many applications. Experiment 2 explored whether a more rapid method, using less intensive testing of a larger sample of subjects, would provide comparable information regarding the slope of the integration function for ethanol.

Materials and methods

Subjects

Four male (aged 25–35) and nine female (aged 24–32) healthy nonsmokers participated. Subjects provided informed consent on forms approved by the IRB of the University of Pennsylvania. Author P.M. Wise was one subject. Analyses with his data excluded support the same conclusions. Others were paid. P.M. Wise and two females had participated in Experiment 1 (the others had not).

Apparatus

Experiment 2 used the olfactometer depicted in Figure 1.

Stimuli

All subjects received ethanol at 1800, 2450, 3300, and 4500 ppm (successive concentration steps increased by a factor of 1.36). Pilot work and Experiment 1 suggested that most subjects could lateralize concentrations in this range with pulses of 10 s or less.

Procedure

For the most part, procedures followed those of Experiment 1. However, an ascending method of limits was used instead of a staircase procedure (Cain *et al.*, 1988). Stimulus duration began at 100 ms for 4500 ppm, 200 ms for 3300 ppm, 400 ms for 2450 ppm, and 800 ms for 1800 ppm. Pilot work suggested these starting points to be more than three duration steps below threshold for the majority of subjects. After each incorrect response, duration increased by a single, 0.10 $\log_{10}$ duration step. Duration remained the same after each correct response. Runs ended when subjects responded correctly in five consecutive trials. If a subject achieved five correct at the starting duration (a rare occurrence), starting duration was halved and the run began again. Runs required about 10–15 min, on average.

After each trial, subjects recorded which nostril received ethanol. Subjects also rated their confidence in the correctness of each response on a 1–4 scale: "1" indicated a total guess, "2" indicated low confidence, "3" indicated moderate confidence, and "4" indicated high confidence. Confidence was collected to help assess validity of threshold runs (see below).

Concentration was fixed within each session. Subjects completed a single session of about 1 h for each concentration, in irregular order. The first session began with one practice run. The concentration presented in the first session differed across subjects. After this single practice run, all runs counted. Most subjects completed three runs in their first session, including the practice run, and two runs in subsequent sessions. Author P.M. Wise completed four runs per session. Three subjects, who finished their two runs quickly, completed an additional run in one session. One subject completed an additional run in two sessions.

Data analysis

For each threshold run, the concentration at which the subject first achieved five consecutive correct responses served as a threshold estimate. The average across runs estimated threshold for each subject and concentration. The imperfect mass-integrator model was fit to the data in two ways. First, thresholds were averaged across subjects and the model was applied to the group data. Second, the model was fit to functions for individual subjects and slopes were averaged across subjects.

To check the validity of thresholds, trials for each concentration were pooled across runs (for each subject separately). Next, trials were divided roughly into quartiles of increasing stimulus duration. Proportion correct and average confidence were calculated for each quartile. Finally, proportion correct and confidence were averaged across subjects for each concentration. The goal of the analysis was to demonstrate that proportion correct and rated confidence, two classic psychophysical measures of detection performance, both increased as stimulus duration increased. Trends in proportion correct and confidence were assessed via linear regression. Monotonic transforms were applied to make functions approximately linear. In one set of analyses, the normal deviate (Z) transform of chance-corrected proportion correct was the dependent variable, and stimulus duration in log ms was the predictor. In the other set of analyses, rated confidence was the dependent variable, and stimulus duration in log ms was the predictor.

Results and discussion

Subjects could lateralize increasingly weaker concentrations if stimulus duration increased (Figure 3). One subject failed to reliably lateralize at 1800 ppm, even for pulses as long

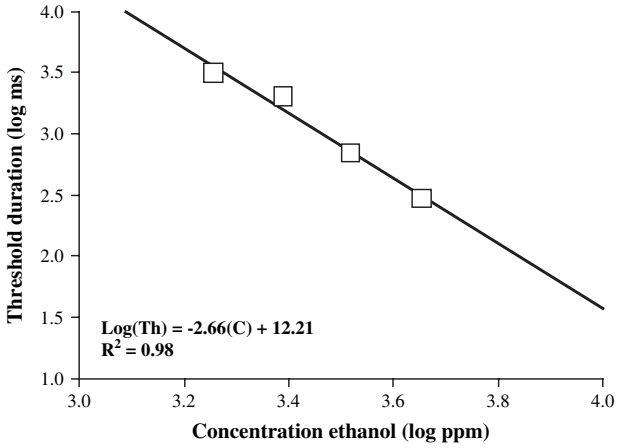

Figure 3 Threshold stimulus duration (log ms) versus concentration of ethanol (log ppm). Thresholds were measured by ascending method of limits. Points represent average values across a group of 13 subjects, except for the lowest concentration, which one subject failed to reliably lateralize. Trend line represents a linear function, fit through least squares regression. The equation for the best-fit function appears above.

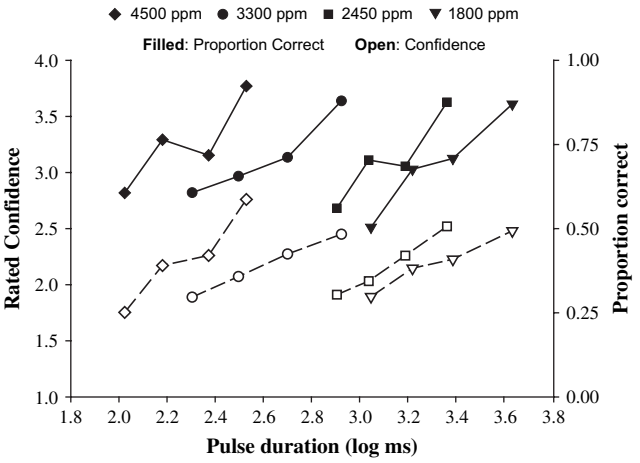

Figure 4 Proportion correct lateralization (filled symbols with solid lines) and rated confidence (open symbols with dashed lines) versus stimulus duration. All trials collected for each subject and concentration were pooled across threshold runs and divided into quartiles of stimulus duration. Values represent averages for each quartile (see text).

as 10 s. Threshold pulse durations follow: 3127 (SD = 904) ms for 1800 ppm, 2013 (SD = 1056) ms for 2450 ppm, 698 (SD = 409) ms for 3300 ppm, and 299 (SD = 118) ms for 4500 ppm. A linear function accounted for 98% of the variance in thresholds for the group function, with a slope of −2.66. Linear fits to data for individual subjects (not shown) accounted for between 69% and 98% (average = 89%) of variance in thresholds. Geometric mean slope, calculated across subjects using absolute values, equaled −2.68 (95% confidence interval from −2.36 to −3.04). On average, an increase in duration of ~6.4-fold was needed to compensate for a twofold decrease in concentration.

Proportion correct lateralization and rated confidence both increased with stimulus duration for all concentrations tested (Figure 4). Proportion correct for the lowest quartiles of stimulus duration was close to chance level, that is, close to 0.50. Proportion correct for the highest quartiles was clearly greater than 0.75, a common definition of threshold. There was a positive association between stimulus duration and proportion correct (significant positive regression coefficient, $P < 0.05$) for 1800, 3300, and 4500 ppm ethanol. For 2450 ppm, the regression coefficient approached significance, $P = 0.06$. There was a significant positive association between rated confidence and stimulus duration for all concentrations. Accordingly, two common measures of detection performance increased with stimulus duration for all four concentrations of ethanol tested. The finding that both proportion correct and rated confidence increased with stimulus duration suggests that responses were under stimulus control, which in turn supports the validity of the results from the ascending method.

Good agreement between Experiments 1 (integration-function slope of −2.54) and 2 (slope of −2.68) also support the validity of the ascending method. This result suggests that the more rapid ascending method, using only one test session per concentration, can yield valid information regarding slopes of time–concentration trading functions. This knowledge can allow studies to progress more rapidly: Experiment 2 involved about 50 h of testing, whereas Experiment 1 required more than 130 h.

General discussion

Short-term integration in nasal chemesthesis

Studies of nasal lateralization of CO_2 (which stimulates by acidifying tissue) and of the base NH_3 found that a simple, but imperfect, mass-integrator model described short-term integration quite well (Wise *et al.*, 2004, 2005). The current results extend these findings to the nonreactive VOC, ethanol. The good linear fits to integration functions in Figures 2 and 3 show that, over some range of concentrations, a fixed-ratio increase in stimulus duration compensated for a fixed-ratio decrease in concentration. That the slopes of the functions were less than −1 indicates imperfect integration, that is, that more than a twofold increase in stimulus duration was needed to compensate for a twofold decrease in concentration. Other studies have demonstrated simple, but imperfect, integration at the suprathreshold level (Cometto-Muñiz and Cain, 1984; Wise *et al.*, 2005). The strong general agreement among the various studies supports the more general form of Haber's rule as a model of short-term integration in nasal chemesthesis (the product of concentration, raised to some exponent, and time is constant for a fixed sensory impact or $C^nT = k$, see Introduction). Pending results on other compounds, the model may well prove widely applicable.

In spite of strong agreement on the general form of time–concentration trading functions, compounds appear to differ in degree of integration. For CO_2 and NH_3, respectively, a 3.1-fold and a 2.5-fold increase in duration compensated for a twofold decrease in concentration. For ethanol, a 5.8-fold increase was required. The reason for the large differences is unclear. Compounds that stimulate through changes in pH, particularly through positive changes, might integrate better over time than nonreactive VOCs. Tests of other acids, bases, and nonreactive VOCs can answer this question. Further, alcohol has broad-ranging effects on the nervous system, including anesthesia (see Weight, 1992; Catlin *et al.*, 1999). According to at least one report, high concentrations of ethanol on the tongue can cause numbing sensations for some subjects (see Green, 1990). If progressive anesthesia occurs, it might undermine integration. Again, studies of other VOCs could help settle the issue.

Only studies of molecules that differ systematically in molecular properties can uncover the structure–activity rules that integration follows. Until further research uncovers such rules, time–concentration trading functions must be determined empirically for each compound. Fortunately, the simple model can allow researchers to characterize time–concentration trading functions by measuring a few, selected points.

Limitations

Previous reports discuss limitations more fully (Wise *et al.*, 2004, 2005). Briefly, the model of integration described above covers all events from injection of the stimulus into the nose to execution of the response. More experiments, for example, studies of dynamics of stimulus concentration in the epithelial or subepithelial layers of the mucosa (Shusterman and Avila, 2003), are needed to elucidate components of this ''black box.'' Further, passive injection of vapor into the nose during velopharyngeal closure is not physiological. The tight experimental control of this method has advantages, but flow rates and patterns of flow through the nasal cavity almost certainly differed from those of natural breathing. Since rates and patterns of flow influence deposition and absorption of volatile compounds in the nasal cavity (e.g., Fredrick *et al.*, 1994, 1998; Morris, 2001; Kurtz *et al.*, 2004), additional studies using natural breathing techniques could help determine how well the current results generalize to natural breathing. Finally, the experiments focus on short-term integration, that is, integration that occurs over less than 10 s. Other rules of integration might apply over minutes or longer.

Basic and practical significance

Previous reports discuss basic significance of psychophysical studies of integration more fully (Cain, 1990; Wise *et al.*, 2004, 2005). Briefly, integration may come from build up over time of stimulus molecules in the mucosa, from neural integrators (either central or peripheral), or from progressive recruitment of different types of fibers. Integration may be imperfect due to clearance or breakdown of molecules, adaptation or saturation of neural integrators, or decreasing numbers of fibers left to be recruited. These possibilities are neither exhaustive nor mutually exclusive. Psychophysics alone cannot relate patterns of perception to specific underlying mechanisms. However, the psychophysical model described above, or extensions thereof, can guide biophysicists, molecular biologists, and physiologists in the effort to uncover the mechanisms that underlie perceived irritation.

Regardless of the underlying mechanisms, the model should prove useful for basic research. Laboratory studies of sensitivity to irritants, both for patients and normal controls, are frequently based on stimulus presentations between 0.25 and 3 s (Hummel, 2000; Shusterman, 2002; Hummel *et al.*, 2003). This includes a good part of the literature on structure–activity relationships in irritant potency (e.g., Abraham *et al.*, 1998, 2003; Cometto-Muñiz *et al.*, 1998b). Often, subjects simply sniff from bottles, with little or no control of sniff duration. Individual differences in sniff duration might become confounded with true differences in sensitivity. Olfactometry can control the duration of stimuli. Nevertheless, individuals might differ in the slopes of their integration functions (see Wise *et al.*, 2004, Figure 1; Wise *et al.*, 2005, Figure 2; Figure 2 above). Researchers who choose different stimulus durations might obtain different pictures of individual differences. Further, if compounds differ in integration, as current results suggest, stimuli of different duration might provide different pictures of differences among compounds. As suggested in Limitations, information gained from letting subjects sample freely may also prove important. It is unclear whether optimal sniffing strategy will prove as important in nasal chemesthesis as it does in olfaction (e.g., Laing, 1983; Sobel *et al.*, 2001), but this possibility exists. Nevertheless, current results on short-term integration suggest that neither free sampling nor stimuli of fixed duration will provide a complete picture of nasal chemesthesis.

The imperfect integration model also has applied significance. Chemesthesis is an important component of the sensory impact of various foods, beverages, and personal products. Imperfect integration means that a given dose, that is, a given number of molecules, will have greater impact when it is delivered over a shorter duration, even within a single, natural inspiration. This may be particularly true for ethanol.

Acknowledgement

This work was supported in part by Kraft Foods through the Term Chair in Chemosensory Psychophysics at Monell awarded to P.M.W.

References

Abraham, M.H., Hassanisadi, M., Jalali-Heravi, M., Ghafourian, T., Cain, W.S. and **Cometto-Muniz, J.E.** (2003) *Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis*. Toxicol. Sci., 76, 384–391.

Abraham, M.H., Kumarsingh, R., Cometto-Muñiz, J.E. and **Cain, W.S.** (1998) *An algorithm for nasal pungency thresholds in man*. Arch. Toxicol., 72, 227–232.

Anton, F., Euchner, I. and **Handwerker, H.O.** (1992) *Psychophysical examination of pain induced by defined CO_2 pulses applied to the nasal mucosa*. Pain, 49, 53–60.

Baumgardt, E. (1972) *Threshold quantal problems*. In Jameson, D. and Hurvich, J. (eds), Visual Psychophysics. Springer-Verlag, New York, pp. 29–55.

Bryant, B.P. and **Silver, W.L.** (2000) *Chemesthesis: the common chemical sense*. In Finger, T.E., Silver, W.L. and Restrepo, D. (eds), The Neurobiology of Taste and Smell, 2nd edn. Wiley-Liss, New York, pp. 73–100.

Cain, W.S. (1990) *Perceptual characteristics of nasal irritation*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses (Vol. 2): Irritation. Marcel Decker, New York, pp. 43–58.

Cain, W.S. (1996) *Overview: odors and irritation in indoor pollution*. In Gammage, R.B. and Berven, B.A (eds), Indoor Air and Human Health, 2nd edn. Lewis, Boca Raton, FL, pp. 23–30.

Cain, W.S., Gent, J.F., Goodspeed, R.B. and **Leonard, G.** (1988) *Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center*. Laryngoscope, 98, 83–88.

Cain, W.S., See, L.C. and **Tosun, T.** (1986) *Irritation and odor from formaldehyde: chamber studies*. In IAQ '86: Managing the Indoor Air for Health and Energy Conservation. ASHRAE, Atlanta, GA, pp. 126–137.

Catlin, M.C., Guizzetti, M. and **Costa, L.G.** (1999) *Effects of ethanol on calcium homeostasis in the nervous system*. Mol. Neurobiol., 19, 1–24.

Cometto-Muñiz, J.E. and **Cain, W.S.** (1984) *Temporal integration of pungency*. Chem. Senses, 8, 315–327.

Cometto-Muñiz, J.E. and **Cain, W.S.** (1998) *Trigeminal and olfactory sensitivity: comparison of modalities and methods of measurement*. Int. Arch. Occup. Environ. Health, 71, 105–110.

Cometto-Muñiz, J.E., Cain, W.S. and **Abraham, M.H.** (1998a) *Nasal pungency and odor of homologous aldehydes and carboxylic acids*. Exp. Brain Res., 118, 180–188.

Cometto-Muniz, J.E., Cain, W.S., Abraham, M.H. and **Kumarsingh, R.** (1998b) *Trigeminal and olfactory chemosensory impact of selected terpenes*. Pharmacol. Biochem. Behav., 60, 765–770.

Doty, R.L. and **Cometto-Muñiz, J.E.** (2003) *Trigeminal chemosensation*. In Doty, R.L. (ed.), Handbook of Olfaction and Gustation, 2nd edn. Marcell Decker, New York, pp. 981–999.

Doty, R.L., Cometto-Muniz, J.E., Jalowayski, A.A., Dalton, P., Kendal-Reed, M. and **Hodgson, M.** (2004) *Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans*. Crit. Rev. Toxicol., 34, 85–142.

Frasnelli, J., Lötsch, J. and **Hummel, T.** (2003) *Event-related potentials to intranasal trigeminal stimuli change in relation to stimulus concentration and stimulus duration*. J. Clin. Neurophysiol., 20, 80–86.

Frederick, C.B., Bush, M.L., Lomax, L.G., Black, K.A., Finch, L., Kimbell, J.S., Morgan, K.T., Subramaniam, R.P., Morris, J.B. and **Ultman, J.S.** (1998) *Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways*. Toxicol. Appl. Pharmacol., 152, 211–231.

Frederick, C.B., Morris, J.B., Kimbell, J.S., Morgan, K.T. and **Scherer, P.T.** (1994) *Comparison of four biologically based dosimetry models for the deposition of rapidly metabolized vapors in the rodent nasal cavity*. Inhal. Toxicol., 6(Suppl.), 135–157.

Garner, W.R. and **Miller, G.A.** (1947) *The masked threshold of pure tones as a function of duration*. J. Exp. Psychol., 37, 293–305.

Green, B.G. (1990) *Effects of thermal, mechanical, and chemical stimulation on the perception of oral irritation*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses (Vol. 2): Irritation. Marcel Decker, New York, pp. 171–192.

Green, B.G., Mason, J.R. and **Kare, M.R.** (1990) *Preface*. In Green, B.G., Mason, J.R. and Kare, M.R. (eds), Chemical Senses (Vol. 2): Irritation. Marcel Decker, New York, pp. 5–7.

Hempel-Jorgensen, A., Kjaergaard, S.K., Molhave, L. and **Hudnell, H.K.** (1999) *Time course of sensory eye irritation in humans exposed to N-butanol and 1-octene*. Arch. Environ. Health, 54, 86–94.

Hummel, T. (2000) *Assessment of intranasal trigeminal function*. Int. J. Psychophysiol., 36, 147–155.

Hummel, T., Mohammadian, P., Marchl, R., Kobal, G. and **Lötsch, J.** (2003) *Irritation of the nasal mucosa using short- and long-lasting painful stimuli*. Int. J. Psychophysiol., 47, 147–158.

Kobal, G. and **Hummel, T.** (1991) *Olfactory evoked potentials in humans*. In Getchell, T.V., Doty, R.L., Bartoshuk, J.B. and Snow, J.B. (eds), Smell and Taste in Health and Disease. Raven Press, New York, pp. 255–275.

Kobal, G., Van Toller, S. and **Hummel, T.** (1989) *Is there directional smelling?* Experientia, 45, 130–132.

Kurtz, D.B., Zhao, K., Hornung, D.E. and **Scherer, P.** (2004) *Experimental and numerical determination of odorant solubility in nasal and olfactory mucosa*. Chem. Senses, 29, 763–773.

Laing, D.G. (1983) *Natural sniffing gives optimum odour perception for humans*. Perception, 12, 99–117.

Miller, F.J., Schlosser, P.M. and **Janszen, D.B.** (2000) *Haber's rule: a special case in a family of curves relating concentration and duration of exposure to a fixed level of response for a given endpoint*. Toxicology, 149, 21–34.

Morris, J.B. (2001) *Overview of upper respiratory tract vapor uptake studies*. Inhal. Toxicol., 13, 335–345.

[NIOSH] National Institute for Occupational Safety and Health (1994) NIOSH pocket guide to chemical hazards (Publication No. 94–116). NIOSH, Cincinnati, OH.

Porter, J., Anand, T., Johnson, B., Khan, R.M. and **Sobel, N.** (2005) *Brain mechanisms for extracting spatial information from smell*. Neuron, 47, 581–592.

Shusterman, D. (2002) *Individual factors in nasal chemesthesis*. Chem. Senses, 27, 551–564.

Shusterman, D. and **Avila, P.C.** (2003) *Real-time monitoring of nasal mucosal pH during carbon dioxide stimulation: implications for stimulus dynamics*. Chem. Senses, 28, 595–601.

Sobel, N., Thomason, M.E., Stappen, I., Tanner, C.M., Tetrud, J.W., Bower, J.M., Sullivan, E.V. and **Gabrieli, J.D.** (2001) *An impairment in sniffing contributes to the olfactory impairment in Parkinson's disease*. Proc. Natl Acad. Sci. USA, 98, 4154–4159.

Weight, E.F. (1992) *Cellular and molecular physiology of alcohol actions in the nervous system*. Int. Rev. Neurobiol., 33, 289–348.

Wetherill, G. and **Levitt, H.** (1965) *Sequential estimation of points on a psychometric function*. Br. J. Math. Stat. Psychol., 18, 1–10.

Wise, P.M., Canty, T.M. and **Wysocki, C.J.** (2005) *Temporal integration of nasal irritation from ammonia at threshold and supra-threshold levels*. Toxicol. Sci., 87, 223–231.

Wise, P.M., Radil, T. and **Wysocki, C.J.** (2004). *Temporal integration in nasal lateralization and nasal detection of carbon dioxide*. Chem. Senses, 29, 137–142.

Wise, P.M., Wysocki, C.J. and **Radil, T.** (2003). *Time–intensity ratings of nasal irritation from carbon dioxide*. Chem. Senses, 28, 751–760.

Wysocki, C.J. and **Wise, P.** (2003) *Methods, approaches, and caveats for functionally evaluating olfaction and chemesthesis*. In Deibler, K. and Delwiche, J.F. (eds), Handbook of Flavor Characterization: Sensory, Chemical and Physiological. Marcel Dekker, New York, pp. 1–40.

Accepted December 6, 2005